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LISTA DE CONTENIDOS

CONTROL TOOLS OF SALMONELLA INFECTIONS IN THE U.S.A.

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Although *Salmonella* enterica serovars are some of the best studied bacterial pathogens, the field still has a long way to go, especially when one considers that (i) they cause significant human morbidity and mortality worldwide; (ii) they have broad host ranges (iii) they are able to establish persistent colonization in some species which serve as reservoirs for transmission/shedding; and (iv) they are increasingly resistant to many antibiotics (Boyle *et al.* 2007). Poultry producers are challenged to improve production while using fewer antibiotics due to increased restriction on antimicrobial usage. Probiotics consisting of live or dead organisms and spores (Patterson and Burkholder, 2003), non-traditional chemicals (Moore *et al.,* 2006), bacteriophages (Higgins *et al.,* 2005), organic acids (Jarquin *et al.*, 2007; Wolfenden, *et al.*, 2007) and others have emerged in the last decades as some of the tools that could be potentially useful in the near future for pathogen control and poultry performance improvement. Our laboratory has evaluated a simple method to select for individual enteric bacteria capable of inhibiting *Salmonella* growth *in vitro* and the ability of selected oxygen tolerant bacteria, in combination, to protect neonatal poults from *Salmonella* infection following challenge (Bielke *et al.*, 2003). We have also been working toward isolation, selection, and further evaluation of probiotic organisms to control food borne pathogens (Tellez, *et al.,* 2006). In the following papers data from the experiments using a lactic-acid bacteria based probiotic and an organic acid mixture to treat and/or prevent *Salmonella* colonization in broiler chicks.

Salmonella is a gram negative rod-shaped bacterium. It is classified as a facultative anaerobe and a member of the family Enterobactericeae. All non-typhoid *Salmonella* serovars, including *Salmonella enterica* serovar Enteritidis (*Salmonella enteritidis*; SE), are motile and have peritricious flagella (Davis et al., 1967). In poultry, infection in young chicks with some SE isolates results in 2% mortality within the first 48 hours of life, and up to 20% morbidity in the first five

days. Clinical signs of infection are anorexia, depression, ruffled feathers, huddling, drowsiness, dehydration, diarrhea, and white pasted vents (McIllroy et al., 1989). *Salmonella enteritidis* can be vertically transmitted from the hen to the egg, or it can be contracted by a young chick from the environment, usually in the hatchery (Borland, 1975). In humans, foodborne salmonellosis can cause diarrhea, fever, and abdominal cramps. Occasionally, the infection can become serious or even fatal, spreading to the blood, bone marrow, or brain. In the United States, it is estimated that 500-1000 persons die each year due to *Salmonella* food borne illness (Foegeding and Roberts, 1994).

Data from the United States' Centers for Disease Control (CDC) (FoodNet) estimates that 76 million people experience foodborne illness every year in the United States. In 2001, surveillance data indicated that the greatest number of foodborne illnesses were caused by *Salmonella*, comprising 40% of all laboratory diagnoses (FoodNet, 2004). Madie et al. (1992) calculated that in the U.S., money lost due to *Salmonella* induced foodborne illness is 1.4 billion dollars annually. This estimate took into account lost human productivity, medical expenses and increased animal production costs. Poultry and poultry products often serve as the vehicle for human *Salmonellosis* (Bean and Griffin, 1990; Persson and Jendteg, 1992), the poultry industry and governmental agencies are focused on reducing or eliminating *Salmonella* both in live birds and in the processing plant (Hargis et al., 2001).

Antibiotic resistance, especially antibiotic resistance due to antibiotics administered to food producing animals, has the attention of both the poultry industry and government agencies. Due to antibiotic resistance of many species of *Salmonella,* antibiotic therapy is frequently not effective at eliminating *Salmonella* infections in poultry (Angulo et al*.,* 2000, Threfall et al*.,* 1997, Manning et al*.,* 1994, Seuna et al*.,* 1980).

In 1990 and 1995, 40% of persons with *Salmonella* infections who required medical attention were treated with antimicrobial agents (CDC, 2000). Even though antibiotics are not required for people with uncomplicated gastrointestinal problems, patients that suffer bacteremia, meningitis, or other

extraintestinal *Salmonella* infections need antibiotics for effective treatment (Angulo et al., 2000). A study, conducted by CDC in 1996, demonstrated that approximately 6% of *Salmonella* culture-confirmed cases involved extraintestinal infection, suggesting that antibiotics were necessary for only 2,400 patients, but as many as 16,000 were treated with antibiotics.

In 1996, 1,272 human *Salmonella* isolates were tested for antimicrobial resistance, 21% were resistant to ampicillin, 10% to chloramphenicol, and 4% to trimethoprim- sulfamethoxazole, but nearly all were susceptible to flouroquinilones and third-generation cephalosporins (CDC, 1996). Thus, flouroquinilones and cephalosporins are frequently the favored treatment for *Salmonellosis* by physicians. Resistance to fluoroquinilones and third-generation drugs could lead to major human health consequences. After the approval of enrofloxacin (a flouroquinilone) for use in food animals in the United Kingdom, *Salmonella typhimurium* DT104 (R-type ACSSuT) isolates resistant to flouroquinolones quickly emerged, mainly among *Salmonella typhimurium* isolates with existing resistance to other antimicrobials. In 1994, none of the *Salmonella typhimurium* DT104 R-type ACSSuT isolates were resistant to flouroquinolones, but by 1996, 14% of the isolates had a decreased susceptibility (Threfall et al., 1997). The CDC found that there was little correlation between the antimicrobial agents used in persons with *Salmonella* infections and development of antimicrobial resistance among human *Salmonella* isolates. The majority of antibiotic resistant *Salmonella* infections are acquired from ingestion of foods contaminated with antibiotic-resistant *Salmonella*e (Angulo et al., 2000).

It was also proposed by Angulo et al., 2000 that there are three ways to support the theory that antimicrobial-resistance among *Salmonella* isolates in humans results from the use of antimicrobial agents in food animals. The first is tracebacks of resistant *Salmonella* in foodborne disease outbreaks to antimicrobial use on farms. The second is increased resistance to flouroquinolones by *Salmonella typhimurium* in the United Kingdom since the approved use of enrofloxacin in veterinary medicine in 1993. The third is similar antimicrobial resistance patterns between humans and animals.

Therapeutic use of several antimicrobial compounds has greatly increased susceptibility of poultry to *Salmonella* infection following withdrawal of antibiotics (Manning et al., 1994; Manning et al., 1992). The use of some antimicrobials such as fosfomycin can cause adverse effects in performance when used in uninfected birds (Fernandez et al., 2001). Seuna and Nurmi (1979) showed that combinations of neomycin/polymixin, or oxytetracyline/neomicin, or dihydrostreptomycin, or furazolidone, and trimethoprim/sulphadiazine proved to be poor in treating intestinal infections caused by the same strain of *Salmonella.* Seuna and Nurmi (1979) also showed that after withdrawal of the drugs a rapid recolonization of *Salmonella* was observed. The use of certain antimicrobial agents increased the number of *Salmonella*-infected chickens (Seuna and Nurmi, 1979).

Barbara et al., (2000) in a human study showed that antibiotic treatment does not affect *Salmonella* excretion and that persistent digestive symptoms are more common among patients treated with antibiotics. Hinton et al., (1990), associated the inclusion of penicillin in a broiler diet with an increase in *Salmonella* shedding. Smith et al., (1965) found that feeding avoparcin and lincomycin to chickens favored colonization of the alimentary tract by *Salmonella typhimurium*, and also enhanced the spread of the organism to contact chickens. Olsen et al., (2001) suggested that the heavy use of antimicrobial agents can cause an increase in the outbreaks of fluoroquinolone-resistant *Salmonella enteritidis.*

In 2001, CDC reported that among the 1419 paratyphoid *Salmonella* isolates reported to the CDC laboratories, 28% were resistant to one or more antimicrobial agents, 22% were resistant to two or more agents, 12% were resistant to five or more agents, and 3% were resistant to eight or more agents. Of the 176 isolates resistant to five or more agents, 114 (65%) were serotype Typhimurium. Of the 40 isolates resistant to eight or more agents, 31 (77%) were serotype Newport. The antimicrobial agents to which the 1419 *Salmonella* demonstrated the highest prevalence of resistance were tetracycline, sulfamethoxazole, streptomycin, and ampicillin: 280 (20%) were resistant to tetracycline, 251 (18%) were resistant to sulfamethoxazole, 241 (17%) were resistant to

streptomycin, and 247 (17%) were resistant to ampicillin. Among the 282 *Salmonella enteritidis* isolates, 40 (14%) were resistant to one or more antimicrobial agents and 16 (6%) were multidrug resistant (NARMS Annual Report, 2001).

Antibiotics can reduce *Salmonella* in infected poultry (Goodnough and Johnson, 1991; Muirhead, 1994). However, Manning et al. (1992, 1994) have reported increased *Salmonella* colonization following treatment of chickens with selected antibiotics, possibly as a result of destroying the normal bacterial flora in the gastrointestinal tract that serve as a natural barrier to *Salmonella* infection. Also, Kobland et al. (1987) and Gast et al. (1988) have recovered antibiotic resistant *Salmonella* from experimentally challenged birds treated with antibiotics. An intense search is currently underway for efficacious non-antibiotic treatments to control bacterial diseases. Immediate concern arises from the significant increase in antibiotic resistant bacteria in the medical environment, rendering even some of the newest anti-microbials ineffective. This increase is attributed to over prescribing of antibiotics by physicians, antibiotic abuse in hospitals, and use of therapeutic and subtherapeutic levels of antibiotics in commercial animal production to promote growth (Brundtland, 2000). Increased political and consumer pressures have resulted in a significant reduction of drug use in the animal industries with potential for completely eliminating the use of antimicrobials in animal production during the next decade.

The United States Food and Drug Administration (FDA) has already proposed the withdrawal of flouroquinolones in poultry production because of the increase in flouroquinolone resistant *Campylobacter* infections in humans (Schwetz, 2002). Additionally, Mølbak et al. (2002) reported an increase in flouroquinolone resistance in foodborne illnesses caused by *Salmonella enterica* serotype enteritidis (SE) from 0.8% in 1995 to 8.5% in 2000. It has become imperative that the commercial poultry industry develop effective and inexpensive methods or products to treat bacterial infections in sick birds in the event that antibiotic use is eliminated completely from animal production, a trend which is currently being realized in Europe. Due to this emergence of microbes resistant to antibiotics ('antimicrobial resistance') that are used to treat human and animal

infections, the European Commission (EC) decided to phase out, and ultimately ban since January 1, 2006, the marketing and use of antibiotics as growth promoters in animal feed. The use of antibiotics is only allowed by veterinary prescription for direct applications or as medicated feed. These restrictions are deemed necessary as antimicrobials may lead to the selection of resistant bacterial strains in animals that could be transferred to humans, by direct contact or via foodstuffs, and subsequently lead to an impairment of the efficacy of antibiotics used in therapy of human infectious diseases (Anadon, 2006).

Competitive exclusion (CE) was first described by Nurmi and Rantala (1973) and has been demonstrated to be an effective method of control of *Salmonellosis* in commercial poultry flocks. Nurmi and Rantala (1973) placed baby chicks in a clean environment and challenged them with *Salmonella*. Some of the chicks then received CE, a crude culture containing adult enteric microflora. The control group that did not receive the CE was very susceptible to the *Salmonella* infection, but in the CE group 77% of the chicks were protected against infection. Other laboratories have extensively studied this concept. In a similar study, host specificity of the CE cultures was suggested because only chicken cecal microflora was able to protect chickens, not horse or cattle feces (Rantala and Nurmi, 1973).

The principle behind CE is based upon competition between normal non-pathogenic gut microflora and pathogenic bacteria for colonization within the host gastrointestinal tract but the specific mechanisms are not well understood. The World Health Organization (1994) defined competitive exclusion as follows:

> "In relation to the avian intestinal tract, 'normal gut flora' is an undefined preparation of live obligate and facultatively anaerobic bacteria, originating from normal, healthy adult individuals of an avian species, which is free from specific pathogenic organisms and is quality controlled. The purpose of such a preparation is to compensate for any deficiencies in the composition of the normal intestinal microbiota that relate to the natural control of undesirable micro-organisms and arise from modern systems of poultry production."

Mead (2000) proposed four ideas with regard to how CE is able to exclude pathogens. The first is competition for receptor sites, a niche, where in the formation of a mat of cells (glycocalyx) with inter-connecting fibers formed by the microflora forms an effective physical barrier against *Salmonella*e. The second is the production of volatile fatty acids (VFAs) by the normal microflora. Volatile fatty acids are produced as the result of metabolism of some intestinal bacteria. In an environment with a pH below 6.0, the VFAs are in an undissociated state. In this state the acetic, proprionic and butyric VFAs are inhibitory to *Salmonella*e. A third possibility is production of bacterosins, but their mode of action and specific properties is still not completely understood. The fourth is that there exists a competition between pathogens and native microflora for limiting nutrients.

The formation of healthy, diverse, and well-established microflora is known to reduce susceptibility of poultry to infection with a variety of *Salmonella* serovars (Baba et al., 1991, Blankenship et al., 1993, Hollister et al., 1994, and Impey et al., 1987). Anderson et al., (1984), and Hollister et al., (1999), showed that a culture of adult chicken cecal contents was protective in turkey poults when the poults were challenge with *Salmonella typhimurium*. Corrier et al., (1993) obtained a reduction range of 30 to 90% on *Salmonella enteritidis* cecal and organ colonization in leghorn chicks when using a CE culture combined with dietary lactose. Blankenship et al, (1993) applied a CE culture in two steps: first, CE was sprayed on broiler chicks in the hatchery followed by administration in the first drinking water. At the end of the grow-out period processed carcasses coming from the treated broiler chickens had significantly less *Salmonella* (from 41% in the controls to 10% in treated). Palmu and Camelin (1997), showed similar reductions of *Salmonella* in the processing plant (neck and skin samples) by treating birds with Broilact^{R} a commercial CE culture. Nuotio et al., (1992) used Broilact^{R} to protect newly hatched chicks against intestinal colonization and invasion by *Salmonella* enteritidis, the treatment proved to effectively prevent both colonization and organ invasion by *Salmonella enteritidis*.

In an experiment by Hume et al., (1997) day-of-hatch broiler chicks were treated by oral gavage with PREEMPT (a commercial CE culture). At 4 hours, one day or two days post-treatment the chicks were challenged by oral gavage with either 10^2 or 10^3 *Salmonella* spp. The results of the study showed an average reduction of *Salmonella* of 6 $\log_{10}$ units and proved that chicks can be protected as early as 4 hours post treatment. A study by Wierup and Wold-Troell (1988), aimed to evaluate the effect of CE if adopted nationwide in the poultry industry and concluded that CE cultures reduce *Salmonella* contamination under field conditions in ways similar to those found earlier in experimental studies. Seo et al., (2000) treated molting birds with antibiotics and then applied CE reducing this way *Salmonella* shedding. In the same study CE alone failed to provide reductions in *Salmonella* shedding.

In order for CE cultures to be safe, they cannot contain antibiotic resistant organisms. Antimicrobial residues present problems with establishment of any effective CE product. Bailey et al., (1987) suggested that the effect of CE is reduced when feed additives that contain antimicrobials such as bacitracin and nicarbazin are used. Other studies have also supported this finding. Anderson and co-workers (1984) showed that antibiotic treatment before administering CE reduced the effectiveness in poults. McReynolds et al., (2000) found that enrofloxacin residues decreased the establishment of PREEMPT while tylosin tartrate, in contrast, increased the effectiveness of the CE microflora in chicks through an unknown mechanism, while sulfadimethoxine residues had no effect. Nurmi, in 1974 found that the use of bacitracin had no effect in the level of protection of a CE culture. Some antibiotics and their residues can have adverse effects on CE cultures, while other antibiotics may have no effect, therefore considerations should be taken with the use of antimicrobials in conjunction with CE treatment.

A probiotic is defined as a live microbial food supplement which benefits the host by improving its intestinal microbial balance (Isolauri et al., 2001). The presence of normal gut microflora may improve the metabolism of the host bird in various ways, including absorptive capacity (Yokota and Coates, 1982), protein metabolism (Salter et al., 1974), energy metabolism and fiber digestion

(Muramatsu et al., 1994), energy conversion (Furuse and Yokota, 1984) and gut maturation (Furuse et al., 1991). Balanced colonic microflora and immunostimulation are major functional effects attributed to the consumption of probiotics (Isolauri et al., 2001). Many probiotic effects are mediated through immune regulation, particularly through balance control of proinflammatory and anti-inflammatory cytokines (Ghosh et al., 1998; Neish et al., 2000).

The GIT serves as the interface between diet and the metabolic events that sustain life. In poultry, intestinal villi, which play a crucial role in digestion and absorption of nutrients, are underdeveloped at hatch (Uni et al., 1995) and maximum absorption capacity is attained by 10 days of age (Noy and Sklan, 1997). Understanding and optimizing the maturation and development of the intestine in poultry will improve feed efficiency, growth and overall health of the bird. In the immediate post hatch period birds must undergo the transition from energy supplied by the endogenous nutrients of the yolk to exogenous carbohydrate rich feed. During that critical time dramatic changes occur both in the intestinal size and morphology (Uni et al., 1995). Maturational changes also affect the epithelial cell membranes, a major mechanical interface between the internal environment of the host and the luminal contents (Rozee et al., 1982). Studies on nutrition and metabolism during the early phase of growth in chicks may help in optimizing nutritional management for maximum growth (Nir, 1995). By dietary means it is possible to affect the development of the gut and the competitiveness of both beneficial and harmful bacteria, which can alter not only gut dynamics, but also many physiologic processes due to the end products metabolized by symbiotic gut microflora. Additives such as enzymes, probiotics and prebiotics are now extensively used throughout the world. The chemical natures of these additives are well understood, but the manner by which they benefit the animal is not (Bedford, 2000).

Administration of CE has been tested using several mechanisms; oral gavage, in the drinking water, and spray or lyophilized alginate beads. Corrier et al., (1993) reported that the methods of administration mentioned before have all proven to be effective in the control of *Salmonella* enteritidis in the cecal contents. In the same study lyophilized alginate beads showed less

protection that the other methods of administration. One method of application that can be easily adopted by the poultry industry is the administration of CE in the drinking water. It was also described that chickens could be reinfected with *Salmonella* right after ceasation of antibiotic treatment (Seuna et al., 1980), thus providing an opportunity for CE to be administered after treatment with antimicrobials to prevent reinfection with *Salmonella*.

Common practice in the poultry industry is to use spray application of certain vaccines, such as Newcastle and infectious bronchitis. These biologics use respiratory and/or ocular portals of entry. Although these vaccines can be effective thru the respiratory tract or ocular system, some biologics must be ingested to be effective. Caldwell and co-workers tested the effectiveness of spraying biologics that require ingestion in order to be effective. They discovered that certain environmental conditions needed to be optimized in order for the birds to preen at sufficient rate for the probiotic to be ingested (Caldwell, 2001).

Many different probiotics have been compared and several labs have shown spray application to be as effective at providing protection as drinking water application (Schneitz, 1992, Corrier, 1994, Blankenship, 1993). In order for many probiotics to be effective at preventing infection, the probiotic must be administered early in life and the application should be uniform. In many cases the drinking water application of probiotics can be compromised by water quality and medicator function and cleanliness. All of these issues can be addressed and minimized if the probiotic was administered at the hatchery by spray application.

In the research preformed by Caldwell and co-workers, these investigations found that a decrease in photointensity prior to spray application increased preening. Also, if the birds were subjected to an increase in photointensity immediately after spray application of the probiotic the chicks preening increased and resulted in more probiotic ingestion (Caldwell et al., 2001).

Another alternative to antibiotics that has been claimed to offer occasional against *Salmonella* in poultry is the use of organic acids. Organic acids are compounds that primarily include the saturated straight-chain monocarboxylic acids and their respective derivatives (unsaturated,

hydroxylic, phenolic, and multicarboxylic versions) and are often referred to as fatty acids, volatile fatty acids, or weak or carboxylic acids (Cherrington et al., 1991; Ricke, 2003). Organic acids are weak acids by nature. The pH of OA solutions are directly related to microbial killing because pH affects the concentration of undissociated acid formed (Davison, 2001; Ricke, 2003). It is believed that undissociated forms of organic acids can easily penetrate the lipid membrane layer of the bacterial cell, and once internalized into the neutral pH of the cell protoplasm, they can dissociate into anions and protons (Eklund, 1983, 1985; Davidson, 2001; Ricke, 2003). This dissociation inside the cell potentially causes problems for bacteria as the organism must maintain a specific internal pH. Thus, organic acids sometimes cause cell death through depletion of energy through the ATP-driven proton pump. The end result can be impaired bacterial cell function and/or lysis. The potential targets of biocidal compounds such as organic acids include the cell wall, cytoplasmic membrane, and specific metabolic functions in the cytoplasm associated with replication, protein synthesis, and function (Denyer and Steward 1998; Davidson 2001; Ricke 2003). Some of the most common organic acids used as food additives are: propionic, acetic, citric, lactic, tannic, and butyric.

Byrd et al., (2001) showed a reduction in *Salmonella typhimurium* recovered from the crop of chickens during an 8-h feed withdrawal period when using 0.5% lactic or formic acid. The study also showed a significant reduction in crop contamination with both *Salmonella* and *Campylobacter*. *Salmonella* isolation incidence in pre-chill carcass rinses was also reduced by 52.4%. The problem with the use of lactic acid at an inclusion rate of 0.5% is that voluntary water consumption is reduced, presumably due to the taste of the acid. Therefore, the use of lactic acid at this concentration is not likely to be widely used in the poultry industry. Thompson and Hinton (1997) included formic and propionic acid in the diets of hens obtaining an *in vivo* reduction of *Salmonella enteritidis*. Chaveeraach et al., (2002) used formic, acetic, propionic and hydrochloric acid, alone and combined (formic, acetic, propionic) administered in the drinking water. These researchers showed bactericidal effects at pH 4.0, but the bactericidal effect was reduced at higher

pH. Barnhart et al. (1999) developed a simulated crop assay to evaluate some disinfectants in the presence of large quantities of organic matter. In this assay, d-Limonene and citric acid among other disinfectants were used to eliminate *Salmonella*. The combination of d-Limonene and citric acid showed the best results of *Salmonella* killing using the simulated crop assay.

Acidifiers have been used by the poultry industry for several years, and there are many putative claims of the effects caused by acids. Recently, the characteristics and qualities of organic acids have been explored in greater detail. Currently, the goal is to use organic acids to reduce *Salmonella* in the crop and the ceca of poultry. The crop and ceca represent the main source of carcass contamination during processing. A reduction in crop and cecal *Salmonella* contamination would likely translate to a reduction of carcass contamination during evisceration in the processing plant, based on previous associations of decreased *Salmonella* burden *in vivo* and reduced recovery at the procession plant (Byrd et al., 2001).

The intestinal microflora is a complex ecosystem and the intestinal mucosa is the largest immune barrier in the body, making it not only important for digestion and absorption, but also for protection of the host from pathogens. An effective method of protecting birds from pathogens such as *Salmonella* is the use of CE and OA. These alternatives also offer promising results in protecting animals from other types of pathogens. Development of defined CE cultures is a step forward in the control of pathogens with CE. A defined CE can be more easily reproduced than undefined cultures. The organisms in a defined culture can selected to be oxygen tolerant and capable of being produce in a mass fermentation culture, thus reducing the cost of amplification. A define culture would also meet safety standards, as well as, improve quality control of the product.

While there are many advantages to CE and OA, there are some disadvantages. First, the preparation and maintenance of competitive exclusion bacteria can be difficult and expensive. The bacteria in these cultures could be aerobic or anaerobic, thus requiring different conditions to be effective. The second disadvantage to CE is the delivery method. Oral gavage is the preferred method because all of the birds receive a consistent amount and concentration of the culture, but

this is the most labor and time intensive method of delivery. Administering the culture in the drinking water is possible, but one cannot be sure all birds are drinking the same amount of the water or that the product is being administered correctly. Administering the culture in the feed is a still under research evaluation. A spray method works well while the birds are small or right after hatch as a prevention measure, but this method poses potential biological hazards and requires significant skill.

The third disadvantage is that the data on CE and OA treatments is somewhat inconsistent; no two studies have produced the same results. Also the effects of the environment are not fully known. The effect of temperature, variations in feed and feed type changes, and the impact of different profiles of native microflora still need further research.

The use of OA can help to create the right acidic environment for some CE cultures to proliferate. OA also inhibits or kills several pathogenic bacteria. The problem with OA is its non-selectivity, foul taste in certain instances, and it can also cause harm to beneficial bacteria or to the host if used inappropriately.

The intense pressure on the poultry industry and the scientific community to find alternatives to antibiotics for food producing animals is the driving force behind this research. The use of CE/probiotics either alone or in combination with organic acids could be a viable alternative for antibiotics.

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BIOSECURITY FOR BROILER BREEDERS AND PRIMARY BREEDING STOCK

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Introduction:

By the time a typical broiler breeder hen lays her first egg over $7.22 has been spent on her (Jones 2002). This cost is the result of the cost of feed, chick cost, vaccine/medication, utilities/fuel, grower payment, service, as well as other miscellaneous costs (Jones 2002). This number does not include the value of future egg production or the value of chicks from this hen. Since "dead hens do not lay eggs," it is important that this substantial investment is properly protected by the use of the best possible management practices including a well managed biosecurity program.

Biosecurity is simply defined as "security of life." In reference to livestock, and poultry in particular, biosecurity is a set of management policies and practices set forth to protect the overall well being of the animals, with specific emphasis on prevention of disease. Since many valuable animals are housed in a single airspace for over a year, precaution must be taken to prevent the outbreak of disease. If a pathogen gains entrance into a facility it may be nearly impossible to stop the spread of that pathogen. Ridding the premises of the pathogen is often difficult to do without depopulating the facility. The result of an infection varies by pathogen, strain, and overall health of the flock. Consequences of infection can range from slightly elevated mortality and a slight decrease in egg production to loss of ability to export egg or chicks or (in the case of a highly pathogenic avian influenza or velogenic New Castle) complete loss of the flock to mortality or depopulation.

The amount of a pathogen (be it bacterial, viral, fungal, or protozoal) an animal must be exposed to in order for that animal to become ill, is known as infectious dose. Typical infectious dose for most pathogens varies from 10^2 to 10^8 organisms, but the infectious dose will vary from pathogen to pathogen and even from strain to strain within a single species. The infectious dose curve is typically of a sigmoidal nature and is generally similar to that depicted in Figure 1.

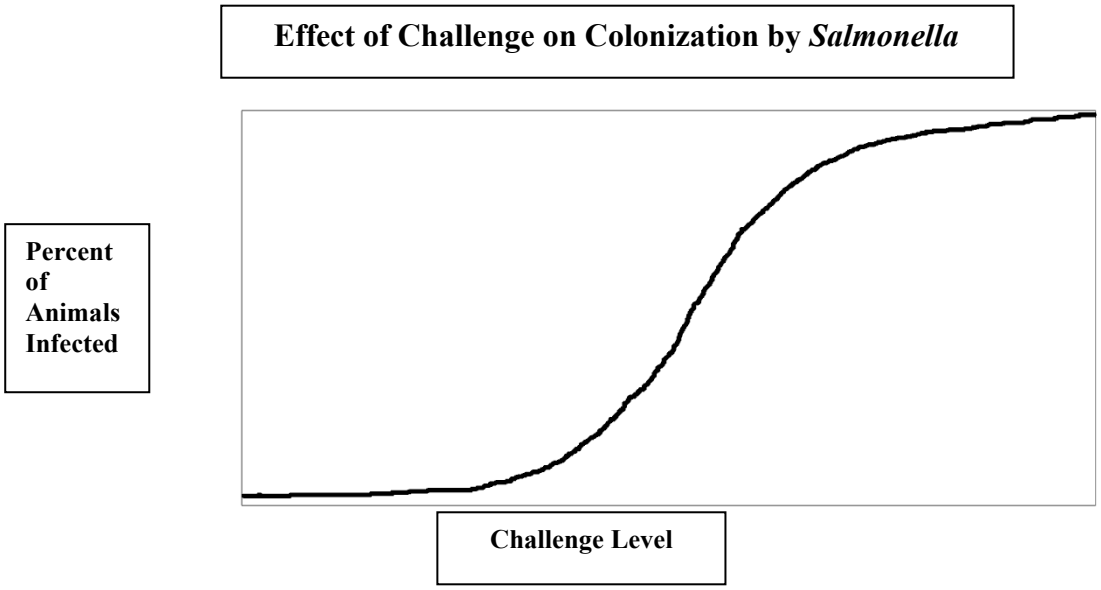

Figure1. Typical infectious dose response resulting from exposure to *Salmonella*

When an animal or flock is exposed to few organisms the chance of infection is low, but as the number of pathogens to which an animal/flock are exposed increases the chance of infection will increase, with the chance of infection approaching 100% at the highest levels of exposure. The goals of a good biosecurity plan should be to lower the probability of infection by lowering the number of pathogens a flock comes in contact with, as well as shifting the infectious dose curve to the left (increasing the number of pathogenic organisms an animal or flock may be exposed to before becoming infected) through immunizations and proper animal husbandry. These goals can be attained through three interconnected principles: isolation/traffic control, sanitation, and resistance (Jeffery 1997, Wolfgang).

Isolation and Traffic Control

The objective of isolation and traffic control is to prevent pathogens from gaining entry into a facility. While it is possible for air-born fomites to infect poultry, the chances of this occurring are relatively low as most pathogens can travel relatively short distance without a vector; this is one reason why poultry production facilities should optimally be spaced 1-2 miles apart (Carey et al L5182). It is much more common for a pathogen to be brought onto a premises on personnel, equipment, vehicles, feed, biological vectors, and new or replacement poultry. To reduce the probability of infection, only those people and things absolutely necessary to the operation of a farm should be allowed in a facility.

People are a common vehicle for poultry pathogens, particularly if those individuals come in contact with poultry, people, or equipment from multiple poultry farms. For this reason only those individuals with an absolute need to come onto a facility should be granted access. Signs should be posted at all access points letting people know they are not to come onto the facility without explicit consent. A fence should surround the perimeter of a facility to block unwanted visitors from entering, and all access points should be well secured.

It is a good practice to ask where a person has recently been prior to coming to that facility, to determine the level of risk they may pose to the poultry. Contractors, salesmen, as well as company personnel are all people who often visit multiple poultry farms. These people often understand and practice good biosecurity practices, such as not visiting other flocks after visiting diseased flocks. It is, however, the responsibility of the farm owner or manager to make sure these people are following the biosecuirty measures put in place by company or facility management. It is also a good practice to maintain a visitor logbook. As most pathogens have a known incubation period, this log may be useful to determine the source of an outbreak. If a person must come onto the facility steps should be taken to shield the poultry from any potential diseases the visitor or employee may carry. The most effective way to lower the potential for introduction of a disease is by having all personnel and visitors shower and change into clean clothing and footwear (preferably laundered on the facility) as they come onto the facility. If this is not possible, a pair of clean coveralls (again preferably laundered on site) or a disposable coverall (such as a Tyvec© suit) as well as durable shoe covers and a hairnet should be provided. Any personal effects should be left outside the perimeter of the farm, and any items which must be brought in should be thoroughly disinfected.

While the above practices help to keep potential pathogens from being transferred into a facility by people, it is also necessary to stop the spread of any known or unknown pathogens by people once they are on a facility. All doors to barns should be locked to keep out unwanted visitors. If an operation has multiple ages of flocks on a single farm, it is advisable to move from younger flocks to older flocks, as this decreases the risk of spreading disease. When houses are entered it is good practice to use foot baths with a disinfectant capable of working in the presence of organic matter. A brush used to remove organic material from boots prior to and after a visit to a barn will decrease the chance of moving around any potential pathogens. If it is possible, changing boots or boot covers upon entry into a barn is advisable. As an extra precaution use of a sanitizing hand gel upon entry and exit may reduce the likelihood of spreading potential pathogens.

Equipment and vehicles are potential mechanical vectors. As with people entering the premises, it is advisable to know where equipment and vehicles have recently been. Prior to entry onto the facility, vehicles and equipment should be thoroughly washed to remove organic matter and then

thoroughly disinfected. While it is common practice for wheels and tires to be sprayed with disinfectant prior to entry onto a farm, this practice is of limited benefit if built up dirt and organic matter is not first removed. A high pressure washer capable of dispensing a disinfectant works much better than a standard water hose or pump sprayer, although these are better than not washing and disinfecting at all. Special attention should be paid to vehicles which visit multiple poultry farms or facilities daily (feed trucks, fuel trucks, company service trucks, etc.) as they pose a greater risk of carrying a disease.

It is a common practice for farm owners to share expensive pieces of equipment between facilities. While this practice does save money on equipment, the cost benefit of this practice must be thoroughly evaluated against the potential risk of spreading a disease. If equipment is moved between farms, steps need to be taken to thoroughly remove all dirt and debris that may be present. A final disinfection after removal of all built-up dirt and organic material may also lower chance of spreading pathogenic organisms. If at all possible, it is advisable to not bring the equipment into contact with the poultry for several days. This serves the dual purposes of reducing the number of pathogens that may be present on the equipment as well as giving more time to monitor the farm from which the equipment came for signs of disease. The same procedures should be followed if any used equipment is brought onto a facility.

In addition to equipment and people, any livestock or pets should not be allowed onto a facility. Both pets and livestock are capable of acting as vectors for disease. In particular they have been known to harbor both pathogenic *E. coli* and *Salmonella* sp. (Kunze et al 2008, Sheng et al 2006, Finely 2007). Both of these organisms are known to be potential pathogens for both people and poultry. It is also not advisable to raise multiple poultry species on the same premises. Raising turkeys nearby or in the presence of chickens often results in outbreaks of Black Head (etiology *Histomonass meleagridis*) in the turkeys (Merck 2008, Helm 2004).

Farms should practice all-in-all-out poultry production whenever possible. This means that all poultry on a farm should come onto a facility at the same time from a common source and them be marketed at the same time at the end of the laying period. This will help eliminate the spread of disease from older to younger birds thus helping to break any cycle of infection on a facility. This practice also allows farms to be completely depopulated for a time so they can be thoroughly cleaned. Since many pathogens decline in numbers rather quickly after poultry are removed from an area (table 1), the simple fact that the farm is empty for period of time will allow the numbers of pathogens to decline thus lowering the probability of infection for the next flock.

Disease	Life Span in Environment
Infectious Bursal Disease	Months
Coccidiosis	Months
Fowl Cholera	Weeks
Infectious Coryza	Hours to Days
Marek's Disease	Months to Years
Newcastle Disease	Days to Weeks
Mycoplasmosis	Hours to Days
Salmonellosis	Days to Weeks
Avian Influenza	Days

Table 1. Life span of important poultry pathogens in the environment (Jeffery 1997)

If replacement poultry must be brought in while the farm is already populated, i.e. replacement males or replacement pullets, these animals should only come from sources that have been extensively tested for common poultry pathogens just prior to shipment. If possible these replacements should be quarantined away from existing flocks for a period of 2-3 weeks and then tested again prior to housing. Extra care should be taken with the movement of spike males between facilities of flocks as they are usually placed into multiple flocks.

Sanitation:

While isolation and traffic control helps keep disease off the premises, proper sanitation will both control and destroy any pathogens that may already be present on a facility. Sanitation can be broken down into three main categories: cleaning and disinfection, pest control, and facility and grounds maintenance.

The actual removal of dirt, debris, and organic matter from equipment and housing will eliminate 95% of pathogens present in a barn (Wolfgang). Without first removing this material, even the most powerful disinfectants cannot effectively sanitize a facility. To truly sanitize a poultry barn, all litter and easily removable equipment (including slats if present) should be removed from the barn. This is the only way to truly get to and remove all organic matter from a poultry house. Equipment should be washed prior to coming back into the house. It is a good practice to sanitize the equipment as it is brought in to kill any pathogens that may have been picked up while outside. High pressure washers work very well to remove even thick layers of organic material from both housing and equipment. As litter is impossible to sanitize, it should be removed to either the bare concrete floor or the hard pack earth and replaced with fresh bedding material

between flocks. Gas powered burners can be brought in to heat sterilize the clean floor in cases where disease is suspected.

Once the barn has been cleaned, it is ready to be sanitized by a disinfectant. Not all disinfectants are created equally, and there is a wide range of both price and effectiveness. Some disinfectants are dangerous to human and animal health and some may even damage housing and equipment. It is important to evaluate all of these factors before deciding on a disinfectant to use (table 2). As resistance may develop to antimicrobials after long periods of use, rotating products every three to six months will help ensure continued effectiveness.

Chemical	Gram+ Bacteria	Gram- Bacteria	Fungi	Virus	Best pH For Activity	Activity in Organic Material	Common Uses
Chlorhexidene	Some Activity	Some Activity	Some Activity	Most	Wide Range	Good	Equipment Premises Footbath
Formaldehydes and aldehydes	++	++	++	++	Wide Range	Good	Equipment Premises Footbath
Chlorine and Chloramines	++	++	++	Some Activity	Acid	Very Poor	Clean Equipment
Iodophores	++	++	++	Some Activity	Acid	Fair to Poor	Clean Equipment
Sodium Hydroxide	++	++	++	++	Alkaline	Good	Premises
Quaternary Ammonias	++	+	Some Activity	Some Activity	Alkaline	Fair	Clean Equipment
Phenols	++	++	Some Activity	Some Activity	Acid	+Good	Equipment Premises Footbath
Potassium peroxymonosulfate	+	+	+	++	Acid	Good	Equipment Premises Footbath

Table 2. General facts about disinfectants (Becker PIH80 , Wolfgang)

Pest control is vital to a good biosecurity program as both rodents and insects have been known to serve as vectors of avian disease. In addition, these pests are capable of causing extensive structural damage to a facility. Farms with large rodent populations have even been known to have noticeably higher feed conversion due to loss of feed to rodents (Carpenter 2000). Pest populations can often be managed with the use of rodenticides and insecticides. Rodenticide bait placed into in simple PVC enclosures and placed at regular intervals in and around poultry houses can often help control the rodent populations. Rodenticide products should be rotated as rodents often learn to avoid baits that are used for long periods of time. Resistance to certain rodenticide poisons may

also develop if baits are not rotated. While it is nearly impossible to make a poultry barn impervious to rodents, steps should be taken to seal all potential entry points to keep out of as many rodents out as possible. In addition to baits and rodent proofing, steps should be taken to eliminate all potential rodent nesting areas. Insecticides should be sprayed on all surfaces of a poultry barn after cleaning to reduce insect numbers. Flies are often controlled through litter management, as keeping litter dry will often keep down the number of flies. If flies become a problem on a facility, some larvacides are able to be added to feed and can effectively control maggots. Pyrethrum fogs or mists will help control adult fly populations (Stringham 1996).

General maintenance of facility grounds is vital to good biosecurity. To help keep rodent populations under control, all vegetation in close proximity to poultry barns should be kept short to decrease the nesting area available. Concrete or gravel placed several feet around poultry barns as a perimeter also acts as a deterrent to rodents as they often do not like to cross open spaces. All feed spills both inside and outside should be quickly cleaned up to cut off potential feed sources for wild birds and rodents. Outside feed spills may be a particular problem as they often attract wild birds which are known to carry ectoparasites, mycoplasmas, and other poultry pathogens (Luttrell et al 2001).

Proper disposal of mortality is necessary to control the spread of disease as well as to control pests. Mortality should be picked up at least daily from poultry houses as they are both potential reservoirs of disease and a food source for pests. Once mortality is removed from a poultry house, the best method of disposal is incineration. This effectively destroys all pathogens that may be carried in a carcass, as well as removing a potential food source for pests. Perhaps the worst option for mortality disposal is open pits, as they are a potential reservoir for poultry pathogens and a food source for pests. Open pits are dangerous not only to the facility they are located on, but also to any nearby poultry farms as pets, pests, and wild animals may carry infected carcasses or pathogenic organisms to nearby facilities.

One of the most often overlooked factors in a biosecurity program is the source of water for the flock. It is often taken for granted that the water source is free of pathogens and contaminants. In many areas this is not so. Open water sources such as ponds or rivers are often contaminated with high levels of bacteria. These open water sources may also contain poultry pathogens such as avian influenza as water fowl which may frequent these water sources are a reservoir for this disease. Closed water sources such as wells or municipal water systems also may be contaminated with high levels coliform bacteria,

E. coli, *Salmonella*, *Pseudomonas*, or other potential pathogenic organisms. It is a good practice to send water samples to a qualified lab for analysis of bacterial levels and identification of potential pathogens. To lower the level of bacteria in the water, a water disinfection system, such as a water

chlorinator, is often of substantial benefit. These systems, if operating properly, can dramatically lower the level of bacteria within a water system. To ensure that the system is working properly water should be monitored for bacterial level before and after the disinfection system. If chlorinators are used, testing the level of free chlorine is also a good indication that the system is functioning properly. Chlorination systems work best if water is acidic, and quickly loose function as water becomes more basic.

Poultry watering systems may also pose a biosecurity hazard. Open systems are hard to maintain in a sanitary manner. Closed systems such as nipple drinker lines are usually preferred. Even closed systems are capable of harboring bacteria, as biofilms can grow unseen inside the pipes of the system (Fairchild 2006). Daily or weekly flushing of the lines as well as a good water disinfection program can help keep these systems free of biofilms and lower bacterial levels within the system.

Resistance to Disease:

The resistance of poultry to disease is an often overlooked component of a biosecurity program. As mentioned previously, the infectious dose of a pathogen required to infect a flock is variable. It depends not only on the pathogen itself, but also on the host. Many factors such as overall health, vaccination, stress, temperature, and environment can play a role in the resistance a flock will have to infection.

One of the most effective ways to increase the disease resistance of a flock is through vaccination. Vaccination programs must be tailored to individual farms and regions as the same disease threats are not present in all areas. Vaccination programs in areas where there is a high density of poultry production often must use a more comprehensive vaccination program than areas where poultry flocks are spread further apart. If the right vaccines are selected and then administered correctly, the infectious dose required for disease will be greatly increased. Mortality and morbidity may also be improved even if a flock does become infected.

Proper management of environmental conditions will allow the flock to have maximum disease resistance. Flocks that have undergone temperature stress often have a lower level of disease resistance. Flocks which are exposed to high levels of dust and ammonia are often more vulnerable to even opportunistic pathogens due to damage to mucus membranes (Oyetund1978). Opportunistic pathogens such as *E. coli* are not usually a problem for poultry producers, but can become a problem when poor ventilation management practices or an underlying disease problem leads to immunosuppression or respiratory damage (Oyetunde 1978).

Conclusion:

A sound biosecurity program is a must to protect not only the substantial investment made to produce a flock of pullets or hens, but also to protect against the potential harm to company reputation and the monetary loses incurred from loss sales of final product. The potential costs of a biosecurity program must be weighed against these losses. Even the cost of an extensive biosecurity program usually amounts to pennies on the dollar when compared to the costs of a serious disease outbreak (Gifford et al 1987). From 1999 thru 2004 over 200 million poultry world-wide were infected with avian influenza. (Capua and Marangon 2007). During the 1994-2003 outbreak of low path avian influenza in Mexico over 1.4 billion turkeys and chickens were vaccinated and millions were tested before the outbreak was stopped (Capua and Alexander 2004, Villareal-Chavez and Rivera-Cruz 2003). The more recent outbreaks of avian influenza in Asia have led to the destruction of millions of poultry and have resulted in financial losses in the billions. As evidenced by the above figures, inadequate biosecuity can lead to serious financial consequences.

At a minimum, a biosecurity program for poultry breeders must contain several fundamental components focusing on traffic control and isolation, sanitation, and disease resistance. All people and things coming onto a poultry farm must be properly cleaned and sanitized before entering the premises. The prior whereabouts of all people and things coming onto a facility should be verified. Any replacement poultry coming onto a farm need to be screened for disease. Doors to houses should be locked to keep out unwanted guests. Houses should be sealed to keep out pests. A pest management program should be implemented to control pest populations. All-in-all-out poultry production should be practiced. At a minimum poultry barns should have all organic material removed between flocks. All mortality should be disposed of in a manner in which animals and pests cannot contaminate other flocks. The water source should be free of pathogenic bacteria. If the source it self is not clean, then the water should be sanitized prior to use. Finally, good environmental management practices and an effective vaccination program should help poultry maintain maximum disease resistance.

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USE OF THE INTESTINAL ECOSYSTEM TO REDUCE FOODBORNE PATHOGENS IN POULTRY

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ABSTRACT: The intestinal tract of poultry is filled with a diverse population of bacteria, protozoa and viruses, and this complex consortium exists in a relatively harmonious ecological balance that affects the health and productivity of the bird, as well as consumers of poultry products. The ecology of microorganisms is similar to that of the larger world where environmental niches are filled by the most fit species. Human illnesses from poultry products have increased in the past 5 years, leading to the recent USDA/FSIS "*Salmonella* Attack Plan" that targets reducing the incidence of *Salmonella* in the live bird. In addition to *Salmonella* spp., *Campylobacter* is the most common cause of bacterial foodborne illness and is commonly found in poultry and their products. Anti-foodborne pathogen intervention strategies for use in poultry must be coupled with knowledge of the intestinal microbial population. Competitive exclusion, sodium chlorate, vaccination, colicins/bacteriocins and bacteriophage are all intervention strategies that can reduce foodborne

pathogenic bacteria in poultry, but it is vital to understand how they function so that we do not produce unintended negative consequences that compromise the safety of our food supply.

INTRODUCTION

Human foodborne illnesses occur around the world, and the most common and serious food-borne pathogenic bacteria are *Campylobacter* spp. and *Salmonella enterica* (Mead et al., 1999). In the U. S. each year, these bacteria strike an estimated 3.3 million people, cause >500 deaths and cost the U. S. economy more than $3.6 billion each year (Mead et al., 1999; USDA-ERS, 2001). *Salmonella enterica* alone are estimated to cause over 30% of all bacterial food-borne deaths in the U.S. (Mead et al., 1999). While recent (2008) widespread human salmonellosis cases have been linked to vegetables and fruits, the most common route of foodborne pathogenic bacterial infection remains via foods of animal origin, especially from poultry (Braden, 2006).

Chickens (Zhao et al., 2001), turkey (Berrang et al., 1998) and eggs (Braden, 2006) can all be contaminated with *Salmonella* and/or *Campylobacter.* *Salmonella* and *Campylobacter* frequently live in poultry as a transient member of the intestinal or crop microbial population without causing any visible disease or impacting animal production. These pathogens are widespread in the environment and within poultry (Rodriguez et al., 2006), thus attempts to control them must be diverse and complex. In order to be able to target these pathogens, we must understand their role in nature and in the gut of food animals, as well as the functioning of the microbial ecology within the poultry gut. Due to the recent implementation of the USDA/FSIS "*Salmonella* attack plan" and the inherent difficulties with *Campylobacter* in poultry, in this manuscript we will focus primarily on *Salmonella*, however the same ecological principles apply to all targeted foodborne pathogenic bacteria.

Salmonella is diverse, widespread and present in many environments

Although there are more than 2,500 serotypes of the Gram-negative bacteria *Salmonella*, only a few serotypes are responsible for the majority of human illnesses (CDC, 2006; USDA-FSIS, 2007). The serotypes isolated most frequently from poultry products and humans pooled across the U.S. are shown in Table 1. The most common serotype found in eggs in the U.S. is *S*. Enteritidis (Braden, 2006), but it is found at a very low prevalence (Ebel and Schlosser, 2000). There is a high degree of correlation between the serotypes found in poultry products and those responsible for a large number of human illnesses (Table 1). Therefore, it is critical to target the sources of these serotypes to interrupt the transmission cycle before they can cause human illnesses, although serotypes involved do vary yearly (Figure 1).

Salmonella spreads easily in both chickens and turkeys via a fecal-oral contamination route (Rodriguez et al., 2006). In a broad study 16% of poultry farms tested positive for *Salmonella* and was present in soil, bedding litter, feces, and feedstuffs (Rodriguez et al., 2006). Fecal *Salmonella* shedding varies by season and there exists a correlation between fecal shedding and human outbreaks. Fecal shedding approaches zero during the winter months and reaches its peak in summer and early fall (McEvoy et al., 2003; Fossler et al., 2005), while human outbreaks peak 13 d after a spike in ambient temperature (Naumova et al., 2007). Although a correlation with environmental temperature exists, the gut temperature is rather consistent so this is unlikely the proximal cause of seasonal shedding.

Salmonella is a serious threat to both broiler and egg production, both as a direct food safety threat in poultry meat and eggs and via vertical transmission to a new generation of infected broilers or layers. Since *Salmonella* can survive in the gut of birds or invade host tissues, it can be transmitted to humans through various routes. *S*. Enteritidis, for instance, can invade the ovaries where it is enclosed in eggs, or it can enter through shell cracks as the egg exits the cloaca (Braden, 2006), or can be transmitted through poultry meat (Kimura et al., 2004). Fertilized eggs can also be infected with *Salmonella* via semen (Reiber et al., 1995). If only 5% of chicks were *Salmonella* positive upon entry to the grower house, the infected population will expand to 72-95% within three

weeks (Byrd et al., 1998). Thus, intervention strategies that are used early in the grow-out period must be complemented by interventions aimed at reducing horizontal *Salmonella* spread in a growing flock.

FOODBORNE PATHOGEN REDUCTION STRATEGIES: PROS AND CONS

Many different strategies have been used epidemiologically over the years to reduce pathogen, both *Salmonella* and the ubiquitous *Campylobacter*, populations in poultry. A test-and-slaughter flock (depopulation of farms positive for *Salmonella*) approach may be effective for eliminating *S.* Enteritidis from parent and grandparent breeder flocks and in layer flocks because it can be vertically transmitted between generations. However, horizontal transmission of other *Salmonella* serotypes within houses or flocks remains a problem, so this proven method is not likely to be a panacea for *Salmonella*.

Reduction strategies that are likely to be successful and acceptable to producers, consumers and regulatory bodies, include a group of "pro-commensal" treatments: probiotics, prebiotics, and competitive exclusion cultures. All of these techniques utilize a non-pathogenic microbial species, or consortium, to establish a stable intestinal/environmental ecosystem (Callaway et al., 2002; Doyle and Erickson, 2006). The best understood pro-commensal strategy is that of competitive exclusion (CE), where day-of-hatch chicks are treated with a defined pathogen-free mixture of normal intestinal bacteria to establish a mature intestinal population that can exclude pathogens from colonizing the gut (Nisbet, 2002; Zhang et al., 2007b, a). The addition of a microbial supplement called a probiotic or a direct-fed-microbial (DFM), that improves gastrointestinal health and the diversity of the intestinal microbial ecology is another pro-commensal technique (Collins and Gibson, 1999). Probiotics/DFM are often a single organism (bacteria or fungi) or products of their fermentation that alter the microbial ecology of the gut when fed to food animals. An exciting concept that is migrating from human to animal health involves the addition of a limiting nutrient (or "prebiotic") that allows an existing microbial population to expand its current niche or increase populations.

Other strategies have focused on working directly against the pathogens themselves. Vaccination of poultry can be used against *S.* Enteritidis; however vaccinated birds are indistinguishable by serum tests from birds infected by *Salmonella*, which poses significant concerns for epidemiological monitoring. Antibiotics have been suggested to be potential methods to reduce specific pathogens such as *Salmonella* or *Campylobacter*; however, due to fears of antibiotic resistance, the use of antibiotics as anti-foodborne pathogen strategies is actively discouraged. In future, it is likely that the prophylactic use of antibiotics in poultry will become more closely regulated, or eliminated. Some natural antimicrobial proteins produced by bacteria may be of use against foodborne pathogens in poultry, which include colicins and bacteriocins. These proteins function by opening channels in the bacterial cell membrane killing the bacterial cell (Jack et al., 1995), and some of these proteins have been scaled up for production in quantities necessary for use in animals. Early studies using these natural proteins in food animals demonstrate that pathogenic *E. coli* populations in the gut of swine were reduced by colicin treatment (Callaway et al., 2004; Stahl et al., 2004; Cutler et al., 2007). The colicins have subsequently been effective against *Listeria* spp. in further studies (Patton et al., 2007).

Sodium chlorate is a chemical that can reduce *Salmonella* in poultry that is currently under regulatory review for use as a feed additive. Chlorate is toxic to some bacteria because of the action of the intracellular enzyme nitrate reductase; but chlorate does not kill all bacteria (Anderson et al., 2000; Anderson et al., 2001). *Salmonella* spp. are equipped with nitrate reductase, and are therefore killed by chlorate treatment. *Campylobacter* populations are not as significantly affected by chlorate treatment (Anderson et al., 2006).

Bacteriophage are viruses that are natural members of the microbial ecosystem of the gut of food animals and act as bacterial "predators" (Callaway et al., 2007; Oot et al., 2007). They can be quite specific which makes them attractive "smart bombs" for use against foodborne pathogenic bacteria. Phages have been used around the world in place of antibiotics in human medicine, and have been used to reduce foodborne and animal pathogens in several species of food-producing

animals (Smith and Huggins, 1983; Huff et al., 2002; Higgins et al., 2005). To date, the effectiveness of phage treatment in the gut of animals has been variable (Kudva et al., 1999; Higgins et al., 2005; Raya et al., 2006). In 2007, a phage that kills *E. coli* O157:H7 on live cattle before slaughter was approved for use by the U.S. FDA as a pre-harvest hide spray. Other researchers have developed phage as an intervention to reduce *Campylobacter* and *Salmonella* in live poultry and swine (Connerton et al., 2004; Loc Carrillo et al., 2005; Callaway et al., 2007) and by spray onto carcasses and/or meat products (Atterbury et al., 2003; Goode et al., 2003). Preliminary results by the present author utilizing phage active against *Salmonella* in newly hatched broiler chicks have been mixed (Callaway, unpublished data).

UNINTENDED CONSEQUENCES: DISASTER ARISES FROM THE BEST INTENTIONS

Any actions we take to reduce *S. enterica* colonization in animals must be taken with the full awareness of unintended consequences. The emergence of *S.* Enteritidis as a human pathogen associated with poultry eggs is a case in point (Kingsley and Baumler, 2000; Rabsch et al., 2001). *Salmonella* Gallinarum/Pullorum cause fowl typhoid which was resulted in morbidity and mortality amongst poultry flocks throughout the 1800's and into the early 20th century; meanwhile *S.* Enteritidis was virtually unknown as a human pathogen. The National Poultry Improvement Program (NPIP) was implemented in 1935 to reduce economic losses caused by fowl typhoid and it was successful since *S.* Gallinarum/Pullorum have only the reservoir of fowl. By the 1970's, *S.* Gallinarum was virtually eliminated; yet, as this incidence decreased, human *S.* Enteritidis illnesses increased. *S.* Enteritidis lived in poultry at low levels, but can also live in rodents. Human cases of *S.* Enteritidis rapidly increased through the 1980s and 1990s, peaking as the most frequently reported serotype isolated from human illnesses; and it is still responsible for > 15% of the reported human salmonellosis cases in the U.S. (CDC, 2006).

Again, bacterial ecology is much like that of the macro world; environmental niches are filled by a succession of species best adapted to each niche. *Salmonella* Gallinarum and Pullorum

filled a niche in the microbial ecology of the gut of chickens, and when it was eliminated, that 'vacuum' was filled by *S*. Enteritidis. When the national poultry flock was emptied of Gallinarum, *S*. Enteritidis was able to jump from its natural rodent reservoir into poultry. The close relationship between these serotypes is demonstrated by the fact that the effective *S*. Enteritidis vaccines used in laying hens are actually made from attenuated strains of *S*. Gallinarum.

The niche-filling capacity of a complex microbial ecosystem poses a troubling concern for all foodborne pathogen-reduction strategies, because there are likely more potential pathogens lurking in the consortium that are present at very low populations . This does not prevent the use of strategies in live poultry, but merely suggests that repercussions should be considered during the early developmental process. Removing a transient or endemic pathogen such as *Campylobacter* or *Salmonella* from the intestinal population will create a vacuum, and at times the most-fit successor could be another virulent pathogen. Any strategy that eliminates foodborne pathogenic bacteria in the live animal should be coupled with a complementary strategy that provides an alternative bacteria, or population, or one that provides limiting nutrients that select for an already existing intestinal population to succeed in the niche occupied by a targeted foodborne pathogen.

CONCLUSIONS

Foodborne pathogenic bacteria are widespread public health issues that are found in all phases of poultry production from the farm to the live animal to the slaughter plant. Several intervention strategies have been brought forward that offer the possibility of reducing foodborne pathogens in poultry before they are slaughtered. Many of these utilize aspects of the microbial ecology to control or replace pathogens in the animal by harnessing the power of commensal organisms. Competitive exclusion, probiotics, prebiotics, vaccination, sodium chlorate, and bacteriophage can indeed impact intestinal and environmental carriage of foodborne pathogens, but they must be used in conjunction with in-plant interventions. Thus 'cleaning up' animals may temporarily reduce the incidence of foodborne pathogenic bacteria, but intestinal populations of other pathogens step into the breach. Therefore it is it critical to include other comprehensive

strategies that provide other intestinal bacteria a selective advantage to occupy the vacated niche. The rise of *Salmonella* Enteritidis following the elimination of fowl typhoid emphasizes the possible unintended consequences of such significant changes in the microbial population of the gut.

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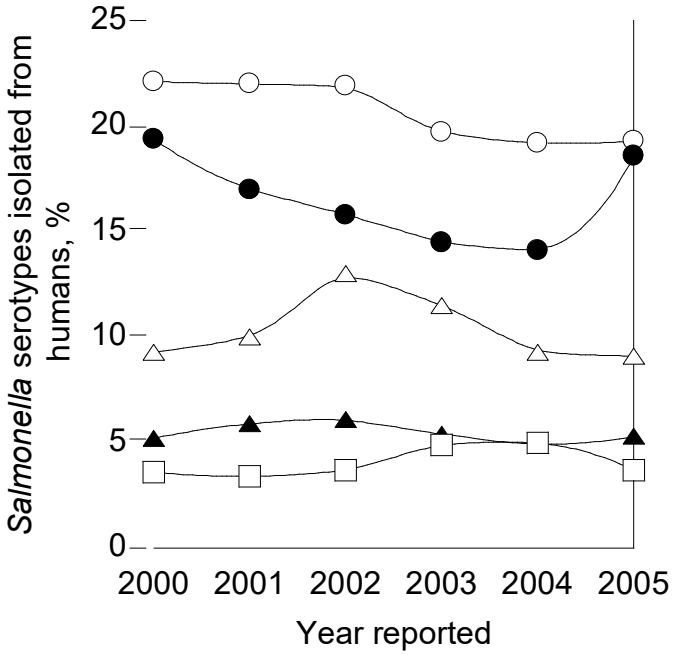
Table 1. Most common *Salmonella* serotypes isolated across the U.S. in 2005 (in order of prevalence). Serotypes in bold represent top five isolates from clinically-ill humans found in poultry products.

Order of prevalence	**Serotypes from broilers in commercial plants (FSIS[1])**	**Serotypes from ground chicken in commercial plants (FSIS[1])**	**Serotypes from clinically-ill humans (CDC[2])**
First	Kentucky	**Enteritidis**	**Typhimurium (includes Copenhagen)**
Second	**Heidelberg**	Kentucky	**Enteritidis**
Third	**Typhimurium (includes Copenhagen)**	**Heidelberg**	**Newport**
Fourth	**Enteritidis**	**Typhimurium (includes Copenhagen)**	**Heidelberg**
Fifth	I 4, 5, 12:i:-	I 4, 5, 12:i:-	Javiana
Total *Salmonella* accounted for by top 5 serovars, %	80%	85%	56%

[1] Food Safety Inspection Service, United States Department of Agriculture (USDA-FSIS, 2006)
[2] Centers for Disease Control and Prevention (CDC, 2006)

Figure 1. *Salmonella* serotypes isolated from humans from 2000 through 2005; data taken from the Centers for Disease Control and Prevention (CDC, 2006). Open circles (○) depict *S.* Typhimurium, (●) represent *S.* Enteritidis, (Δ) indicate *S.* Newport, (▲) show *S.* Heidelberg, and (□) indicate *S.* Javiana.



DIAGNOSIS AND CONTROL OF RESPIRATORY DISEASES OF POULTRY WITH SPECIAL ATTENTION TO AVIAN INFLUENZA AND *Mycoplasma* spp

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Respiratory diseases of poultry remain of major economic and public health importance. Many pathogenic microorganisms are present to a limited degree under most management conditions. If conditions favourable for multiplication of the specific pathogen exist, an active disease outbreak may occur in apparently healthy flocks. The severity and course of any respiratory disease is influenced by virulence of the agent, immune status of the birds and management.
Respiratory diseases of poultry are associated with severe economic losses, due to high mortality, high medication cost, drop in egg production in layer and breeder flocks and in many case low fertility and hatchability. In breeder flocks attention must be paid to prevent infections with vertically transmitted agents. Early recognition and monitoring programmes are essential in managing the infections and minimizing the economic impacts. Many of these diseases once introduced into a geographic area, can explode into an epidemic and may have a significant negative effect on national and international trade.

Several pathogens are incriminated as possible cause either alone (mono-causal) or in synergy with different other micro-organisms (multi-causal) or accompanied by non-infectious factors such as climatic conditions and management related problems (Fig.1).

Worldwide the emerging and re-emerging respiratory diseases and or infections of poultry are Infectious Bronchitis (IB), Infectious laryngotracheitis, Avian Metapneumovirus (aMPV) and Ornithobacterium rhinotracheale (ORT) infections. In addition, Avian Influenza (AI), Newcastle disease (ND) and Mycoplasma infections appear to causing problem in some countries.

The severity of clinical signs, duration of the disease and mortality are extremely variable and are influenced by kind, virulence and the pathogenicity of the infectious agent as well as by many environmental factors such as poor management, inadequate ventilation, high stocking density, poor litter conditions, poor hygiene, high ammonia level, concurrent diseases and the type of secondary infection.

The diagnosis of the disease complexes is usually not a straightforward business. Basically the diagnosis consists of case history as well as management and environmental investigation on spot. In addition, clinical investigations and post-mortem examination done on the farm is an important step toward disease diagnosis. However, clinical signs and necropsies are mostly not the final step of the diagnosis. The final diagnosis can be reached by laboratory diagnosis.

Fig 1: Some possible cause of respiratory disease in poultry

Non infectious	**Infectious**
Management Litter quality Stocking density Ventilation rate Temperature High ammonia level High dust concentration ***Feed*** High dust content Vitamin A deficiency	***Viral agents*** IB, ILT, ND, Influenza A, aMPV, PMV3, Pox ***Bacterial agents*** ORT, P. multocida, Mycoplasma, Chlamydia, E. coli, Haemophilus, Bordetella avium, Streptococci, Staphylococci ***Mycotic agents*** Aspergillus fumigatus ***Parasites*** Syngamus, Cryptosporidium

In the space available, it is not possible to review extensively the entire field of respiratory diseases. Instead, this paper is limited to Avian influenza and Infectious bronchitis.

AVIAN INFLUENZA

AI viruses are members of the Orthomyxoviridae family. Influenza A viruses can be divided into subtypes on the base of the antigenic relationships of the two surface glycoproteins haemagglutinin (HA) and neuraminidase (N). The haemagglutinin and neuraminidase are respectively important in the attachment and release of the virus from the host cell. At present 16 H subtypes and 9 N subtypes are known. All subtypes can infect wild waterfowl (Anon., 2000; Fouchier et al., 2005). In poultry the most virulent form of avian influenza was designated as fowl plague. Recently the term „highly pathogenic avian influenza (HPAI)" based on the results of different laboratory tests and pathotyping is suggested to be used (Anon. 2000). Currently, only the viruses of H5 and H7 subtype have been shown to cause HPAI in susceptible species, but not all H5 and H7 viruses are highly pathogenic. However, it has been proven that highly pathogenic avian influenza (HPAI) viruses emerge in domestic poultry from low pathogenicity (LPAI) progenitors of the H5 and H7 subtypes (Garcia et al., 1996; Senne et al., 1996; Perdue et al., 1997). It was recently shown, that H5N1 influenza A can also infect pigs, cats leopards, tigers, civets, dogs, mink and a stone marten. Since December 2003, epidemic influenza due to a highly pathogenic H5N1 virus strain has devastated the poultry industry. As of 7th January 2009, H5N1 virus infection, mainly due to direct transmission from birds, have been confirmed in 395 humans, of whom 249 had died (WHO, 2008).

Recent genetic characterization of H5N1 viruses has demonstrated two distinct phylogenetic clades: Clade 1 viruses have circulated primarily in Cambodia, Thailand, and Vietnam and clade 2

viruses have circulated primarily in China and Indonesia and have spread westward to the Middle East, Europe, and Africa. Six different subclades of clade 2 have been recognized (Webster, 2006).

The disease can be transmitted directly through contact with infected birds or indirectly through contaminated equipment. Wild and domestic waterfowl are the major natural reservoir of influenza viruses. The currently circulating H5N1 appears to be virulent for a variety of wild bird species (Chen et al., 2005; Liu et al., 2005; Olsen et al., 2006). Studies on the nucleoprotein and other gene lineages in avian species show separate sublineages of influenza in Eurasia and the Americas, indicating that migratory birds moving between these continents (latitudinal migration) have little or no role in the transmission of influenza, while birds that migrate longitudinally appear to play a key role in the continuing process of virus evolution (Webster, 1998). The continuing spread of H5N1 appears to be related to two factors: spread through movement of poultry (legal as well as illegal) and spread through wild migratory birds (FAO, 2006a,b; Liu et al., 2005). Free-ranging backyard chickens, illegal transportation of domestic birds, and cockfighting also have been shown to contribute to spread of the virus (Tiensin et al., 2005).

Kilpatrick et al. (2006) investigated the pathways by which the virus has and will spread between countries. They integrated data on phylogenetic relationships of virus isolates, migratory bird movements, and trade in poultry and wild birds to determine the pathway for 52 individual introduction events into countries and predict future spread. The results show that 9 of 21 of H5N1 introductions to countries in Asia were most likely through poultry, and 3 of 21 were most likely through migrating birds. In contrast, spread to most (20/23) countries in Europe was most likely through migratory birds. Spread in Africa was likely partly by poultry (2/8 introductions) and partly by migrating birds (3/8). The obtained results predict that H5N1 is more likely to be introduced into the Western Hemisphere through infected poultry and into the mainland United States by subsequent movement of migrating birds from neighbouring countries, rather than from eastern Siberia. These results highlight the potential synergism between trade and wild animal movement in the emergence and pandemic spread of pathogens and demonstrate the value of predictive models for disease control.

The diagnosis of poultry diseases is based on the case history, clinical signs, gross lesions and laboratory diagnosis. In case of the avian influenza in the EU the diagnosis of primary infection with highly pathogenic avian influenza (HPAI) outbreak must be carried out in accordance with the "Council directive 2005/94/EC of 20 December 2005 on Community measures for the control of avian influenza and repealing Directive 92/40/EEC (EC, 2005).

The confirmation of avian influenza should be carried out with appropriate laboratory tests, in accordance with the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2005). This include samples collection and in the primary outbreak in given country virus isolation and identification and assessment of the pathogenicity. The Council directive 2005/94/EC (EC, 2005) defined avian influenza as follows:

1. Avian influenza: means an infection of poultry or other captive birds caused by any influenza A virus:

(a) of the subtypes H5 or H7; or
(b) with an intravenous pathogenicity index (IVPI) in six-week old chickens greater than 1.2.

2. Highly pathogenic avian influenza (HPAI): means an infection of poultry or other captive birds caused by:

(a) avian influenza viruses of the subtypes H5 or H7 with genome

sequences codifying for multiple basic amino acids at the cleavage site of the haemagglutinin molecule similar to that observed for other HPAI viruses, indicating that the haemagglutinin molecule can be cleaved by a host ubiquitous protease; or

(b) avian influenza viruses with an intravenous pathogenicity index in six-week old chickens greater than 1.2.

3. low pathogenic avian influenza (LPAI): means an infection of poultry or other captive birds caused by avian influenza viruses of subtypes H5 or H7 that do not come within the definition in paragraph 2.

In addition, RT-PCR techniques on clinical specimens could result in rapid detection and subtype (at least H5 and H7) identification. Direct RT-PCR tests may be useful for rapidly identifying subsequent outbreaks in flocks once the primary infected premises have been identified and the virus characterized. Furthermore, several commercially available rapid tests are available. The main advantage of the test is that it can demonstrate the presence of avian influenza within 15 -30 minutes. The disadvantages are that it may lack sensitivity, it has not been validated for different species of birds and subtype identification is not achieved.

Several serologic tests can be useful to diagnose the disease such as. AGID, ELISA, Haemagglutination-inhibition (HI) tests and Neuraminidase-inhibition test. The last mentioned test requires specialized expertise and reagents; consequently this testing is usually done in an OIE Reference Laboratory.

Control approaches

Bio-security is the first defence line to prevent the introduction of an infectious disease in an area. In case of present an infectious disease in an area several measures can be adopted in aim to prevent further spread this can include beside bio-security measures the culling of infected flock and vaccination of non exposed flocks.

The community measures for the control of highly pathogenic avian influenza are based first on the depopulation of the infected flocks, in accordance with community legislation on animal welfare. If an outbreak occurs, it is necessary to prevent any further spread of infection by carefully monitoring and restricting movements of poultry and by tightening biosecurity measures at all levels of poultry production, by cleaning and disinfecting the infected holding, by establishing protection based on a minimum radius of three kilometres around the infected holding itself contained in a surveillance zone based on a minimum radius of 10 kilometres and, if necessary, by vaccination (EC, 2005).

Once the presence of highly pathogenic avian influenza has been officially confirmed all poultry and other captive birds on the holding shall be killed without delay under official supervision. The killing shall be carried out in such a way as to avoid the risk of spread of avian influenza, in particular during transport.

In accordance with Directive 2005/94/EC, vaccination against avian influenza is generally prohibited in the EU. However, under certain circumstances a member state can introduce ***emergency vaccination*** as a short term measure or may also introduce ***preventative vaccination*** in poultry or other captive birds as a long term measure. The Commission shall immediately examine and approve the vaccination plan. The vaccination strategy should allow differentiation between infected and vaccinated animals. Products of vaccinated poultry, such as meat and table eggs, can then be placed on the market in accordance with the relevant Community legislation.

Vaccination, when used properly seems to be an effective tool to in prevention and control of AI (Capua et al., 2003; Capua and Marangon, 2006, Swayne, 2006). Currently several commercial and

experimental inactivated and recombinant vectored AI vaccines showed to be able to protect against clinical signs, mortality, reduces virus shedding, increases resistance to infection and reduces contact transmission of challenge virus. However, the virus is still able to infect and replicate in clinically healthy vaccinated birds and silent spread can occur after vaccination (van der Goot et al., 2007). In addition, the circulation of the virus in a vaccinated population for a long period may result in antigenic drift away from the vaccine strain as reported for H5N2 in Mexico (Lee et al 2004) and speculated for H5N1 in China (Smith et al., 2006, Wei, 2007).

Despite the above mentioned disadvantages in countries where H5N1 virus infections are endemic vaccination may be helpful and can protect food supplies and economic well-being of poultry owning families as well as industrial concerns (OIE, 2007).

Generally, the concept of vaccination against avian influenza is complex and there is no simple solution for all given epidemiological situations and differences existing in bird and poultry species (Anon, 2007).

In most of countries inactivated, adjuvanted ***homologous*** /or ***heterologous vaccine*** vaccines are used. The protection seems to depend to a large extent on antibodies which neutralise the virus and are predominantly directed against the haemagglutinin (HA). However, also antibodies against neuraminidase may neutralise the virus (McNulty, 1986). On the other hand Sylte et al. (2007) were reported that with NA alone, multiple doses are needed to give protection and it is inferior to HA induced antibodies.

For both homologous and heterologous vaccines, the degree of clinical protection and the reduction in viral shedding are improved by a higher antigen mass in the vaccine (Swayne et al.,1999).

For heterologous vaccines the degree of protection is not strictly correlated to the degree of homology between the haemagglutinin genes of the vaccine and challenge strains (Swayne and Suarez, 2000).

Several ***novel vaccines*** either have been developed or are under development e.g. recombinant fowl pox viruses expressing the H5 or H7 antigen or other vectors such as infectious laryngotracheitis virus (Beard et al., 1992; Swayne et al., 2000; Lüschow et al., 2001). The only field experience with a recombinant Poxvirus-H5 to control AI has been obtained in Mexico Villareal-Chaves and Rivera-Cruz (2003). No such product has been licensed in the EU to date. Additional Examples include, DNA vaccines, Subunit vaccines, Vaccines based on reverse genetics, Adenovirus-vectored vaccine, Newcastle disease-vectored vaccine and Newcastle disease virus–based bivalent live attenuated vaccine (Lipatov et al., 2005; Veits et al., 2006; Gao et al., 2006; Ge et al., 2007).The effects of presence of anti-vector and/or anti-AI active and/or passive antibodies due to natural infection or vaccination on the efficacy of these vaccines required more investigation.

Generally, the immunity induced by vaccination is of short duration and it is necessary to apply the vaccines several times during one rearing period. There are little or no data available about the frequency of the vaccinations require keeping the breeder and layer flocks protected during the entire production period. Vaccination strategies alone are not sufficient to stop farm-to-farm spread of HPAI. Furthermore, emergency vaccination is also hindered by practical difficulties related to the administration of the vaccine (each single bird must be injected). Generally the efforts to control the disease by vaccination alone, without depopulation of affected birds to reduce the virus load in the environment, will probably not be successful.

Vaccinal breaks were observed in some vaccinated flocks in some countries (Aly et al., 2008). Vaccinal break defined as sub-optimal vaccinal protection of a flock and can have several causes. The efficacy of vaccine is very much dependent on the quality of the product as well as the quality of the manufacturing process and quality control procedures. In addition, the antigen concentration is very important. According to Gardin (2007) The reduction of the antigen content leads to a reduction of the capacity of the vaccine to prevent the shedding although antibody response to

vaccination remains almost identical (within a certain range of concentrations) and although the monitoring of antibody response in the fields is useful to check the quality of the vaccination, but is not a very accurate and sensitive way to evaluate the level of protection. Inappropriate storage, handling and improper administration are further factors. The quality of the vaccines application is crucial since all non injected chickens are not protected, and improperly injected chicks will be poorly protected. Using post-vaccination necropsy (residue of oil at the site of injection) or serological testing demonstrated, that it is not uncommon to see as much as 20% or 30% or even more of chickens that were not injected (Gardin, 2007).

In conclusion avian influenza infections in poultry are associated with severe economic losses, early recognition and monitoring programmes are essential in managing the infections and a universal solution for prevention and control of avian influenza does not exist. Generally, one of the above mentioned measures alone is of little value, unless they are accompanied by improvements in all aspects of management and bio-security. In countries in which the infection become endemic and when other control measures such as stamping out, movement restriction of poultry and bio-security cannot stop the spread of the infection poultry flocks should be vaccinated using a vaccine of high quality.

Finally, since the success of any control program depends on the hygiene practices of the personnel, it is essential to incorporate education programs about micro-organisms and their modes of transmission, as well as awareness of the reasons behind such control programs for all people involved throughout the poultry production chain.

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AVIAN MYCOPLASMOSIS

Mycoplasmas have affected the industry for many years and effective control of Mycoplasma infection has been a fundamental milestone to improve performance and productivity. However, infections appear to make a comeback (Kleven, 1997, Bradbury, 1999, Hafez et al., 2006). Numerous species of mycoplasmas have been isolated from avian sources. Two species are recognized as predominantly pathogenic to chickens and turkeys. *Mycoplasma gallisepticum* (MG) affects the respiratory system and is referred as chronic respiratory disease (CRD) in chickens, and infectious sinusitis in turkeys. *Mycoplasma synoviae* (MS) may cause either respiratory diseases and / or joint diseases. Two additional species are known to be pathogenic to turkeys. *Mycoplasma meleagridis* (MM) causes airsacculitis, and *Mycoplasma iowae* (MI) causes decreases in hatchability.
Mycoplasmas are the smallest self replicating procaryotic organisms. They have no cell wall but are bounded by a triple layered plasma membrane which is composed mainly of lipids and proteins. The genus Mycoplasma belongs to the family Mycoplasmataceae, order Mycoplasmatales of the class Mollicutes (Razin, 1992).

Mycoplasmas are sensitive in the environment and susceptible to the most commonly used chemical disinfectants. The viability of Mycoplasmas outside the host is of short duration: at 5-10 °C nearly 2-8 days. Sunlight kills the organism in 20-30 min. They remain stable in faeces at 20 °C for 3 days, in hatchery fluff for 5 days and at -20°C for several months (Chandiramani et al., 1966). Mycoplasma can survive in the human nose for 24 hours up to 4 days, on human hair 3 days, and on feathers for 2 to 4 days (Christensen et al., 1994, Lierz et al., 2008a). They may exist for very long periods in the respiratory tract of infected birds and these apparently healthy carriers are essential for mycoplasma survival in poultry populations.

The disease spreads by vertical transmission through infected eggs. Infected progeny then transmit the agent horizontally either by direct bird-to-bird contact or by indirect contact through contaminated feed, water and equipment. Concerning vertical transmission, hens which become infected before the onset of laying tends to egg transmit at a lower rate than hens initially infected during egg production. Generally egg transmission is intermittent and the rate is variable (1-10%) and very low. The spread of infection from bird to bird within one pen is usually rapid but it is rarely transmitted from one pen to another. However, in continuous production complexes (multiple-age) with chronic apparent healthy carriers the spread of infection is difficult to control since the cycle of infection can not be broken without complete depopulation (Yoder, 1991). The agent also can be transmitted by other species of birds as well as mechanically by other animals and man. Bradbury (1999) reported on the problems related to re- emergence of mycoplasma infections. For example: In the past mycoplasmas appeared to have a restricted host range, which should help to limit their lateral spread. This does not seem to be true for MG or MS, both of which have been found in a number of avian hosts. A widespread epidemic of MG infection also occurred in North American finches (Ley et al., 1996). In addition, MM was isolated from raptors in Germany (Lierz et al., 2000) and MI from chickens and from wild and exotic birds (Bozeman et al., 1984). Recently, Lierz et al (2007c) isolated *M. lipofaciens* from an egg of an imprinted 4 year old Northern Goshhawk (*Accipiter genitilis*). The pathogenicity of this strain to chicken embryos as well as for turkey embryos was demonstrated by Lierz et al (2007a,b). The authors described severe tissue edema with infiltrates of heterophils, necrosis in liver, intestine and CAM in the embryos died.

The clinical signs and the course of the disease are influenced by several factors such as the presence of concurrent microorganisms such as (TRT, Influenza, Reo, ORT and E.coli) and/ or improper management (increased dust and ammonia levels in the environment).
The clinical manifestation due to **MG** may include drops in feed consumption, coughing, sneezing, rales, ocular and nasal discharge and swelling of sinuses. In some cases, sinusitis may be absent and only rhinitis, tracheitis und airsacculitis could be found accompanied with fibrinous pneumonia. **MS** has the affinity for synovial membranes and may infect the membranes of joints resulting in swelling of the joints followed by lameness. Hocks, footpads, wing joints and the sternal bursa are most frequently involved. Affected birds are not able to move and lose weight or fail to gain. MS may also cause respiratory disease and airsac lesions similar to MG. The role of MS as a primary pathogen in both respiratory and locomotory disease of turkeys is less clear and other factors may be involved and responsible for the onset of clinical signs.
MM causes embryo deaths, sinusitis, stunting, airsacculitis, and occasionally bone defects as well as swollen hock joints. **MI** causes mortality of turkey embryos and some strains may cause inflammation of the joints, but the organism appears to be rarely encountered since its eradication by the primary breeders (Ley, 2003).

Diagnosis of mycoplasma on the basis of clinical features and pathological lesions is often difficult, since these signs may be confused with other infectious diseases. Proof of infection therefore must be confirmed by laboratory diagnosis either by direct detection, direct isolation or indirectly using serological methods. During the acute stages of infection the number of mycoplasma in the respiratory tract is very high. In such cases 5 - 10 tracheal or chonal cleft cotton swabs are sufficient for mycoplasma isolation. In chronic cases however, a high number of samples is essential. In dead birds culture of lesions should be carried out. For culturing embryonated eggs, samples of yolk and yolk membrane should be included. The samples should be cultured immediately after sampling using liquid and agar Mycoplasma media (Frey et al.1968, Bradbury ,1998)
The isolation is accompanied by several disadvantages as follows: 1) the organism is relatively fastidious and slow growing and culturing requires about 21 - 28 days confirming a negative result. 2) the growth of mycoplasmas may be inhibited by damage during sample transport to laboratory or by growth inhibitors in the medium. 3) in some flocks several different mycoplasmas (non

pathogenic) can be grown from a single sample and compete regularly with the growth of a pathogenic one.

To avoid such difficulties MG and MS DNA-probe test kits have been developed and are now commercially available. The test is highly sensitive and specific and is able to detect small amount of mycoplasmas even in long-term frozen samples within 8 hours. The cost is comparable to isolation procedures. In addition, several PCR-based tests have also been published for MG and are cited by Kempf (1998) including a multiplex PCR, which is designed to detect all four avian mycoplasma pathogens (Wang et al., 1997). Furthermore, a manual published by Lauerman (1998) contains a validated PCR assay for avian mycoplasmas. Recently Lierz et al. (2008b) reported on the use of polymerase chain reactions to detect *Mycoplasma gallisepticum, Mycoplasma imitans, Mycoplasma iowae, Mycoplasma meleagridis* and *Mycoplasma synoviae* in birds of prey.

For serological examinations the most commonly used initial screening test is the *rapid serum plate agglutination test (SPA)*. The test is based on the use of specific stained antigen for MG, MS and MM that are commercially available. The test is quick, inexpensive and highly sensitive (Kleven, 1975), but there are also variations between batches in sensitivity and specificity (Kleven and Levisohn, 1996). Serum plate agglutination suspected reactors generally must be confirmed by other tests such as a Haemagglution inhibition test (HI) using fresh culture antigen (Kleven et al., 1996). The corresponding titres are 1:40 and 1:80 or above respectively. The HI-test appears to be more specific than SPA, but still shows wide variations in results due to lack of uniformity between antigen preparations and that some isolates fail to agglutinate red blood cells and do not stimulate the production of HI- antibody. ELISA-kits have also been developed for detection of antibodies against MG, MS, and MM. These kits are commercially available. ELISA is sensitive and specific but also can be prone to false positive and negative results. Generally, significant antigenic variability among strains also exists, which could affect the sensitivity of serological tests (Kleven et al., 1988, 2001). In all cases confirmation of positive cases can be carried out only by retesting the birds after about a month, or by culture.

Treatment and control

Several drugs have been found useful for reducing clinical signs and shedding in infected flocks. However, no known antibiotic regardless of dosage or length of treatment can eliminate the infection in birds and hatching eggs.
Among the more common antibiotics are tylosin, spiramycin, tetracycline, quinolones (enrofloxacin, flumequin), spectinomycin and lincomycin. The drugs can be administered by numerous routes (Injection, feed, water). Treatment is able to reduce the losses; but relapses may occur when treatment is discontinued. Since the isolation of the causative agent is difficult and time consuming, currently little is known about the susceptibility of recent isolates.

Eradication of mycoplasma in breeder flocks through testing and slaughter is the preferred method to clean the production chain from the top and to prevent mycoplasma introduction through primary and commercial breeder flocks. However, in places with intensive continuous poultry production and in valuable pedigree lines it has been determined that this method is too expensive and impractical (Ley, 2003).

Hatching egg treatments with antibiotics for the control of egg transmitted bacterial pathogens has been widely investigated and seems to be of great value. Different methods of egg treatment have been used such **egg dipping** in antibiotics using pressure differential dipping or temperature differential dipping (Olsen et al., 1962, Hall et al., 1963). These methods greatly reduce the mycoplasma egg transmission, but do not always completely eliminate it.

Dipping solutions can become excessively contaminated with resistant microorganisms such as pseudomonads and organic material. To prevent bacterial contamination of the solution filtering with subsequent cool storage and/or addition of disinfectants is the most effective method. Thorough and continuous bacteriological monitoring of dip solution is also required. The concentration of the antibiotics must be examined regularly and renewed routinely. By using enrofloxacin the pH-value of the dipping solution can be corrected during storage. The use of egg dipping in antimicrobials should be critically evaluated, because of the irregular uptake of dip solution, uneven distribution of active substance in the egg compartments and lack of standardization in dipping technique. Additionally, it is known that different disinfectants used for washing can influence negatively the antibiotic uptake of hatching eggs (Hafez, 1997). Therefore it is recommended that the compatibility of different disinfectants used for egg washing and/or used in dipping solution has to be examined before application (Bickford et al., 1973, Horrox, 1987).
As the uptake of active substance by the hatching egg can be very irregular during dipping, individual egg injection with accurate delivery of the proper dose is preferred in elite and grandparent stock breeding. Automated systems for *in ovo* drug disposition before hatch are being developed (Ghazikhanian et al., 1980; Froyman, 1996).

Generally in commercial flocks kept in endemic area application of vaccines against MG and/ or MS may help prevent clinical disease. The subject of MG vaccination has been reviewed by Whithear (1996).
Vaccination against MG infection using inactivated culture was attempted in the early 1950`s without success until oil emulsion inactivated vaccine ***"bacterins"*** were introduced. The inactivated bacterins came into wide spread use in the early 1980's and were primarily used in commercial layer flocks and now are used in broiler breeder flocks in many areas world-wide. The bacterins provide good protection against egg production losses (Hildebrand et al., 1983). In vaccinated flocks feed conversion improvements and reductions of medication costs have been demonstrated. In vaccinated breeder flocks a drastic reduction of egg transmission also has been observed. The lag period between infection and egg shedding in vaccinated flocks is longer, and this period could be prolonged by revaccination of the birds during production. However, inactivated vaccines have some disadvantages: they are expensive; they must be applied by injection which leads to higher labour costs, and do not provide optimal protection against infection and tracheal colonization by field strains.

A live MG vaccine called ***6/85 strain*** has been introduced (Evans and Hafez, 1992, Evans et al., 1992). It is apparently less pathogenic for both chickens and turkeys providing a significant protection against airsacculitis and egg losses. Also a live vaccine based on a ***Temperature-sensitive (TS-11)*** mutant strain of MG has been developed and is widely used in Australia and licensed in some other countries (Whithear et al., 1990). MG vaccines have had less use in turkeys. The F strain is too pathogenic for consideration in turkeys, but 6/85 or ts-11 strains may have potential use under very limited circumstances. There has been relatively little work on MS vaccines. There has been one MS bacterin licensed in the USA, but it apparently has had little field use (Kleven, 2004).

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PHOSPHORUS: AN EXPENSIVE NECESSITY, IS PHYTASE THE ANSWER?

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Introduction

Phosphorus is an essential nutrient for growth, development, and maintenance of the skeleton of animals. Because of the demands for adequate skeletal development of rapidly growing birds and the sensitivity to deficiencies of phosphorus, it is necessary to provide an adequate margin of safety for this nutrient in broiler diets (Waldroup, et al., 2000). However, there is a concern worldwide regarding the quantity of phosphorus that is released into the environment generated from land application of poultry litter from commercial houses (Powell et al., 2008). The environmental concerns in combination with the increase in cost of supplemental phosphorus have led to the inclusion of phytase enzymes into poultry diets. Published reports indicate that the minimum level of non-phytate phosphorus needed in a starter diets range of 0.37% - 0.39% (Waldroup et al., 2000) which is higher than 0.45% which is recommended in the NRC (1994) and Lesson and Summers (2005). Due to rising dietary costs including supplemental phosphorus, allowing a margin of safety with regards to dietary phosphorus concentration may no longer be practical, and increasing the demand of phosphorus release with phytase inclusion may decrease cost.

Therefore, two experiments were designed to investigate the minimum level of non-phytate phosphorus needed to maximize growth performance and determine the amount of phosphorus release from phytate with the inclusion of three commercially available phytase enzymes.

Experiment 1

Objective:

The objective of this experimental design was to determine the minimum non-phytate phosphorus level in a two dietary phase program needed to maximize growth in straight-run broilers through 28 days of age reared in two different rearing environments.

Experimental Design:

The experimental design consisted of a total of six dietary treatments with selected calculated non-phytate phosphorus concentrations in the started diet of 0.30, 0.35, 0.40, 0.45, 0.50, and 0.55%. Each treatment consisted of five replicate pens containing 40 straight-run chicks per pen for a total of 1200 chicks placed. Broilers were fed the starter until 14 days of age and then switched to a grower diet. The grower diet was fed through the termination of the study at 28 days of age. The high nutrient density diet for raising straight-run market broilers found in Leeson and Summers (2005) with an available phosphorus level of 0.45% was used as the reference diet. Available phosphorus levels in all grower diets were decreased by 9% from the initial level present in the starter diet. This level also corresponds to Leeson and Summers (2005) which is being used as the control diet. Chicks were reared in floor pens with fresh pine shavings as litter material, given age appropriate supplemental heat, and given access to feed and water *ad libitum*.

In a parallel study, this entire experimental design was duplicated in battery cages. There were five replicates of each of the six dietary treatments. Ten birds/pen were placed in battery units and

identical to that of the chicks raised in floor pen. Chicks for both studies were obtained from the same source and fed the same dietary treatments. Weigh days and dietary changes corresponded with the floor trial as well.

On day of placement, chicks were weighed, banded, and randomly placed in treatment groups. Bulk pen weights and feed consumptions were determined on day 7, 14, 21, and 28 for the calculation of average bird weight and feed conversion ratios. On day 28, broilers in each pen were separated by phenotypical differences on the basis of sex to determine the minimum available phosphorus level needed to maximize growth for each sex.

Results

Floor-Reared Broilers

Body weight tended to be a more predicted factor than feed conversion ratio when determining minimum available phosphorus requirements for floor and battery reared broilers. In floor reared broilers, growth depression was observed at 7 days of age with the lowest percentage yielding the lowest average body weight (Table 1). This trend continued through day 28 with the 0.30% level yielding the lowest body weight. At the conclusion of the starter diet (Day 14), the 0.45% diet appeared to be the minimum available phosphorus level needed to maximize growth being significantly higher than all of the lower levels. However, this trend did not continue once the dietary change was made. The broilers fed the 0.40% dietary treatment had similar body weights compared to the 0.45% level at 21 and 28 days of age. Differences in feed conversion ratios were only seen in cumulative ratio for the starter period and for the duration of the study (Table 2). At both of these time periods, the 0.40 and 0.45% level performed similarly. Upon separating sexes at day 28, data did not indicate any differences among sexes.

Battery-Reared Broilers

Similar to floor reared broilers, battery reared-broilers fed the lowest level of available phosphorus, 0.30%, yielded the lowest body weight throughout the duration of the study (Table 3). Through day 21, the minimum available phosphorus level needed to maximize growth appeared to be the 0.40% level; however continuation of the study through day 28 resulted in similar body weights for 0.40% and the 0.35% levels at the conclusion of the study. Upon separation of the male and female weights on day 28 indicate that the minimum level needed in males was the 0.35% level however the minimum level for females to maximize growth was slightly higher at 0.40%. This observation was not detected in the floor pen broilers.

In combination, these data indicate that the available phosphorus level needed for maximizing growth performance is dependent on rearing environment.

Table 1. The effect of calculated available phosphorus level on body weight through 28 days of age reared in floor pens.

	Available Phosphorus (%)					
	0.30%	0.35%	0.40%	0.45%	0.50%	0.55%
Day 7	141.66^{c}	150.78^{b}	149.92^{b}	155.01^{ab}	159.08^{a}	154.04^{ab}
Day 14	340.54^{d}	375.57^{c}	388.83^{c}	412.58^{ab}	424.51^{a}	391.95^{bc}
Day 21	646.40^{c}	738.65^{b}	769.73^{ab}	816.28^{a}	818.05^{a}	790.40^{ab}
Day 28	1076.75^{c}	1231.84^{b}	1316.53^{a}	1344.67^{a}	1338.90^{a}	1300.44^{a}
Day 28 (females)	1015.76^{d}	1139.25^{c}	1239.90^{ab}	1252.56^{ab}	1259.21^{a}	1194.36^{bc}
Day 28 (males)	1145.97^{c}	1321.14^{b}	1393.78^{ab}	1450.68^{a}	1408.77^{a}	1418.53^{a}

a-d Means in rows with different superscripts differ significantly at $P<0.05$.

Table 2. The effect of calculated available phosphorus level on mortality corrected feed conversion ratio through 28 days of age reared in floor pens.

	0.30%	0.35%	0.40%	0.45%	0.50%	0.55%
Day 1-7	1.38	1.27	1.36	1.32	1.26	1.32
Day 7-14	1.33	1.41	1.34	1.29	1.25	1.37
Day 1-14	1.34[a]	1.36[a]	1.34[a]	1.30[ab]	1.25[b]	1.36[a]
Day 14-21	1.44	1.39	1.37	1.39	1.47	1.40
Day 1-21	1.39	1.38	1.35	1.35	1.35	1.38
Day 21-28	1.66	1.60	1.58	1.66	1.65	1.70
Day 1-28	1.50[ab]	1.47[bc]	1.45[c]	1.48[abc]	1.47[bc]	1.50[a]

a,b Means in rows with different superscripts differ significantly at P<0.05.

Table 3. The effect of calculated available phosphorus level on body weight through 28 days of age reared in battery pens.

	0.30%	0.35%	0.40%	0.45%	0.50%	0.55%
Day 7	149.83[c]	159.25[b]	165.71[ab]	168.70[a]	164.53[ab]	171.11[a]
Day 14	448.93[c]	499.41[b]	516.33[ab]	529.53[a]	522.51[ab]	536.38[a]
Day 21	713.30[c]	827.88[b]	882.47[a]	897.43[a]	880.01[a]	900.33[a]
Day 28	1129.90[b]	1337.16[a]	1391.10[a]	1404.33[a]	1359.21[a]	1387.37[a]
Day 28 (females)	1038.49[c]	1210.63[b]	1306.47[ab]	1274.89[ab]	1305.96[ab]	1344.66[a]
Day 28 (males)	1220.43[b]	1496.59[a]	1540.59[a]	1490.80[a]	1461.37[a]	1446.90[a]

a-c Means in rows with different superscripts differ significantly at P<0.05.

Table 4. The effect of calculated available phosphorus level on mortality corrected feed conversion ratio through 28 days of age reared in battery pens.

	0.30%	0.35%	0.40%	0.45%	0.50%	0.55%
Day 1-7	1.24	1.13	1.25	1.10	1.26	1.10
Day 7-14	1.32	1.31	1.35	1.33	1.29	1.31
Day 1-14	1.30	1.25	1.32	1.26	1.28	1.24
Day 14-21	1.47	1.38	1.37	1.38	1.35	1.45
Day 1-21	1.34	1.32	1.35	1.32	1.32	1.35
Day 21-28	1.76[a]	1.60[b]	1.66[ab]	1.58[b]	1.76[a]	1.77[a]
Day 1-28	1.48	1.43	1.47	1.42	1.48	1.50

a,b Means in rows with different superscripts differ significantly at P<0.05.

Experiment 2

Objectives

The objective of the this design was the development of a dose response curve to selected levels of non-phytate phosphorus and the evaluation of three commercially available heat stable phytase enzymes on broiler growth performance and bone ash when fed diets deficient in available phosphorus.

Materials and Methods

The experimental design consisted of a total of 10 dietary treatments which are listed in Table 5. Each treatment consisted of six replicate pens containing 50 straight-run chicks. Broilers were fed the starter until 14 days of age and then switched to a grower diet for the remainder of the experiment at 35 days of age. Chicks were reared in floor pens with fresh pine shavings as litter material, given age appropriate supplemental heat, and given access to feed and water *ad libitum*.

Table 5. Dietary Treatments fed to straight-run market broilers which varied in available phosphorus concentration and supplemented with one of three commercially available phytase enzymes.

Trt #	Treatment	Starter Available P (%)	Starter Phytase FTU/kg	Grower Available P (%)	Grower Phytase FTU/kg
1	0.225	0.225	0	0.175	0
2	0.275	0.275	0	0.225	0
3	0.325	0.325	0	0.275	0
4	0.375	0.375	0	0.325	0
5	Phytase A	0.175	250	0.125	250
6	Phytase B	0.175	1850	0.125	1850
7	Phytase C	0.175	400	0.125	400
8	Phytase B	0.145	3700	0.145	3700
9	Phytase A	0.125	1000	0.125	750
10	Phytase C	0.125	1000	0.125	750

Results

With regards to body weight on day 14, a linear relationship was observed with significant increases with each incremental increase in available phosphorus (Table 6). Mortality corrected feed conversion (FCR) through 14 days of age following a similar pattern however improvement was only observed through 0.325% available phosphorus diet. Boilers fed the lowest level of available phosphorus with out supplemental phytase (Trt 1) were terminated prior to the end of the study due to decreased mobility and observed morbidity. Body weights for day 35, followed a similar linear pattern as on day 14 with incremental increases with increases available phosphorus inclusion. FCR also maintained a similar pattern with the lowest remaining available phosphorus level yielding the highest FCR while the two highest levels were similar. Tibia weights and ash percent following a similar linear pattern as body weight.

Broilers fed phytase supplemented diets (Trt 5-10) that were lower available phosphorus than those not supplemented (Trt 1-4) yield performance characteristics similar to that of Trt 1 and 2. However, evaluate parameters varied amongst the three commercially available phytase enzymes with regard to body weight, FCR, tibia weight, and tibia ash.

These data indicate that the commercially available phytase enzymes can be an effective means to reduce the amount of dietary supplemental phosphorus used in poultry production and high levels of supplementation can be used to gain greater that a 0.10% phosphorus release if cost effective.

Table 6. Body weight, mortality corrected feed conversion, tibia weight, and tibia ash of straight-run market broilers fed diets containing selected concentrations of available phosphorus and supplemented with one of three commercially available phytase enzymes.

Trt #	Body Weight Day 14 (g)	Feed:Gain Day 1-14	Body Weight Day 35 (kg)	Feed:Gain Day 1-35	Tibia Weight (g)	Ash (%)
1	209.7[e]	1.41[a]				
2	272.8[d]	1.36[b]	1.11[de]	1.69[b]	8.12[de]	40.57[c]
3	331.1[b]	1.30[cd]	1.47[b]	1.63[c]	9.54[b]	42.79[b]
4	369.5[a]	1.29[d]	1.68[a]	1.62[c]	10.53[a]	44.20[a]
5	297.2[c]	1.33[bcd]	1.09[e]	1.71[b]	8.32[d]	41.42[c]
6	310.0[c]	1.34[bc]	1.18[d]	1.72[ab]	8.58[cd]	40.98[c]
7	264.8[d]	1.36[b]	0.95[f]	1.76[a]	7.19[f]	39.44[d]
8	303.0[c]	1.35[bc]	1.38[c]	1.64[c]	8.92[c]	42.56[b]
9	310.5[c]	1.32[bcd]	1.37[c]	1.65[c]	9.77[b]	42.94[b]
10	268.9[d]	1.32[bcd]	1.05[e]	1.70[b]	7.75[e]	41.03[c]

a-f Means in rows with different superscripts differ significantly at $P<0.05$.

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PATOLOGÍA AVIAR DEBIDA A MICOTOXINAS

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Introducción

Las micotoxinas son un grande grupo de productos tóxicos para los animales, plantas y seres humanos que ocasionan diferentes patologías, dependiendo de su estructura, interferido en las diferentes vías del metabolismo de los animales. Hasta ahora, son conocidas cerca de 300 diferentes micotoxinas y estudios de la FAO mostraron que ellas están presentes en más de 30% de los cereales producidos en el mundo.

El grande desafío de las micotoxicosis es la falta de un diagnostico preciso, sabiendo los productores de aves que las micotoxinas están íntimamente relacionadas con bajas de desempeño. De esta manera, grande parte de los casos de diagnósticos de micotoxicosis están basados en la historia y signos clínicos, y principalmente lesiones hepáticas, el órgano más perjudicado. Ya que no es fácil la detección de las micotoxinas en los alimentos para los animales que presentan problemas de supresión inmunológica, mal desempeño o lesiones clásicas de micotoxinas.

Se sabe que el muestreo inadecuado es la causa más común de la variable de los análisis de micotoxinas. Las micotoxinas nunca son distribuidas uniformemente en todos los cereales o alimentos almacenados, ellas están más concentradas en áreas con mayor humedad o con mayor nivel de oxigeno. En la mayor parte de los casos, la colecta de la muestra es realizada en una pequeña porción del silo.

Consecuentemente, los resultados de los análisis pueden ser negativos o con bajos niveles de micotoxinas, dependiendo del lugar de muestreo. Por otro lado en situaciones donde hay la notificación de micotoxinas de campo, generalmente, los animales ya consumieron todo el alimento, no restando la cantidad suficiente para el análisis. Además de eso, en situaciones de campo, más de un tipo de micotoxinas están presentes en los alimentos al mismo tiempo, y aunque los análisis muestren bajos niveles de una determinada toxina, en la misma ración, se podría encontrar otra, creando un efecto sinérgico.

Del punto de vista epidemiológico, el crecimiento fúngico puede ocurrir en diferentes fases de la explotación vegetal y animal. Ellos pueden invadir las semillas antes de la retirada, o se desarrollar en el almacenamiento de los granos. Otro punto crítico es el procesamiento de las raciones, principalmente en la fase donde la temperatura y humedad están elevadas. Y, por último, el crecimiento fúngico y las micotoxinas son presentes en locales de la fábrica de ración, silos de granjas y comederos donde no hay higiene y limpieza adecuadas.

En realidad, las perdidas provenientes del crecimiento fúngico sobre el desempeño animal no son solo debido a la producción de micotoxinas. Los hongos también causan daños físicos y pérdidas en la calidad nutricional de los granos, siendo un efecto aditivo a la severidad de las micotoxicosis. La actividad metabólica de los hongos está asociada a la respiración aeróbica, a si la deterioración del grano de una reacción de oxidación de gorduras y hidratos de carbono, en la presencia de oxigeno dando como resultado un acido carbónico, agua, calor y estructuras fúngicas (Dixon & Hamilton, 1981). Además de eso el nivel de lípidos es muy reducido en los granos infectados por los hongos y eso esta relacionado con la reducción de energía disponible. Esta situación podría tener una gran influencia sobre la gravedad de las micotoxicosis en los animales especialmente si este nivel de energía no sea corregido por un nutricionista.

Considerando este panorama sobre los problemas del crecimiento de los hongos y micotoxinas, se torna claro que para comprender esa temática en la producción avícola es necesario

ampliar el conocimiento sobre su epidemiologia. Con base en este conocimiento será posible establecer algunas acciones para prevenir la ocurrencia de micotoxicosis en estos animales. Es necesario saber cómo evaluar la relación costo/beneficio en la prevención de esta enfermedad tan peligrosa y silenciosa en la salud animal.

Efecto de las micotoxinas en la salud de las aves

El efecto de las micotoxinas en la avicultura está relacionado directamente al tipo y la cantidad de toxinas, ya que existen cerca de 300 tipos conocidos. Las micotoxinas conocidas por afectar más la salud y el desempeño de las aves están presentadas en el cuadro:

Cuadro: Micotoxinas más estudiadas en la avicultura

Micotoxina	Género fúngico productor
Aflatoxina B1, B2, G1, G2	*Aspergillus* y *Penicillium*
Tricotecenos (T2, DON, DAS, nivalenol, etc)	*Fusarium*
Ocratoxina	*Aspergillus y Penicillium*
Patulina	*Aspergillus y Penicillium*
Zearalenona	*Fusarium*
Citrinina	*Aspergillus y Penicillium*
Fumonisina	*Fusarium*
Gliotoxina	*Aspergillus*
Ácido penicílico	*Penicillium*
Moniliformina	*Fusarium*
Ergotamina	*Claviceps*
Ácido ciclopiazonico	*Aspergillus*
Ácido fusárico	*Fusarium*

Efecto de micotoxinas sobre el comportamiento y desempeño animal.

Normalmente, los casos comunes de micotoxicosis a campo (crónica), las aves no poseen ninguna señal o comportamiento típico de la enfermedad. Los signos más comunes son caracterizados por apatía y pueden ser observados en estados avanzados de la enfermedad, por lo que existe la posibilidad de ser confundida con otras patologías aviarias. En algunos casos, el principal efecto de la micotoxinas es la inmunosupresión, que resulta en infecciones bacterianas secundarias. La mayor parte de estas lesiones están relacionadas a las infecciones secundarias con *E. coli,* como aerosaculitis y celulitis. El efecto de las toxinas sobre el desempeño animal dependerá del tipo y de la cantidad y su asociación con factores externos, como malo manejo de las aves. Las micotoxinas de mayor importancia y gravedad son las aflatoxinas y ocratoxinas. Ellas causan disminución en la pigmentación del pico y patas (bien como reducción en el crecimiento). Como gran parte de las micotoxinas afectan el sistema digestivo y absorción de nutrientes, la presencia de los alimentos no digeridos en las heces puede ser un síntoma clínico común. Lesiones renales promovida por ocratoxina, patulina y citrinina también pueden resultar en heces liquidas con aumento de humedad y presencia de urato caracterizada por heces blancas, lo que puede resultar en lesión de patas por la mala calidad de la cama.

Efecto de las micotoxinas en el tracto gastrointestinal y renal de aves

Logo de la ingestión de las micotoxinas con el alimento contaminado, las células epiteliales del intestino son expuestas a gran cantidad de esos metabolitos de hongos. Algunas micotoxinas pueden causar necrosis por contacto como es el caso de los Tricotecenos, como T2. Toxinas como el acido ciclopiazónico pueden también causar necrosis en las mollejas.

Por otro lado, las fumonisinas pueden afectar la resistencia entre las células intestinales (Bouhet et al, 2004) disminuyendo la barrera intestinal contra los patógenos e regulación del equilibrio osmótico de las células. Todos esos eventos pueden ser clínicamente presentados como una severa enteritis que tornase más severa con la presencia de bacterias oportunistas. Lesiones microscópicas pueden ser caracterizadas por reducción de las vellosidades intestinales y con microscopia electrónica se observa extrusión de las células.

Muchas de las micotoxinas son liposolubles y rápidamente absorbidas por las células intestinales. Una vez absorbida llegan al hígado y circulación sanguínea sistémica. La mayor parte afecta el metabolismo del hígado. Pero, cada micotoxina puede ter distintos mecanismos de acción. Aflatoxina y ocratoxina son las más toxica para el hígado provocando de forma aguda alteración del color para roja intenso, incremento del tamaño del hígado y de la vesícula biliar. La lesión crónica puede promover una disminución del hígado con coloración amarilla y la vesícula biliar disminuida o ausente, donde en la microscopia se observa vacuolización de hepatocitos y proliferación de los ductos biliares (Santin et al, 2002). La ocratoxina (OA), tiene DL50 más baja que la aflatoxina (lo que significa que es más toxica en niveles mucho más bajos) y además de la lesión de hígado, causa incremento de tamaño de riñones. Reducción en la productividad, incremento en el consumo de agua y de la humedad en la cama, son las principales señales de la ocratoxicosis. Aflatoxina y ocratoxina causan lesiones hepáticas severas que incrementan los niveles de la enzimas AST y GGT en sangre con reducción en los niveles de proteína y calcio séricos (Santin et al, 2002b).

Tricotecenos son un gran grupo de más de 100 metabolitos de hongos con la misma estructura básica. Las más conocidas son T2, DAS y deoxinivalenol (DON). La lesión oral es la más común asociada a los tricotecenos además de la diminución en el consumo, producción y calidad de huevos. DON y T2 en la dieta disminuyen los valores de hematocrito, leucocitos, como linfocitos T CD4+, CD8+ y B y la concentración de IgA biliar.

Zearalenona también es una fusariotoxina con potente acción estrogénica. La inducción de señales de anormalidades reproductivas en cerdas es mucho conocido como causa de esa micotoxicosis, mas su efecto en aves no está muy claro. Malekinejad *et al* (2006) informan que la zearalenona puede ser metabolizada en el hígado en dos diferentes metabolitos: alfa o beta-zearalenol. Alfa-zearalenol presenta alto potencial estrogénico y beta presenta poco potencial. Los

autores informaron que el hígado de aves, diferente a lo que sucede con cerdos, produce más beta-zearalenol y por eso serian necesarios niveles más altos de esa toxina para causar efectos deletéreos en aves. Por lo que en aves; el efecto de la zearalenona, así como su efecto sinérgico con otras toxinas, está muy poco esclarecido. De facto, se sugiere que la detección de esa toxina en alimento de aves solo puede servir de biomarcador para otras fusariotoxinas.

Fumonisinas son las micotoxinas asociadas primariamente a leucoencefalomalacia equina y el edema pulmonar porcino. En aves puede causar severa diarrea, hepatomegalia, alta mortalidad y reducción en la productividad (Ledoux et al). Estas toxinas han sido muy encontradas en varias materias primas y el conocimiento de los problemas que causan no han sido esclarecidos por completo; sin embargo, sus efectos en el sistema inmunitario de los animales han sido muy informados en varios estudios.

Los alcaloides de ergot, son otra clase de micotoxinas producida por *Claviceps sp* y causa isquemia, necrosis y gangrena de las extremidades. Los alcaloides son muy comunes en sorgo y trigo.

Además todos los problemas relatados en estudios con toxinas purificada el efecto de la asociación de las toxinas son muy variables y pueden dificultar mucho el diagnostico sobre todo cuando hay interacción con otras enfermedades infecciosas.

Efecto de las micotoxinas en la inmunidad de las aves

Las micotoxinas causan regresión y despoblación celular en los órganos linfoides. En el caso de aflatoxina y ocratoxina es muy probable que la interferencia con la síntesis proteica sea la principal causa de la inmunosupresión. La respuesta inmune necesita de intensa síntesis de proteína y si hay algo que no permita esto, el sistema inmune reduce su función. En un estudio con aflatoxina y ocratoxina se observó reducción en la cantidad de células en mitosis en la bolsa de Fabricio y reducción en los títulos vacúnales contra enfermedad de Newcastle en pollos (Santin *et al*, 2002). Eso sugiere que hay reducción en la eficacia de las vacunas, lo que, en caso de reproductoras puede reducir la inmunidad pasiva y afectar la viabilidad de pollitos en la primera semana.

Las fumonisinas por su lado, son potentes inhibidores de la enzima sphinganine N-acyl transferasa. La actividad biológica de la enzima está relacionada con la integridad de las membranas celulares que parecen ser el principal tejido blanco de las fumonisinas *in vivo*. Ledoux *et al,* observaron disminución en inmunidad humoral y supresión en la proliferación de linfocitos y en la eliminación de bacterias.

Sin embargo, la inmunosupresión es la forma más común de efecto de micotoxinas en el sistema inmune, pero un estudio de Petska *et al* (2004) encontraron que DON y otros tricotecenos

pueden exacerbar la respuesta inmune. En el estudio, bajos niveles de tricotecenos incrementaron la expresión de las citocinas y genes inflamatorios que estimulan la respuesta inmune. Sin embargo, las mismas toxinas en altas dosis resultan en apoptosis de leucocitos resultando en inmunodepresión. Estos resultados deben ser evaluados más cautelosamente. En realidad que bajos niveles de tricotecenos causen estimulación inmune, no significa que sea bueno para la salud y desarrollo animal. Koutsos y Klasing (2001), demostraran que cada vez que la respuesta inmune es estimulada se torna muy costoso para el animal, además se pueden desarrollar muchas formas de alergias.

Casos de campo de micotoxicosis están relacionados con reducción de títulos vacúnales y aparición de infecciones oportunistas como *E. coli* o *Clostridium sp*; así como el incremento de pérdidas en la planta de proceso, debidas a lesiones septicémicas. Asimismo, incremento de lesiones no específicas en las mucosas orales e intestinales, así como en el índice de conversión alimenticio. Estos trastornos pueden estar asociados a la estimulación de la respuesta inmune.

Costo de las micotoxicosis en aves

Es muy difícil de calcular los costos de las micotoxicosis en aves, principalmente debido a diferentes condiciones ambientales y de manejo. En reproductoras el principal impacto económico de las micotoxinas es calculado con los índices de viabilidad, producción de huevos, incubabilidad y calidad de pollitos. En pollos micotoxicosis afectan su desarrollo, incrementan la medicación y, principalmente, disminuyen la calidad de canales debido a enfermedades septicémicas.

En verdad hay otros factores que interfieren con la severidad de las micotoxicosis en los animales. Los más importantes son malas condiciones de manejo e higiene. Hay muchas evaluaciones prácticas que sugieren que malas condiciones de manejo incrementan estrés y la mala higiene incrementa las exposiciones de los animales a patógenos. Como estos animales ya presentan sistema inmune afectado, aumenta la probabilidad de la aparición de lesiones graves. Así que en casos de campo en muchas ocasiones se observa que el mismo alimento provoca casos severos de micotoxicosis en unos animales, mientras que en otros casos las lesiones y signos son más leves o no se presentan. Por lo que en la producción avícola es un sistema muy complejo, donde condiciones de estrés, mal manejo de campo y nutricional, exposición a patógenos pueden afectar el desempeño y la salud de los animales. La combinación de los diferentes factores, está directamente relacionado con la severidad de la micotoxicosis.

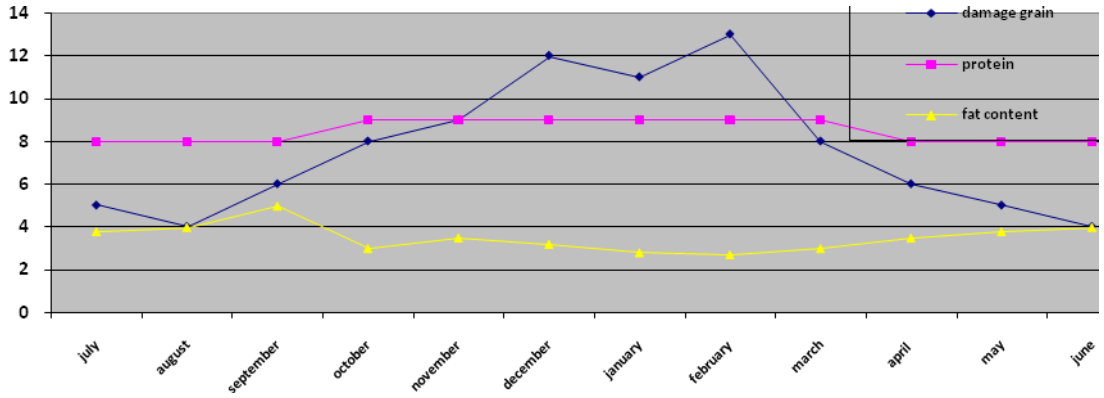
Programa de monitoria para valorar el costo de las micotoxicosis en salud y desempeño animal.

El programa de monitoreo para valorar el costo de las micotoxicosis considera los efectos de las micotoxinas en la salud aviar (inmunosupresión, lesiones, etc) y la calidad de los ingredientes de la dieta.

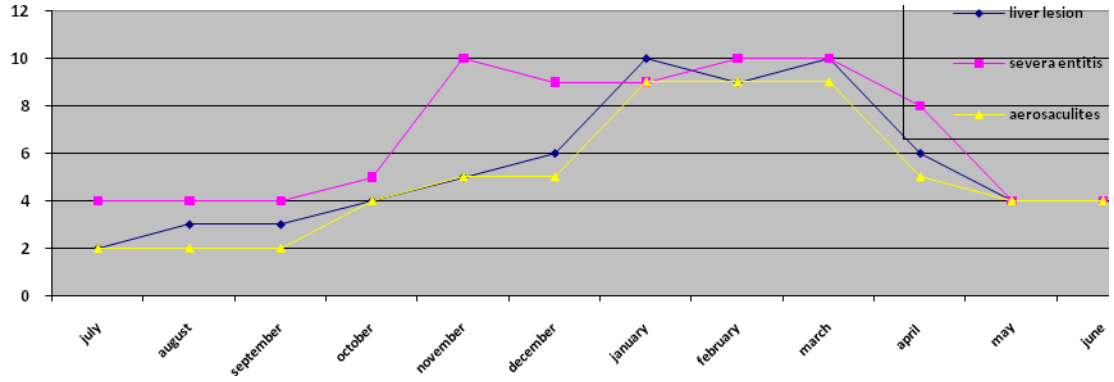
El análisis de micotoxinas en cereal y alimento es muy difícil debido a dificultades en muestreo y metodología de análisis. Mientras tanto, hay alta correlación entre granos dañados, pérdidas nutricionales y niveles de micotoxinas. Cuan alta es la cantidad de granos dañados, más alta la probabilidad de producción de micotoxinas y bajos valores nutricionales del cereal. De esa manera, aplicando constante monitoreo del porcentaje de daños físicos de granos (quebrados, con hongos, quemados, etc) con elaboración de gráficas van a permitir evaluar el periodo de mayor probabilidad de producción de micotoxinas en alimento (Gráfica 1). Siguiendo el ejemplo en gráfica 1 es posible observar un incremento de granos dañados y reducción de lípidos en los meses de diciembre e febrero.

Para relacionar los resultados de calidad de granos y problemas con los animales, es necesario se generar un mismo grafico que valore los índices de performance de los animales en campo, títulos vacúnales, lesiones de matadero, eclosión de huevos, medicación de las aves, etc. En la gráfica 2, comparada con la gráfica 1, es posible observar que la enteritis y otras lesiones están asociadas a la misma época del año donde los granos disminuyen su calidad física y nutricional (Gráfica 1 y 2)

Gráfica 1- Porcentaje de granos dañados, niveles de proteína y de lípido a lo largo del año.



Gráfica 2 – Resultado del monitoreo de lesiones observadas en campo en pollos.



Prevención de las micotoxicosis

Una vez que se considera las micotoxicosis como importante en el costo de producción animal, es muy importante establecer un plan de control de esas micotoxinas para los próximos lotes, una vez que nunca se puede recuperar las pérdidas referentes a las micotoxinas.

Ya que más probablemente las micotoxinas se encuentran en granos dañados; también se tiene que considerar que esos granos tienen perdidas nutricionales. Así que el primer punto de prevención de las micotoxicosis es la corrección de las fórmulas con la calidad de los granos no que se refiere a la energía, pero es necesario mucho cuidado con el uso de grasa oxidada que puede agravar los casos. En caso de almacenamiento de granos es muy importante controlar la humedad, actividad de agua, temperatura y preferencialmente usar inhibidores de hongos a base de ácidos orgánicos. Una práctica muy común en casos de granos de mala calidad es hacer una dilución de los mismos con granos de buena calidad; sin embargo, esta práctica es muy riesgosa, porque no se sabe exactamente las cantidades de micotoxinas y puede suceder que toda la mezcla se torne peligrosa.

Durante el procesamiento del alimento, la limpieza de los equipamientos es muy importante para evitar el desarrollo de hongos y la producción de toxinas, dentro de esos, lo mismo se aplica para los equipos en las granjas, como silos y comederos.

El uso de adsorbentes efectivos y de amplio espectro, como los orgánicos, son una buena alternativa también para reducir los niveles de micotoxinas a niveles non tóxicos a los animales. Pero esa adsorción va ser limitada a la capacidad del adsorbente y la cantidad de toxina de la dieta. Se hay gran niveles de micotoxinas, muchas veces el efecto de los adsorbentes no es capaz de controlar micotoxicosis.

Lo mejor es un plan de prevención y control con base en los principios de HACCP que reduzca los riesgos de desarrollo de hongos en cereales y alimento; además permita rápidas medidas correctivas cuando los problemas son detectados.

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BACILLUS SPORES FOR VACCINE DELIVERY

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1. The genus ***Bacillus***

Bacteria belonging to the genus Bacillus are Gram-positive rods that form a single endospore (spore). The genus comprises 69 species approved in the International Journal of Systematic Bacteriology (IJSB) until 1998, which group into 3–4 major clusters. This diversity, documented by ribosomal 16S rRNA typing, underlines the fact that the genus Bacillus comprises a wide variety of species whose taxonomic position in a single genus is unsatisfactory and needs revision. Bacillus subtilis, Bacillus licheniformis, Bacillus coagulans, Bacillus cereus, Bacillus thuringiensis and Bacillus anthracis, all fall within the well-defined group I [1]. Members of the B. cereus/B. thuringiensis phylogenetic group, which includes B. anthracis, are virtually indistinguishable by 16S rRNA sequence analysis, with as much variability present among the multiple rDNA operons of an individual species as among different isolates [2]. Multi-locus enzyme electrophoresis and analysis of nucleotide polymorphisms suggest they may represent a single species [3 and 4]. Most isolates of the species B. cereus, B. thuringiensis, and B. anthracis carry plasmids that vary in number and size, with some plasmids exceeding 500 kb [2]. In fact, the virulence factors of B. anthracis and B. thuringiensis are encoded by plasmid-carried genes [5, 6 and 7].

2. Bacillus spores as probiotics

"Probiotic" is a word derived from the Greek, meaning 'for life'. It was first used in the modern context to describe "organisms which contribute to intestinal microbial balance" by Parker in 1974 [8]. Probiotics maintain or enhance the indigenous defence mechanisms in the animal without disturbing normal physiological or biochemical functions. The definition was later revised as "a live microbial feed supplement which beneficially affects the animal host by improving its intestinal microbial balance" [9]. This revised definition stressed the need for the probiotic to contain live micro-organisms. The benefits on human health include antagonistic activity against pathogens, anti¬allergic and other effects on the immune system. More recently, the concept of probiotics evolved from food with beneficial, even if not clearly established, effects on well-being, to the new area of functional foods and biotherapeutics [10 and 11]. Probiotic micro¬organisms in use, licensed for human and veterinary applications as prescription and non¬prescription products, comprise a wide variety of micro-organisms including members of the genus Bacillus, Lactobacillus, Streptococcus, Enterococcus, and yeasts [11]. In this group the position of members of the genus Bacillus is unique since they are delivered as spores. This feature makes them of particular industrial interest due to the long shelf life of the respective products and the uncomplicated conditions for storage and transport.
The use of Bacillus spp. as probiotics is not restricted to a single species of this genera, but includes B. subtilis, B. cereus, B. coagulans, B. clausii, B. megaterium and B. licheniformis [12, 13 and 14]. The administration of many spore-based products by the oral route is considered safe due to the widespread consumption of Bacillus spp. through many foods. Alkaline-fermented foods, achieved by mixing bacterial cultures including B. subtilis, are widely consumed in Southeast Asia and African countries. It is the case of "natto", a popular fermented soybean food used as a condiment in Japan, obtained by using B. subtilis var. natto [15]. Also in Western countries, Bacillus spp. are

frequently ingested, as demonstrated for white and wholemeal wheat bread produced without preservatives and a variety of other foods [16].

The effects of these spore preparations on well-being of humans or animals are not clearly established. An immune-stimulating activity has been reported [17 and 18], but the mechanism of stimulation is not fully explained. The lack of information on the biological background for the spore-based probiotic "phenomenon" is due to the fact that it is generally perceived that probiotics act upon the host organism through metabolic products or cell components of actively multiplying vegetative cells. In the case of Bacillus spore probiotics, this aspect is more complex, since no unequivocal data on the growth of bacilli in the human and animal gut are published [19, 20, 21, 22 and 23]. It should be noted that Bacillus spores are part of the normal intake of food and feed [24, 25, 26 and 27]. Despite this daily uptake of spores, vegetative forms of any Bacillus are not part of the normal flora of humans and animals [23 and 27].

3. Spore-based vaccines against anthrax

Anthrax is an acute infectious disease caused by the spore-forming bacterium B. anthracis. Anthrax most commonly occurs in livestock (cattle, sheep, goats, bison, and other herbivores), but it can also infect humans exposed to infected animals (e.g. farmers) or contaminated tissues (e.g. mill workers). Human anthrax has three major clinical forms: cutaneous, inhaled, and gastrointestinal. Pathogenesis of B. anthracis depends upon three plasmid-encoded virulence factors: the anti-phagocytic poly -glutamic acid capsule and two proteinaceous toxins termed edema toxin and lethal toxin (LeTx) [1 and 7].

It is over 120 years that the protection against anthrax, conferred by spores of B. anthracis, has been described (Greenfield in 1880, and Pasteur in 1881). Initially the vaccine schedule of Pasteur was adopted (two doses of partially heat-inactivated spores), but variability in potency and virulence stimulated the search for a more effective and stable vaccine [28]. The success of the attenuated Sterne veterinary vaccine in the 1930 determined a global reduction of anthrax cases in livestock in response to national programmes [29]. This decrease in animal cases determined a consequent decrease in human cases. Unfortunately there are still regions were anthrax is endemic or hyper¬endemic. This fact generally parallels the quality of public health and veterinary services in the respective regions [30] (http://www.vetmed.lsu.edu/whocc/mp_world.htm).

Presently the most widely used vaccine for the prevention of anthrax in animals is the Sterne strain vaccine. This vaccine is based on a toxigenic live attenuated variant of B. anthracis developed by Sterne in 1937 (strain 34F2). This non-encapsulated strain lacks plasmid pOX2 that codes for capsule formation. In Russia and China, live spore vaccines equivalent to the Sterne strain are in use (strain 55), while in Italy animals are vaccinated with a strain (Carbosap) which still carries both virulence plasmids, being nevertheless attenuated in virulence [31 and 32]. General procedures for manufacturing all these vaccines are equivalent and are described by a FAO position paper [33]. After a single subcutaneous vaccination (5000–10,000 live spores per dose depending on vaccinated animal in 0.1% saponin), immunity develops following 7–10 days. While a single dose will provide immunity for about a year, repeated vaccinations (six doses) are required for long-term protection [28].

The residual virulence of all these live vaccine strains (local side reactions and occasional casualties of vaccinated animals) discouraged their use in humans [29]. To overcome this problem, acellular vaccines have been developed as response to threats of biological warfare or bioterrorism since the end of World War II. In the USA a cell-free culture filtrate adsorbed onto aluminium hydroxide from non-encapsulated non-proteolytic strain V770-NP-R is used for human vaccination. The UK vaccine is an alum precipitate of cell-free culture supernatant of the non-encapsulated toxigenic

strain 34F2 (Sterne). Vaccination consists, in both cases, of six initial doses followed by a yearly booster. Present work, aimed to develop alternative vaccines, includes (i) production of purified antigenic polypeptides, (ii) attenuated strains, (iii) recombinant vaccine vectors, and (iv) DNA vaccines [34, 35 and 36]. Ideally such a vaccine should be given orally, or intranasally, and should induce rapid immunity following a single dose.

The identification of immunological correlates of protection in the case of anthrax vaccines is difficult to obtain due to the low incidence of disease, which hampers vaccine efficacy studies. All data reported underline that the toxin-neutralising-antibody titre, and possibly the titre of anti-protective antigen (PA), are predictors of survival and could be used as surrogate markers for protection [29, 37, 38, 39, 40 and 41]. Still it should not be underestimated that spore vaccines appear to be more efficacious than vegetative-cell-vaccines or rPA-vaccines, indicating that spore antigens contribute to protection [42, 43, 44 and 45] or that a spore-linked physical characteristic (targeting of antigens to immune cells, enhancement of phagocytosis of vaccine by antigen presenting cells, etc.) could enhance vaccine efficacy.

4. Bacillus spores and the immune system

Many studies of interaction between Bacillus spores and the immune system have been centred on B. anthracis because of its pathogenicity and danger for human beings. An early report [46] showed that spores have a high affinity for the regional macrophages and are efficiently and rapidly phagocytosed in vivo. During the migration of spore-containing macrophages toward lymph nodes, germination occurs. After spore germination and intracellular multiplication, vegetative bacilli kill the macrophage and are released into the bloodstream, where they live as extracellular multiplying pathogens.

These observations have been recently confirmed by studies of immunofluorescence staining, confocal scanning laser microscopy and image cytometry [47]. The efficient germination of B. anthracis spores within phagosomes of the bronchoalveolar macrophages constitutes the strategy developed by the bacterium to exploit host immune cells to its advantage. Alveolar macrophages play, indeed, a central role in the cell-mediated immune response, assuring the clearance of invading micro-organisms from the lung alveoli. Guidi-Rontani et al. [48] have investigated the fate of germinated spores (Sterne strain) and peritoneal macrophages using fluorescence staining techniques, showing that the survival of spores accompanied loss of macrophage integrity and vitality. The cytotoxicity is a result of the activity of the lethal factor (LF, a zinc-endopeptidase) that is synthesised within the macrophage upon spore germination and is released by lysis of the infected cells [48]. As demonstrated by Hanna et al. [49], macrophages serve as cellular mediators of the action of lethal toxin (PA+LF; LeTx) in vivo. They also observed that at sub-lytic concentrations, LeTx stimulated the release of proinflammatory cytochines such as TNF-α and IL-1 that declined at higher lytic concentrations of LF. Their conclusion was that death from systemic anthrax can be directly attributed to the effects of these cytokines, especially IL-1, produced by LeTx-stimulated macrophages [49]. A completely opposite result has been recently obtained by Pellizzari et al. [50] and Erwin et al. [51]. They found that sub-lytic doses of LF cause a reduction in the production of TNF-α and NO, suggesting that this reduction of the inflammatory response would permit growth and diffusion of the bacterium during the first stages of B. anthracis infection [50].

Besides B. anthracis spores, there are many other spore-forming Bacillus spp. showing immunostimulating and immunomodulating activity, which are however devoid of pathogenicity. The mechanisms responsible for their immunomodulatory properties are still not clear, and few data are available [18].

Since cytokines constitute important soluble mediators with immunomodulatory ability, we have investigated the production of pro-inflammatory cytokines, such as TNF-α and IL-1β, in human purified monocytes stimulated with B. subtilis spores. In vivo, monocytes continuously exit the bloodstream and enter body tissues, where they undergo differentiation [52] toward macrophages or dendritic cells according to the local cytokine environment and the presence of other stimulatory signals [53]. Upon interaction with B. subtilis spores, the percentage of monocytes synthesising TNF-α (13.3%) or IL-1β (14.9%) or both (20.7%) was significantly higher than in untreated monocytes. Interaction of monocytes with other non spore-forming Gram-positive bacteria (such as Streptococcus gordonii) did not significantly stimulated IL-1β and only 4.8% of cells were positive for TNF-α production (data not shown).

5. Engineering the B. subtilis spore

To obtain surface display of heterologous antigens on the spore of B. subtilis [54], the spore coat protein CotB [55] was used as a fusion partner. CotB (380 amino acids) is the most abundant protein of the outer spore coat of B. subtilis, localised on the spore surface [54]. Inactivation of the cotB gene, with consequent absence of CotB from the spore, does not yield any phenotypic variation [55].

The genetic construct for display of a model heterologous protein is described in Fig. 1. Recombinant strains are merodiploid for cotB, since genetic constructs are performed in the amyE locus (Fig. 1) [56] in order to permit easy selection and to guarantee the expression of wild type cotB from its original locus. As a model system, we analysed the surface display of the 459-amino acid-long fragment C of tetanus toxin (TTFC) [57], a well-characterised and highly immunogenic 51.8 kDa peptide encoded by the tetC gene of Clostridium tetani. Display of CotB–TTFC fusion proteins on the surface of recombinant spores was confirmed by FACS, Dot blot and Western blot with specific antibodies. Growth, sporulation and germination parameters were comparable to wild type cells for all recombinants. The system described [54] is the first evidence that a heterologous

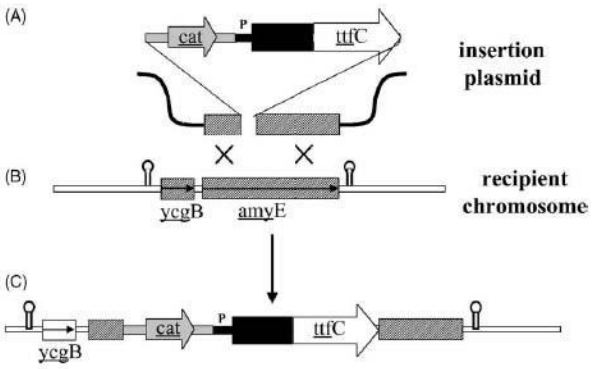

Fig. 2. Schematic representation of the genetic constructs for display of heterologous proteins on the surface of *B. subtilis* spores. (A) Gene fusions of fragment C of tetanus toxin (*ttf*C; white) to the 5′ part of the *cot*B gene are constructed on *E. coli* plasmids carrying *cot*B sequences (black) and a *cat* marker (chloramphenicol acetyl transferase) (grey) within sequences of the *amy*E gene (α-amylase; genome location at 327.201kb; AL009126) (striped). (B) Recombinant plasmids are then transformed into the *B. subtilis* chromosome by natural transformation. Integration by double crossing over is guided by the homologous *amy*E sequences flanking the recombinant insert [56]. The gene *ycg*B, coding for a protein of unknown function, is located in a putative transcriptional unit together with *amy*E (shown for completeness only). Putative terminator hairpins are shown. (C) Representative transformants carry the *cot*B–*ttf*C gene fusions, which are transcribed by the *cot*B promoter (P), integrated on the chromosome within the interrupted *amy*E gene (α-amylase deficient clones appear blue on starch-containing plates flooded with iodine solution).

protein can be expressed on the surface of a bacterial spore and points to this peculiar cell form as a novel and potentially powerful system to display bioactive molecules.

6. Immune response to recombinant spores

The possibility of using recombinant spores as vaccine vectors was investigated using a recombinant strain of B. subtilis engineered to express TTFC on the spore surface. Expression of the heterologous protein on the surface of recombinant spores was demonstrated by flow-cytometric analysis and the amount of TTFC expressed was
2.2 µg/1010 spores as quantified by Dot blot assay.

Subcutaneous injection of BALB/c mice with recombinant spores (1010 per mouse) induced high levels TTFC-specific IgG in serum. The concentration of antigen-specific antibodies was 8.2±2 µg/ml of serum, with controls showing levels of 0.004±0.0007 µg/ml [54]. The neutralising ability of the antibodies was verified in vivo by challenging mice with lethal tetanus toxin. When mice were injected with 10 times the LD50 of soluble tetanus toxin (LD50=2 ng per mouse), all immunised mice were protected, while the control group rapidly died (unpublished results). Recombinant spores of B. subtilis were also shown to be able to prime mice by the mucosal route. Animals immunised by the oral route with spores expressing TTFC developed an efficient secondary response following a subcutaneous injection with soluble TTFC [58] (unpublished results).

7. Perspectives

The main goal in vaccine development is the design of heat-stable, non-parenteral vaccines, which simplify vaccine delivery. Yet, correct targeting of antigens to the most appropriate cellular compartment for generation of a protective immune response is still an unresolved task. Bacillus spores, used widely as probiotics, have an excellent safety record in humans when taken orally. Due to the physical and biological characteristics of the spore, these preparations (powders or spore suspensions in distilled water) are extremely resistant and have a prolonged shelf life. Also the cost of production of spores for oral bacteriotherapy is low with respect to any production of purified vaccine components. The option to use these organisms for generation of recombinant vaccines is made even more feasible by the well-described systems available for genetic engineering of B. subtilis.

The immunostimulating properties of spores, their interaction with antigen-presenting cells (APC), and induction of pro-inflammatory cytokines are characteristics which taken together make spores interesting vectors for targeting vaccine antigens to the APC and the secondary lymphoid organs. The capacity of recombinant B. subtilis spores, expressing heterologous antigens, to induce an antigen-specific immune response (following subcutaneous injection) and to prime the immune system also by the oral route are important further characteristics. Taken together all these features propose recombinant Bacillus spores as vector for vaccine delivery.

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AVIAN ADENOVIRUSES INFECTIONS WITH SPECIAL ATTENTION TO INCLUSION BODY HEPATITIS/ HYDROPERICARDIUM SYNDROME AND EGG DROP SYNDROME

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The first avian adenovirus (AAV) associated with clinical disease was isolated from an outbreak of respiratory disease in quail in 1950 (Olson, 1950). Since that time, AAVs have been found in all types and breeds of chickens and from a variety of other avian species. The infections may be asymptomatic or associated with several clinical and pathological conditions.

Aetiology

The avian adenoviruses are non-enveloped DNA viruses. Adenoviruses are resistant to many several disinfectants and are relatively tolerant to heat and pH changes. Iodophor and aldehyde disinfectants seem to be effective if they are allowed to have contact to the virus for longer time. Composting infected chicken carcasses for 20 days completely inactivates the virus (Senne et al., 1994).

In the past the family *Adenoviridae* was divided by host range and antibody reactivity into two genera: the genus *Mastadenovirus* for viruses isolated from mammalian and the genus *Aviadenovirus* for viruses isolated from birds. Recently, The International Committee on Taxonomy of Viruses has divided the member of the family adenoviridiae in four genera (Benkő et al., 2005). The ***Mastadenovirus*** genus contains the mammalian adenoviruses such as the human, simian, bovine, porcine, equine murine, porcine, ovine and caprine adenoviruses. The genus ***Aviadenovirus*,** formerly designated as group I avian adenoviruses (AAV), contains 11 of the 12 recognized European adenovirus serotypes classified in five (A to E) molecular groups and other related viruses. The genus ***Siadenovirus***, formerly designated as group II avian adenoviruses, includes the Haemorrhagic enteritis virus of turkeys (HEV), Marble spleen disease of pheasants (MSDV) and Avian adenovirus splenomegaly virus of chickens (AASV). The genus *Atadenovirus*, formerly designated as group III avian adenoviruses, includes the Egg drop syndrome virus (EDS) (Table 1).

According to Jensen and Villegas (2005) the nomenclature used for the serotypes of avian adenoviruses from chicken has created some confusion as different systems have been used in Europe and the U.S.; however, a revised nomenclature system has been published (Benkő et al., 2005) that, if adopted, will clarify matters (Table 2).

Table 1: Classification of adenoviruses from birds (Smyth and McNulty, 2008)

Genus	Species	Serotype
Aviadenovirus	*Fowl adenovirus A*	*FAdV-1*
	Fowl adenovirus B	*FAdV-5*
	Fowl adenovirus C	*FAdV-4*
		FAdV-10
	Fowl adenovirus D	*FAdV-2*
		FAdV-3
		FAdV-9
		FAdV-11
	Fowl adenovirus E	*FAdV-6*
		FAdV-7
		FAdV-8a
		FAdV-8b
	Goose adenovirus	*GoAdV-1*
		GoAdV-2
		GoAdV-3
	(Duck adenovirus B)	DAdV-2
	(Pigeon adenovirus B)	PiAdV
	(Turkey adenovirus B)	TAdV-1
		TAdV-2
Siadenovirus	Turkey adenovirus A	TAdV-3
Atadenovirus	Duck adenovirus A	DAdV-1

Table 2: Classification of fowl adenoviruses (Jensen and Villegas (2005))

Species	Serotype number			Proposed type strains	
	Europe	USA	ICTV	Europe	USA
A	1	1	1	CELO	QBV/Phelps
B	5	8	5	340	M2/Tipton
C	4	4	4	KR5	J2
C	11	10	10	C2B	C2B
D	2	2	2	GAL-1	P7
D	3	3	3	SR49	--
D	10	9	9	A2	A2
D	12	12	11	380	--
E	6	5	6	CR119	--
E	7	11	7	YR36/X11	X11
E	8	6	8a	TR59	T8
E	9	7	8b	764	B3

Inclusion body hepatitis (IBH) / Hydropericardium syndrome (HPS)

IBH was first described in 1963 in the USA (Helmboldt and Frazier, 1963). Then after, the disease has been reported in many countries worldwide. It is a sporadic disease condition caused by several serotypes of fowl adenoviruses (Fitzgerald, 2008, Smyth and McNulty, 2008).

In 1988 a new broiler disease was reported in Pakistan and called Angara Disease. The clinical signs and the course of the disease are similar to IBH. The main pathological finding is the accumulation of a clear, straw coloured fluid in the pericardial sac, wherefore the disease was called **hydropericardium syndrome** "HPS". At the same time, several outbreaks of HPS were reported from Asia, central and South America (e.g. Mexico, Ecuador, Peru and Chile) with severe losses (Toro et al., 1999). An adenovirus was detected and later isolated (Rabbani and Naeem 1996, Voss et al., 1996, Mazaheri et al., 1998, Singh et al., 2002).The inclusion body hepatitis/hydropericardium syndrome (IBH/HP) has been reported to occur in both broilers and layers (Cowen, 1992). It seems that immunosuppression, prior to or concurrently with a FAdV infections, is necessary to develop IBH. Infectious bursal disease virus (IBDV), chicken anaemia virus (CAV) and mycotoxins are known to increase the pathogenicity of FAV infections (Fadly *et al.,* 1976; Bülow et al., 1986, Rosenberg et al, 1975, Toro et al., 2000; Shivachandra et al., 2003). However, several cases of IBH occurred without obvious influence of infectious immunosuppression (Reece et al*.,* 1986; Christensen and Saifuddin, 1989). On the other hand Zavala et al. (2002) infected 1-day-old grandparent meat-type chickens carrying maternal antibodies against FAV with a field isolate of FAV associated with inclusion body hepatitis in broilers, avian leukosis virus subgroup J ALV-J, or both FAV and ALV-J and they found no significant differences in the dually infected birds in comparison with chickens that received a monovalent challenge with either FAV or ALV-J.

The infection is transmitted by vertical and horizontal means. Vertical transmission is reported as an important feature of fowl adenovirus (FAV) to spread from parent birds to progenies. Infected breeder shed virus to their progeny for three to six weeks until development of immunity occurs (Toro et al., 2001, Mazaheri et al., 2003). There is evidence that adenovirus infections can become latent and that periods of stress, such as the onset of egg production, will reactivate viral shedding. Girshick et al. (1980) reported that there is evidence that adenovirus infection can remain latent and undetected for at least one generation in a specific-pathogen-free flock.
The bird-to-bird transmission of the virus in a flock occurs horizontally by the oral-faecal route and further spread take place by mechanical means and by contamination with infected faeces. Commercial hatching eggs may be a mechanism of spread of AAV from one country to another.

Mazaheri et al (1998) tested the pathogenicity of FAV serotype - 4 isolated from typical field cases of hydropericardium syndrome in Pakistan and Ecuador in one day old specific pathogen free (SPF) chicks. Infected chickens as well as their contact sentinels, showed depression and reduced flight reactions between day 6 and 11 p.i. After this period no further clinical signs were seen. Mortality commenced 7 days p.i. and continued for 5 days. It was impossible to isolate adenovirus from the livers from any killed chicks at 3 days p.i.. Isolation of virus was possible from the livers of following oral infection of 1-day-old chicks with purified field isolates.

Under field condition the disease is characterized by sudden onset of mortality in chickens < 6 weeks old and as young as 4 days of age. Mortality normally ranges from 2-40 percent, especially when birds are < 3 weeks of age. However, there have been outbreaks in which mortality has reached 80 % depending on the pathogenicity of the virus, immune status of the chicks and concurrent secondary infections. Mortality generally peaks within three to four days and ceases within 9-14 days. Clinically the birds showed lethargy, huddling with ruffled feathers, inappetence

and yellow, mucoid droppings may be seen. The infection can be accompanied with bad feed conversion and a reduced weight gain.

Gross lesions include an enlarged pale friable liver sometimes with necrotic foci also haemorrhages may be present in the liver and muscle. The heart can be flabby with a mild hydropericardium. In case HPS a straw-coloured transudate is present in the pericardial sac. In addition, nephritis, enlarged spleens and thymus atrophy could be observed in most dead birds. Histopathological lesions include necrotic focal lesions and some of the livers had basophilic intranuclear inclusion bodies. Haemorrhages under the epicardium with multifocal necrosis in the myocard are the major findings in the hearts and lymphoid depletion of spleen, thymus and bursa of Fabricius could be observed.

Egg drop syndrome (EDS)

EDS is a disease characterised by a drastic drop in egg production as well as the production of abnormal eggs in apparently healthy chickens and quails. The disease was firstly described in 1976 by Van Eck et al. (1976) in The Netherlands. Then after, the disease was observed in several countries around the world.
The initial outbreak in chickens was probably caused by a contaminated vaccine grown in duck embryo fibroblasts (McFerran, 1979). In addition, the EDS outbreaks observed in the quail flocks, which were reared together with infected chickens, resulted in the fall of the egg production and in the increase of number of soft-shelled eggs (Das and Pradhan 1992).

In spite of the fact that the disease outbreaks were mostly recorded in laying hens only and some time in quails, EDSV or the antibodies against the virus have been detected in ducks and geese (Schlör 1980), pheasants, guinea fowls (Zanella et al., 1980), pigeon (Durojaiye et al., 1992) and in wild birds (Malkinson and Weisman, 1980).

In 2001 EDSV showed to cause a severe acute respiratory disease of the young goslings in Hungary. The disease affected goslings between 4 and 20 days of age. The symptoms included anorexia, depression, sneezing, coughing, dyspnoea, and rales (Ivanics et al., 2001). Recently, Biđin et al., (2007) reported on a naturally occurring EDS in turkey breeder flocks in Croatia, which were accompanied with a significant decrease in both egg quality and production.

The disease caused is by duck adenovirus a member of genus *Atadenovirus*. The virus has haemagglutination activity and has its reservoir in ducks and geese. The complete nucleotide sequence data reveal that it is an intermediate virus between mammalian and avian adenoviruses (Hess et al., 1997).

EDS virus transmits vertically from hens to chicks and also horizontally from chicken to chicken (Cook and Darbyshire, 1980, 1981, Darbyshire and Peters, 1980). Contaminated eggs as well as egg trays or faeces seem to be the main sources for virus spread (Smyth and Adair, 1988). However, some outbreaks have been attributed to contact with wild birds or water contaminated by faeces from wild birds.

Smyth et al. (1988) carried out an investigation on the pathogenesis of EDS in laying hen. After experimental infection viral antigen and intranuclear inclusion bodies were detected in the surface epithelium of the nasal cavity of conventional hens 2 to 6 days p.i. Low levels of viral antigen were detected in lymphoid tissue throughout the body 2 to 5 days p.i. and inflammatory lesions and viral antigen were observed in the infundibulum 3 to 5 days p.i.. Viral replication was first detected in the pouch shell gland (PSG) 8 days p.i.. Viral antigen was never detected in the surface epithelium of the alimentary tract.

The disease is most severe in broiler breeders and brown egg layers. White layer lines are less affected. The mortality is usually negligible. Birds infected vertically can remain asymptomatic until the bird become sexually mature. The eggs from infected birds are mostly shell-less, thin-shelled, discoloured or misshapen and have a poor internal quality. It take mostly 4-10 weeks till the birds re-start to produce normal.

At necropsy there is no specific lesion, but a slight atrophy of ovary and oviduct can be observed. Histopathological changes can be seen in the oviduct and uterus (shell gland). There may be severe degeneration and desquamation of the epithelial cells, atrophy of the uterine glands, and infiltration of heterophils, lymphocytes, and plasmacytes. Intranuclear inclusion bodies may be found in the epithelial cells of the uterus, isthmus, and vaginal gland region (Adair and Smyth, 2008, Smyth and McNulty, 2008).

Diagnosis of Adenovirus infections

Basically the diagnosis of poultry diseases based on case history, clinical signs and post-mortem examination as important steps toward disease diagnosis, but it should not be the final step. In most cases clinical signs and lesions of many diseases are similar and laboratory tests are required to identify the specific cause (Fig. 1).

The laboratory diagnosis can be applied to direct detection as well as for isolation and identification of the causative agent or indirectly to detect antibodies (Hafez and Hess, 1999).

The diagnosis of adenovirus infection in poultry is in most cases based on histological investigations and detection of intranuclear inclusion bodies in hepatocytes or on detection of the antigen or virus particles using Immunofluorescence test or electron microscopy. In the last years several molecular biological tools such as PCR, Real-time PCR and REA were developed allow the detection of the Virus – DNA as well as the further identification and typing of adenoviruses (Erny et al., 1991; Raue and Hess, 1998, Hess et al., 1999, Raue et al., 1999, Hess, 2000, Lüschow et al., 2007).

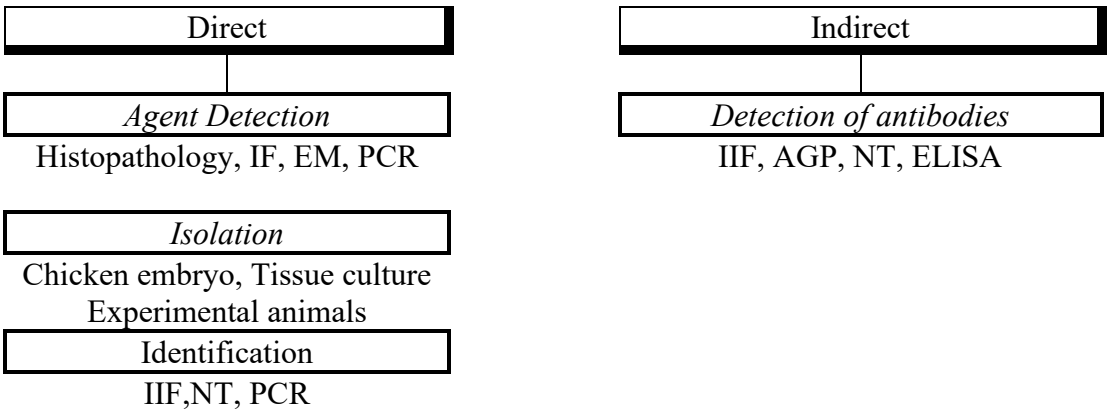
However the isolation of the **aviadenoviruses** using chicken embryo liver (CEL) cell culture and chicken embryo fibroblast cell culture with further identification and determination of the pathogenicity seems to be very important, since the pathogenicity of the isolates within the same serotype can be widely differ. Cross neutralization tests are necessary to serotype the isolated virus and to determine a new serotype.

EDSV can be isolated in embryonated duck or goose eggs, and in cell cultures. Susceptible cell lines include duck and chick embryo liver, duck kidney, and fibroblast cells. The virus may be isolated directly from the reproductive tract of affected hens. Alternatively, abnormal eggs may be fed to naive hens; virus isolation is attempted from the shell gland of these hens when they produce abnormal eggs.

The most common serologic test is the immunodiffusion test that detects the group specific antigen. This test is not sensitive enough. A group specific ELISA and IIF tests are more sensitive. The serum neutralization test has been used to detect serotype-specific antibody but is labour intensive and expensive. In general the interpretation of serologic tests is difficult because antibodies against AAVs can be found in both healthy and diseased birds.

In addition for the detection of antibodies against **EDSV** haemagglutination inhibition using fowl RBC can also use. Dhinakar Raj et al. (2007) developed immunofiltration (flow through) test to detect the presence of antibodies to egg drop syndrome 76 (EDS) virus in chicken sera and compared it with HI and ELISA. In total, the immunofiltration test could detect EDS antibodies with a sensitivity and specificity of 90.14% and 92.86% respectively as compared to the HI test. Compared to ELISA, the sensitivity and specificity of the developed immunofiltration assay was 79.45% and 94.58% respectively. The disadvantage of this test is the qualitative detection of antibodies in the serum, which may not be highly informative on all occasions and this test can be used as a preliminary test before confirmation can be done by another more sophisticated laboratory based assay.

Fig. 1: Laboratory diagnosis of poultry diseases



Control of Adenovirus infections of poultry

Control of IBH / HPS

Biosecurity practices are the primary and essential step to prevent the infection. Proper management, cleaning and disinfection of premises and equipment, restricted entry of visitors and vaccination crews in the poultry houses play a significant role in prevention of the disease. However, in countries with high infectious pressure the disease has been brought under control by formalin-inactivated vaccines prepared from liver homogenates from infected birds or by inactivated cell culture–derived vaccines. The vaccines are effective in the face of natural outbreaks or experimental challenge and significantly reduce mortality (Balamurugan and Kataria, 2004)

In general, the control can be achieved by the use of an autogenous inactivated vaccine prepared from a homogenate of an infected liver or inactivated cell culture vaccine (Balamurugan and Kataria, 2004)

Balamurugan and Kataria (2004) review the experiences of several authors using the vaccines to control HPS in poultry. In two field trials, involving 570 000 birds on 128 farms, the overall mortality ranged from 0.77% to 3.8% in vaccinated and from 11.11% to 30% in unvaccinated birds (Ahmad et al., 1990). In another trial, the mortality in vaccinated birds was 0.52% compared to 5.34% in unvaccinated birds kept on the same premises. Vaccination was also effective when carried out in the face of an outbreak; mortality in the vaccinated infected birds being 2.33% compared with 10.27% in unvaccinated infected birds (Afzal and Ahmad, 1990). Shane (1996) evaluated five inactivated vaccines used in Mexico. Complete protection, with an absence of histological changes in chicks challenged with $10^{3.5}LD_{50}$ of the DCV-94 adenovirus strain, was observed. Icochea et al. (2001) evaluated the efficacy of three inactivated vaccines against IBH / HPS in Peru in two different experiments and concluded that the protective effect of a commercial oil-adjuvanted cell culture IBH vaccine was superior to the autogenous vaccines and that the mortality rates were not dose-dependent. As most cases of IBH are the result of vertical transmission, vaccines have been proved to be highly successful at controlling IBH by preventing vertical transmission and inducing maternal immunity (Toro et al., 2002; Alvarado et al., 2007). Toro et al. (2002) reported that effective protection of the progeny of chickens against IBH-HPS could be achieved by dual vaccination of breeders with FAV-4 and CAV.

Alvarado et al. (2007) isolated pathogenic adenovirus, identified as Stanford strain and characterized as European serotype 9. The level of protection against IBH was evaluated in two broiler-breeder progenies from AAV 8/11– vaccinated grandparent flocks and a commercial broiler flock by challenge at 1 or 7 days of age with the AAV 8 and 11 serotypes and/or the Stanford strain. The broiler-breeder progenies and the commercial broiler flock exhibited protection against IBH after challenge. They conclude that broiler-breeder progenies from 30- to 50-wk-old grandparents vaccinated with the AAV 8/11 vaccine were adequately protected against challenge with the AAV 8 and 11 serotypes and the Stanford strain (serotype 9).

Control of EDS

Beside biosecurity, vaccination with an inactivated vaccine prior to lay is mostly important to prevent egg production losses and reduced egg shell quality in commercial layer and breeder flocks. Initial vaccination occurs between 14 and 16 weeks of age. However, vaccination cannot completely inhibit virus excretion in faeces, but decrease virus shedding (Heffels et al., 1982).

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COCCIDIOSIS: WHAT DO WE TRULY KNOW ABOUT THIS DISEASE?

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Summary:
The coccidian parasites invade the intestines and cecas of the host. Birds of all ages are susceptible to one or more species of coccidia, unless immunized. Eimeria species have had predictable behaviors or patterns which were described and accepted, but recently un-characteristic patterns such as cryptic and variant organisms are being observed. Is it then that these observations were not recorded or they are new and now being recorded. The pathology and pathogenicity of the coccidia species are characteristic traits of the species; *Eimeria praecox* is relatively benign whereas *E. necatrix* is highly pathogenic. The fecundity of the organisms, chicken house management practices and the methods of coccidia control may influence the prevalence of the coccidia species in the environment. The most pathogenic species *E. necatrix* is the least prevalent or even extinct from broiler chicken houses. Coccidia damage host tissue and the sequel is weight loss, impaired_feed utilization, poor pigmentation, and mortality. Coccidia are ubiquitous and are in most all commercial poultry houses and pose a threat to the health of the animals. These prolific organisms are resistant several environmental conditions, but susceptible to desiccation. Host tissue is destroyed during the development of various stages; the damage is directly related to the number of coccidia and species of eimeria ingested. Controlling coccidiosis is essential and is achieved via facilitate low parasite burden resulting in minimal cells destroyed through the use of effective drugs and biological means.

Introduction:
Avian coccidiosis is a disease of enormous economic importance and estimated to cost the world poultry industry billion of dollars annually. In the presence of loss of drug effectiveness and an explosion in the available coccidia vaccines; we are still in fairly high learning curve when it comes to avian coccidiosis. Many of the changes that have occurred with intensive poultry production practices, such as re-using litter for several flocks (breeders and broilers), restricting food and water for specific periods, using selective drugs for extensive periods due to cost and even removing the drugs from the feed for extended periods to control cost. These practices may have facilitated some of the un-characterized patterns or behavior of coccidia being observed in recent times. It is for us to use all the past and new information about this dynamic and diverse disease for a better understanding and management.

Parasite development: The intact sporulated oocysts enter the host via ingestion, the gizzard and digestive juices aid in the rupture, excystation and subsequent release of the sporozoites. These sporozoites are the invasive units of the oocysts. These protozoans enter the enterocytes of the intestines and or ceca where they develop and complete the asexual and sexual cycles. The end products of these replications lead to unsporulated oocysts. It is believed that these parasites develop only in the enteric cells, but several endogenous stages meronts, gamonts and oocysts have been found in non-enterocytes epithelial cells in such places as the proventriculus and ventriculus.

Pathology and pathogenicity: The pathology of coccidia is dependent on the species and strains involved, dose level ingested and how deep in the tissues these parasites develop. The most pathogenic is *E. necatrix,* because it develops deep in the sub-epithelial tissues. Whereas *E. praecox* is less pathogenic and develops primarily above the host cell nucleus and just beneath the brush border of the cells. Parasite fecundity plays a role in the pathology and pathogenicity; however, the species that are highly pathogenic, tend to produce a lower number of offsprings primarily due to

the tissue damage and loss during the earlier replication stages. *E. necatrix* is unique, in that the asexual stages occur in the small intestine and the sexual development occur in the ceca, this creates for a lower fecundity rate as compared to say *E. acervulina* in which all the endogenous stages occur in the upper small intestine. Laboratory altered strains such as the precocious and attenuated organisms are less pathogenic and also demonstrate less or lowered degree of pathology than their parent or non-altered strains. This is due to the reduction in the number of meront stages. Innate host susceptibility plays a role in the pathology and pathogenicity; some commercial strains of broilers are more tolerant to coccidia that other strains (personal data). The parameters that are routinely monitored to determine pathogenicity are growth rate, feed utilization and skin pigmentation.

Factors affecting coccidiosis: Several factors may affect the incidence and severity of coccidiosis in floor raised commercial poultry. Excess litter moisture, 60% and above and high levels of ammonia negatively affect the sporulation rate of the *E. maxima* oocysts. The *E. maxima* oocysts also sporulate fairly well in new litter as compared to used litter during the same tme frame allowed. The number of birds per unit area has a great influence on the number of oocysts per gram litter (OPG). A density of more than 22 birds per m^2 for an extended time can increase the oocysts concentration in the litter to alarmingly high numbers to cause an outbreak. Infectious agents such as IBDV and CAV affect bird health and immune status thus affecting host susceptibility to coccidiosis. The selection and use of overly used anticoccidials can lead to poor coccidia control and outbreaks. Improper mixing or improperly blending of efficacious compounds may contribute significantly to clinical coccidiosis. It is extremely important for judicious use and calculated non-use periods of these compounds to spare the effectiveness.

Immunogenicity and immunity: The coccidia species are good sources of antigens; when the host is exposed to the specific antigens, in approximately three week the host develops a solid immunity to those antigens. The immunity to coccidia is stable, but wane over time if the animal is maintained in a coccidia free environment. This immunity is species specific, in that birds immunized against *E. tenella* will not be protected against *E. maxima* and *visa versa*. The immunity to coccidiosis is primarily cellularly mediated with some secondary humoral responses. Birds recovered from an infection will have some measurable immunity (active); however, it has been demonstrated that passive immunity is also associated with coccidiosis. A vaccine (Coxabic) is available in some countries using this methodology; immunity is obtained through vaccinating the dam against coccidia and the protection is passively passed on to the off-springs. With this type of protection, it is very possible that the broilers may obtain an active infection during the growing period with some parasitemia and eventually immunity develops.

Diagnosis: The methods used for many years diagnosing and identifying coccidia species are oocysts morphology and parasite biology. These methods are effective; nine species of chicken and seven turkey Eimeria have been named and described via these methods. However, there are limitations, such as there are overlaps in the size and shapes and regions of the intestines parasitized. With someone skilled in the arts, these limitations are miniscule; but might be a challenge for the less experienced person. In recent times, enzyme electrophoresis the Polymerase Chain Reaction (PCR) methodology has become another tool in diagnosing and identifying the species coccidia. These techniques are not without limitations. It must be assumed that the standards used to develop these metrics for the described coccidia species were obtained from pure samples that conformed to the species not based on one criterion but several criteria.

Control: Pharmaceutics: During the early years of trying to control coccidsiosis, there have been many unsuccessful attempts. For example, the use of whey/milk by-products and home remedies were used in the early years of coccidiosis control. These attempts paved the way for the sulfa-based products. However, toxicities became an issue with the sulfas; synthetic anti-parasitic agents replaced the sulfas. Early synthetic anticoccidials were highly efficacious; the mindset was to

eradicate coccidia. Resistance to anticoccidial became a major problem with the highly effective products. The next group of products discovered was the forgiving "ionophores". These products became the primary method for coccidiosis control, with a slower rate for drug resistance to develop. To reduce or delay the onset of resistance; shuttle programs and rotation of anticoccidials have been used in coccidiosis control programs. Recently, vaccinating birds with drug sensitive strains of coccidia has shown great promise. This practice also enables the addition of drug sensitive organisms to the coccidia population. These drug sensitive organisms have shown to enhance the efficacy of the anticoccidials. Mathis *et al,* 2003, demonstrated that there are benefits for using Coccivac-B® in a rotational program with Clinacox™ (diclazuril) to restore the sensitivity of resistant species of chicken Eimeria to Clinacox™. The mode of action of the anticoccidial compounds allow the products to be used primarily as prophylactics and or therapeutics. The ionophores are primarily used as prophylactics and products such as amprolium and azauridines (Baycox) may be used as prophylactics and therapeutics. To maintain the efficacy of the anticoccidial drugs, a more judicious use of these products is required. This should include the incorporation of the biological systems such as the host and vaccines into the strategies for coccidiosis management.

Biologics: From the early work of Johnson and Edgar, many years ago; immunizing chickens against coccidiosis eventually lead to the first commercial coccidiosis vaccine (Coccivac®). During the early years, the acceptance of vaccine usage was inconsistent, probably due to application issues. However, in recent years, the acceptance has grown considerably, probably because of the methods of applications. These vaccines can be applied to the host in a variety of ways; feed, water, eye, nostril, injection, coarse spray that encourages preening and in-ovo application. There are several types of products; such as killed products (subunits, IGY and recombinants) and live products. Two types of live products are available; non-attenuated (Coccivac, Immucox, Eimeria, Advent and Inovocox) and the attenuated (Paracox, Livacox, Eimeria Pty, Hipracox and Hatch Pac). Specific claims are made for each product by the manufacturers. The benefit of using vaccines with drug sensitive strains of coccidia is that the anticoccidial drugs can be physically rested. This practice may extend/preserve or prolonged the life of the anticoccidials by judiciously using them in rotation with drug sensitive vaccines.

In a relatively poor drug program, the non-immune animals become carriers of the infectious agents and play a major role in the spread of the disease. However, in the immune animal, the parasitemia keeps the body alert and therefore keeps the immune system in a defensive mode. Eventually, the animal becomes solidly immune to the coccidia infection. Recently, the practice to "manage" coccidia, involves the host-parasite and environment relationship. By managining coccidiosis, the infestation pressure is kept relatively low to avoid or prevent devastating losses and to minimizing the buildup of infection pressure.

Variant coccidia: There are now several citations concerning the evidence of drug resistance to several species of chicken and turkey Eimeria. This decline in drug efficacy has occurs over time and may be exacerbated by the over use period or in-discriminate use of these drugs. All species of *Eimeria* are fairly antigenic and may offer protection to different strains within each species, but offer no cross species protection. There are reports of poor protection or variability in cross protection among strains of *E. maxima* (Norton and Hein, 1976, Long and Millard, 1979, Fitz-Coy, 1993, Smith, A. L. et. al. 2002, Jenkins et at 2004). But the question that has not been answered is how prevalent or wide-spread are these variant organisms. Some strains or isolates within a species may be better antigens for a vaccine candidate than others. Some vaccines use multiple *E. maxima* antigens to enhance the antigen capabilities of that species. Many of these reports might not have address vaccines antigens and might have also challenged birds when they were poorly immunized or due to timing of the challenge of the birds.

There have been reports concerning organisms within a species that behave uniquely different, but are these one and the same species or are other species involved. Researchers recently shared data on the genetic characterization of coccidia from chickens, and revealed more taxonomic confusions about the species of chicken Eimeria. These unknown agents are referred to as cryptic species. These findings are agreeing with normal species diversity. How important are these organisms? Within these diverse populations what is the relative abundance of these organisms?

Things to ponder:

1) Conformation on the validity of the species – some references acknowledge seven species, other acknowledge nine species. Based on my working experiences with coccidia of chicken, there are nine species, (*E. acervulina, E. mivati, E. hagani, E .praecox, E. brunetti, E. mitis, E. maxima E. tenella* and *E .necatrix).*
2) The process of immunizing the birds – it is known that a single exposure does not confirm an immunity, but what is critical the initial exposure. As early as this can occur it should, ideally from the immunization process and not from hatch-mates. The idea is to get as uniform an initial exposure as possible.
3) Misconceptions about vaccinations – there is a belief that this practice of coccidia control is less ideal than using pharmaceutical agents. In fact this practice of vaccinating birds is very effective. It is no different immunizing your child against childhood diseases. You would rather have your child immunized early in life against the disease than going to school and be exposed to these agents from classmates.
4) Misconceptions that the drugs are as effective as when they were first introduced years ago – it has become more apparent that some of the commonly used anticoccidial drugs have lost some if not all of their effectiveness against some field strains.
5) Misconceptions about anticoccidial sensitivity tests (AST) – there is a strong belief by some that these tests are not valuable. Before each drug gets an approval from the federal government, a series of the exact tests are conducted to demonstrate that these products are effective against field isolates. Why then are these tests not valid?
6) Pullet house management – lighting programs, feed restrictions, keeping pullets for 20-22 weeks before transferring to layer house and re-using litter; these management tools have added new variances to the already complex issues. These management practices do impact bird behavior, which consequently influence coccidia ingestion.

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GENE EXPRESSION IN THE HYPOTHALAMUS OF FASTED AND FED NEONATAL CHICKS

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INTRODUCTION

The availability of chicken genomic data and the significant number of chicken expressed sequence tags (EST's) entered into GenBank (599,383 entries as of May 2008, http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html) has provided new opportunities to understand the gene networks utilized in chickens. Further, the application of microarray technology allows for a transcriptional snapshot of gene expression (which genes are 'on' and which genes are 'off') on a nearly genome-wide basis. Exploration of differential expression of genes in this experiment using a strong metabolic perturbation (fasting and refeeding) is contributing to our understanding of how the interactions of genes contribute to hunger and satiety in the chicken. This experiment evaluated the gene expression of 20,120 genes in chicks during feeding, fasting or delayed feeding over the first four days of life.

FASTING OF NEONATAL CHICKS

When chicks are hatched, they are commonly held from 24 up to 72 hours for many reasons, including accumulating a large enough population to be shipped, administration of vaccinations, beak trimming and decombing, and transportation (particularly international shipping). In recent years, it has become more apparent that the first few days of life are potentially the most important for poultry in a production setting. Research has shown that withholding nutrients for more than 24 hours can have lasting negative effects on both broilers and turkeys (Tarvid, 1994; Knight, 1998; Noy et al., 2001; Batal and Parsons, 2002; Juul-Madsen et al., 2004). Delaying access to feed and water causes hatchlings to be more susceptible to pathogens, causes weight loss, and restricts the development of critical tissues.

Early research focusing on glucose metabolism in the fasted chicken indicated that after 24 or 72 hours of fasting, glucose utilization was reduced and chickens appeared to efficiently reutilize glucose-carbon sources to maintain blood glucose levels during fasting (Belo, et al., 1976). Noy and Sklan (1999b) observed a rapid yolk weight decrease in both fed and fasted chicks, with the fed chicks showing a more rapid decrease. Another study comparing chicks provided feed, water, or both revealed that by 21 days of age, chicks that received feed and water or feed alone for the first 34 hours post hatch showed weight increases of 5 and 8%, respectively, compared with fasted controls. However, fasted chicks lost 15-20% of body weight (Geyra et al., 2002). Importantly, delay of feeding newly hatched chicks for 48 hours not only decreases body growth, but also breast muscle weight over a 6-week period, potentially due to reduced proliferation of muscle satellite cells as compared to fed control chicks (Halevy et al., 2000).

Early fasting also affects development of the gastrointestinal tract. Delayed feeding for 24 hours caused a decrease in villus surface area, a decrease in enterocytes, and an increase in intracellular mucins in the intestinal tract (Uni et al., 2003). Gonzales et al. (2003) also observed reduced villus height along with reduced weight and reduced length of intestine in fasted neonatal chicks. Another study examined the effects of delayed feeding for 48 hours post-hatch and observed reduced body growth, enterocyte proliferation, and expression of the transcription factors cdx A and cdx B in the small intestine of broiler chicks (Geyra et al., 2002).

HYPOTHALAMUS AND FEED INTAKE

The hypothalamus is part of the brain that is recognized to play a major part in regulation of feed intake in animals. However, genome wide evaluation of the hypothalamus has not previously been evaluated. The hypothalamus is responsive to both neuronal and endocrine signals within the brain, and is responsible for stimulation of the pituitary gland to produce and release many endocrine hormones. Regions of the hypothalamus that have been determined to participate in regulation of feed intake include the arcuate nucleus (ARC), paraventricular nucleus (PVN), and lateral hypothalamic area (LHA). Neurons in these areas associated with feed intake are commonly classified as orexigenic (increasing feed intake) and anorexigenic (decreasing feed intake). The ARC orexigenic neurons produce neuropeptide Y (NPY) and agouti-related protein, which have increased expression in response to fasting and decreased expression in response to leptin, a hormone produced by adipocytes. Anorexigenic neurons in the ARC include pro-opiomelanocortin (POMC) and cocaine- and amphetamine- regulated transcript (CART) neurons. These genes have decreased expression as a result of fasting, and increase expression in response to leptin from adipocytes (Coll et al, 2007). Other neurons that play a part in regulation of feed intake include corticotropin releasing hormone (CRH) and thyrotropin releasing hormone (TRH), which are anorexigenic hormones in the PVN, and also the orexigenic neurons containing melanin concentrating hormone (MCH) and orexin (ORX) in the lateral hypothalamic area (Remmers et al., 2008).

EXPERIMENTAL DESIGN

This experiment used the metabolic perturbation of fasting in order to disrupt the gene pathways associated with metabolism and nutrient utilization. Perturbation is a method that is used widely for modeling of functional networks of genes (Wagner, 2001; de la Fuente et al., 2002). Additionally, fasting during the neonatal period is common in poultry production, due to the fact that many chicks may spend extended periods of time in the hatching cabinet prior to removal. As hatchery processing (vaccination, beak trimming) and transportation also may take considerable time, it may be up to 2 days after hatch before a chick receives its first meal. As described above, delay in feeding for over 24 hours results in decreased growth and performance over the life of the chicken. The expression of genes regulating metabolism in the early post-hatch time period are not well understood. In these experiments our goal was to evaluate differences in gene expression in the hypothalamus of neonatal chicks after fasting post-hatch and followed by delayed feeding.

Fertile broiler eggs (Ross x Cobb) were obtained from a local hatchery, and were incubated in our laboratory at the University of Maryland. The eggs were set immediately upon arrival at the laboratory. Eggs were incubated under standard conditions (37.5 C and 60% relative humidity), with turning every hour for 18 days. On day 18, eggs were transferred to a hatching cabinet and were no longer rotated. Male chicks were identified by feather sexing at hatching, which was confirmed by visual inspection of the gonads at the time of dissection. All chicks were brooded in cages with 24 hours light and continual access to water during this experiment. Hypothalamus samples from 8 chicks were taken from each experimental group on the designated day, and immediately snap frozen in liquid nitrogen for subsequent RNA isolation and microarray analysis. Ten groups total are included in this experiment (8 chicks per group, 80 chicks total), which are described in Table 1. These groups were chosen in order to maximize the data obtained from chicks that were fasted and refed, with minimal experimental groups (The microarray analysis is very expensive, and therefore the number of samples had to be limited).

Table 1. Experimental Design

Treatment Group	Days of Sampling d0	d1	d2	d3	d4
Hatch	-			Feed Provided	
24h Fed	+	+			Fasted
24h Fast	-	-			
48h Fed	+	+	+	Samples Collected	
48h Fast	-	-	-		
48h Fast, 4h Fed	-	-	+		
72h Fed	+	+	+	+	
48h Fast, 24h Fed	-	-	+	+	
96h Fed	+	+	+	+	+
48h Fed, 48h Fast	-	-	+	+	+

Briefly, eight chicks were sampled at hatch, and on day 1 both fed and fasted chicks were sampled. On day 2, fed chicks, fasted chicks, and chicks fasted for 48 hours and refed for 4 hours were sampled. Chicks fed *ad libitum* and chicks fasted and refed for 24 or 48 hours respectively were sampled on days 3 and 4 respectively. All feed was provided to refed chicks at 8 am.

MICROARRAY ANALYSIS

The microarray platform used for these experiments was the Chicken Oligo Microarray (Operon) containing 70mer probes, which was printed at the University of Arizona. This array contains 21,120 elements (or spots), and covers much of the chicken genome. Prior to hybridization, total cellular RNA was isolated from the hypothalamus tissue using the RNeasy Mini Kit (Qiagen, Valencia, CA) according to manufacturer's protocol. Quantification of RNA was performed by measuring absorbance at 260nm, and quality was evaluated using a bioanalyzer (Agilent Technologies, Palo Alto, CA). Due to the fact that the hypothalamus yields insufficient mRNA for microarray analysis, amplification of the mRNA was accomplished using Amino Allyl MessageAmp™ II aRNA Amplification Kit (Ambion, Austin, TX). Resulting aRNA was purified and quantified.

Samples were hybridized to the microarrays using a reference design (Simon et al., 2002). An internal reference standard was created by pooling aRNA from all samples within the experiment and labeling it with Alexa fluor 647 9 (red). Experimental samples were labeled with Alexa fluor 555 (green) and then hybridized to individual microarrays along with the Cy5-labeled reference pool. Four arrays per group were utilized for this study (40 arrays total). Labeling of cDNA with Alexa fluors, microarray hybridization, and image scanning was performed at the University of Delaware.

The data analysis was performed according to established protocols in the Porter laboratory as previously described (Ellestad et al., 2006, http://physiolgenomics.physiology.org/cgi/content/full/25/3/414). Data were initially processed in Dr. Porter's laboratory using GenePix 6.0 software. The data were then normalized using freely available software that is part of the TM4 suite of microarray data analysis applications from The Institute for Genomic Research (TIGR, Rockville, MD). The data were then analyzed statistically by determining the $\log_2$ ratio (normalized Alexa 555/ Alexa 647) for each spot. Spots determined to be statistically significant ($p<0.05$) (One-Way ANOVA, SAS) among the 10 treatment groups were analyzed further.

FURTHER DATA ANALYSIS

Genes in clusters exhibiting specific and reciprocal responses to fasting and refeeding are currently being evaluated. Gene Ontology (biological process) analysis has been completed for these genes in http://www.agbase.msstate.edu/. Gene ontology is a list of terms that are assigned to genes using a common vocabulary between species to describe the biological process, molecular function, and cellular processes that the gene is involved in. Additionally, for genes that were significant ($p<0.05$) and differed in expression by at least 2-fold (119 genes), we determined the human orthologues, and submitted our genes for analysis through Pathway Miner, a freely available program through the Bioresource for Array Genes (http://www.biorag.org/). This analysis utilizes three available databases to search for genes within a list of submitted genes that are present in a common biological pathway. This analysis identified six genes within our list of differentially regulated genes that were associated within a pathway: pro-opiomelanocortin, adrenergic receptor beta 2, somatostatin receptor 5, neuropeptide Y receptor 5, glutamate receptor 8, and relaxin 3.

qRT-PCR

Microarrays yield expression data for thousands of genes simultaneously. However, the large number of statistical comparisons made can lead a proportionately large number of false positives. Therefore, it is important to confirm key findings by a second technique. Two-step quantitative reverse transcription - real time polymerase chain reaction (qRT-PCR) was performed for at least 16 genes to confirm expression patterns in this experiment. All 8 samples for all treatment groups were assayed using qRT-PCR, due to the fact that the cost and time required are much less for qRT-PCR than for the microarray analysis. An oligo(dT) primer was combined with 1µg of total RNA for the RT reaction. A negative control for genomic DNA contamination was performed without the addition of reverse transcriptase. PCR primers were designed utilizing Primer 3 (freely available at http://fokker.wi.mit.edu/primer3/input.htm), based on the full-length mRNA sequence predicted from the chicken genome sequence available through ENSEMBL (http://www.ensembl.org/Gallus_gallus/index.html). mRNA levels were quantified using the MyiQ Single-Color Real-Time PCR Detection System (Bio-Rad) and the 2X Quantitect SYBR Green PCR Master Mix (Qiagen). Cycles were performed as follows: denaturation at 95C for 15 min to activate the polymerase, followed by 40 cycles of 95C for 15 s, and 60C for 60 s. Data were transformed using the equation 2^{-Ct} where Ct represents the fractional cycle number when the amount of amplified product reaches a threshold for fluorescence. Results were analyzed statistically (ANOVA) to confirm reciprocal effects of fasting or refeeding on gene expression. This type of analysis is performed routinely in the Porter laboratory (see Ellestad et al., 2006). The gene expression patterns observed by qRT-PCR were compared with the patterns observed by microarray analysis to confirm the accuracy of the microarray data.

RESULTS

Physiological Parameters

In addition to gene expression differences, the chicks for these experiments were weighed, yolk sacs were weighed, blood glucose, triglyceride, and non-esterified fatty acid levels were determined. These data indicate that there are significant physiological differences between groups due to fasting. Mean body weight at hatch was 46 g, and this did not significantly change within 24 hours in fed or fasted chicks (Table 2). However, by day 2, fed chicks were significantly heavier than chicks fasted or fasted and refed for 4 h. On days 3 and 4, fed chicks were again heavier ($p<0.05$) than their fasted and refed counterparts. Remarkably, by day 4, fed chicks had effectively doubled their hatching body weight. When yolk sac weights were compared between treatment groups, we observed that the weight decreased with time, to less than 1g by day 4 in both fed and fasted-refed groups.

Table 2. Physiological parameters measured in fasted, fed, and delayed fed chicks.

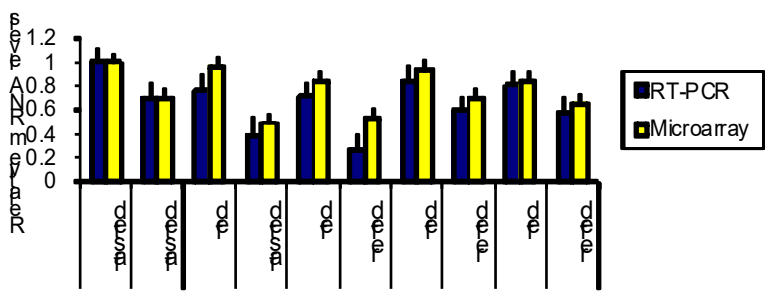
Treatment	Body Weight (g)	Yolk Sac Weight (g)	Glucose (mg/dL)	Triglycerides (mg/dL)	NEFA (mEq/L)
Hatch	40.15 ± 3.88[f]	4.66 ± 0.38[a]	221.15[cd]	56.021[de]	0.32[a]
24h Fed	42.12 ± 7.15[ef]	2.64 ± 0.19[b]	261.96[cd]	120.895[a]	0.17[bc]
24h Fast	39.11 ± 2.86[f]	3.14 ± 0.30[b]	237.60[cd]	54.878[de]	0.29[a]
48h Fed	58.27 ± 3.97[d]	1.54 ± 0.17[cd]	418.54[a]	97.324[abc]	0.18[b]
48h Fast	37.90 ± 3.44[f]	1.80 ± 0.17[cd]	185.68[d]	33.673[e]	0.31[a]
48h Fast, 4h Fed	43.44 ± 5.74[ef]	1.51 ± 0.13[cd]	289.63[bc]	91.174[bc]	0.13[c]
72h Fed	74.13 ± 6.30[b]	0.93 ± 0.10[de]	390.21[a]	102.736[ab]	0.20[b]
48h Fast, 24h Fed	53.17 ± 3.94ef	0.80 ± 0.06[e]	357.50[ab]	73.201[cd]	0.18[bc]
96h Fed	95.77 ± 8.35[a]	0.61 ± 0.05[e]	391.49[a]	120.753[a]	0.16[bc]
48h Fast, 48h Fed	66.09 ± 6.20[c]	0.59 ± 0.06[e]	361.26[ab]	101.506[ab]	0.16[bc]

Circulating glucose levels were < 300 mg/dL in chicks at hatch and in both fed and fasted chicks on day 1 (Table 2). By day 2, fed chicks had higher ($p<0.05$) glucose levels than fasted chicks or chicks refed for 4 h. However, refeeding for 4 hours caused glucose levels to increase ($p<0.05$) compared with chicks fasted for 48 h. Samples compared on day 3 and day 4 (following refeeding for 24 or 48 h, respectively), revealed no differences in glucose levels between full-fed chicks and refed chicks. In contrast, triglyceride levels were very different on day 1, with fed chicks having twice the concentration compared with fasted chicks (120.9 mg/dL vs. 54.9 mg/dL) (Table 3). Chicks fasted for 24 or 48 hours did not have differences in triglycerides compared with chicks at hatch. Interestingly, chicks fasted for 48 hours and refed for 4 hours had levels of triglycerides that matched full-fed chicks, and were higher ($p<0.05$) than fasted chicks. On day 3, 24 hours of refeeding still resulted in somewhat lower ($p<0.05$) triglyceride levels compared with full fed, but these were not different on day 4 following 48 hours of refeeding.

Gene Expression

Microarray analysis of gene expression in the hypothalamus resulted in a total of 119 genes identified that were differentially expressed by at least 2 fold and significantly different as determined by ANOVA ($p<0.05$). Genes that were significantly upregulated by fasting at 48h and were confirmed with qRT-PCR were cytochrome p450, FK506 binding protein 51, coagulation Factor C, deiodinase type II, neuropeptide Y receptor 5, and somatostatin receptor 5. Genes that were significantly downregulated by fasting at 48 h and

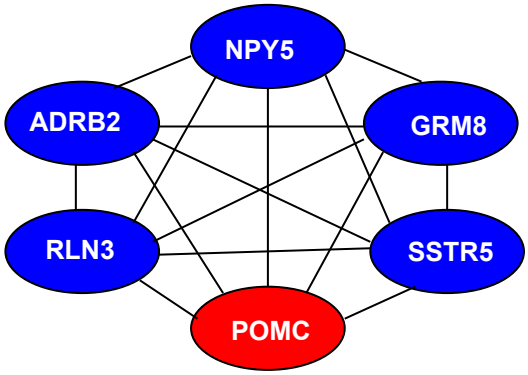
Figure 1. Relative Gene Expression of Pro-opiomelanocortin (POMC)



confirmed by qRT-PCR include: fatty acid binding protein 7, sal-like 3, protein kinase C iota, cytochrome 39a, and proopiomelanocortin (POMC) (Figure 1). The list of 119 differentially expressed candidate genes was further analyzed using Pathway Miner (http://www.biorag.org/pathway.php), a freely available program designed to detect genes that interact within the same pathway from comprehensive lists of genes. The Pathway Miner analysis detected that six of the 119 candidate genes were linked (Figure 2). The six genes include five that were upregulated: relaxin 3 (RLN3), adrenergic receptor β 2 (ADRB2), neuropeptide Y receptor 5 (NPYR5), glutamate receptor 8 (GRM8), and somatostatin receptor 5 (SSTR5). One gene was associated that was downregulated by fasting, proopiomelanocortin (POMC).

Due to the differences in all evaluated physiological indicators utilizing this model of fasting and refeeding, we believe that the metabolic perturbance of fasting followed by refeeding did cause physiological changes which are likely a result of gene expression changes between treatment groups. The differences in expression of 119 genes in the hypothalamus also confirm that this model results in differential gene expression.

Figure 2. Analysis of Candidate Genes with Pathway Miner



http://www.biorag.org/pathway.ph

We have identified many new genes that are expressed in the hypothalamus in response to fasting or feeding of newly hatched chicks. Some of these are likely to control feed intake or metabolism during this critical period of development. The knowledge gained from this work will be valuable in future efforts to improve our understanding of appetite and metabolic regulation in poultry and in efforts to improve production performance.

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DETERMINACIÓN DEL EFECTO DE LA ADMINISTRACIÓN EN AEROSOL DE UNA MEZCLA DE ÁCIDOS ORGÁNICOS (ETANOCARBÓNICO, FÓRMICO Y LÁCTICO) SOBRE LA REACCIÓN ADVERSA EN POLLOS DE ENGORDA INOCULADOS CON UNA VACUNA ACTIVA CONTRA LA ENFERMEDAD DE NEWCASTLE

Jesús Alejandro Castillo Contreras, Ricardo Salado Carbajal, Álvaro Vera Noguez y Celestino Gallego Vargas

Las reacciones post-vacunales si no son controladas, causan grandes pérdidas económicas en la avicultura, especialmente en granjas con parvadas de edades múltiples y diferente estatus inmunológico, éstas asociadas a patógenos oportunistas y al ambiente. Por tanto, se determinó el efecto que tienen estos ácidos orgánicos, mediante la administración en aerosol, evaluando la severidad de las reacciones adversas por medio del grado de severidad de los signos clínicos y, mortalidad. Se usaron 160 pollos de engorda de un día de edad línea cobb y, se dividieron en cuatro grupos de 40 pollos cada uno: control negativo; un grupo vacunado y con tratamiento; un grupo vacunado y sin tratamiento; y un grupo sin vacunación y con tratamiento de ácidos orgánicos. Se corrieron pruebas de aglutinación rápida en placa para detectar micoplasma en los pollos llegados de incubadora y pruebas de inhibición de la hemoaglutinación después del tratamiento en aerosol de ácidos orgánicos, el tratamiento con los ácidos no demerito la respuesta inmune a la vacunación, los grupos de aves tratadas con los ácidos mostraron una severidad y frecuencia menor en las reacciones posvacunales, que los pollos sin tratamiento y el grupo control.

Palabras clave: ácidos orgánicos, reacciones post-vacunales, enfermedad de newcastle, aerosol terapia, pollos de engorda.

DEGRADACIÓN DE AFLATOXINAS EN RACIONES DESTINADAS PARA AVES CON UN TRATAMIENTO DE ÁCIDO CÍTRICO ACUOSO

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Las aflatoxinas son metabolitos secundarios producidos por ciertos hongos pertenecientes principalmente al género *Aspergillus*. Este tipo de compuestos suelen producirse en una gran variedad de productos agrícolas, y son los contaminantes naturales de los alimentos mas extendidos a nivel mundial, siendo altamente tóxicos, mutágenos, cancerígenos, teratógenos e inmunosupresores. Debido a la gran variedad de efectos tóxicos y sobre todo a su extrema termoresitencia, la presencia de las aflatoxinas en los alimentos es considerada de alto riesgo para la salud del hombre y de sus animales. Consecuentemente, en el presente trabajo se evaluó la degradación de las aflatoxinas en las raciones destinadas para las aves, mediante un tratamiento con ácido cítrico acuoso, confirmando la detoxificación con el método de inmunoafinidad con columna de anticuerpos monoclonales, el ensayo de Ames y con patos de un día de edad (Peking White). Las determinaciones de inmunoafinidad mostraron que la aflatoxina a una concentración inicial de 110 ng/g fue parcialmente destoxificada (86%) por el tratamiento acido. Los resultados de la prueba de Ames indicaron que la mutagenicidad de la aflatoxina tratada fue grandemente reducida o inactivada, basándose en las reversiones $his^- \rightarrow his^+$ en la cepa de *Salmonella typhimurium* TA100. Los estudios de toxicología y patología mostraron que la protección en los animales, debido al procedimiento de acidificación de la ración, no fue completa. En conclusión, los resultados indican que el ácido cítrico posee actividad destoxificante y además, puede proteger a los animales de la toxicidad crónica causada por las aflatoxinas.

Palabras clave: Aflatoxinas, ácido cítrico, raciones contaminadas para aves, destoxificación.

EL AGUA EN LA AVICULTURA

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En la industria avícola la calidad y cantidad de agua que se utiliza en toda la cadena productiva esta subestimada; sin embargo, es importante tener en cuenta que algunos problemas de desempeño productivo y productividad de las empresas avícolas pueden estar atribuidos a esos factores. El agua es un requerimiento importante, se dice que bajo condiciones normales los animales pueden consumir el doble de agua que de alimento, esta cantidad varía con la edad, estado de salud y época del año. El agua no solo es un nutriente de vital importancia, el agua es esencial para el funcionamiento del organismo, es el principal componente de las células y la sangre, se encarga de transportar nutrientes y desechos metabólicos juega un rol importante en el proceso de digestión y absorción de nutrientes, actúa también como reguladora de la temperatura corporal debido a su calor específico y características de evaporación. El agua es esencial para el mantenimiento de la homeostasis controlando el pH, la presión osmótica, la concentración de electrolitos, etc. El agua en la avicultura es indispensable en toda la cadena productiva ya que forma parte de las aves, es pieza fundamental en el proceso de desinfección del huevo, incubación, limpieza y desinfección de instalaciones avícolas, vacunación, medicación y desde luego es pieza clave a la hora del sacrificio y procesamiento de las aves. Debido a todo esto el objetivo de este trabajo fue hacer una revisión del uso del agua y la calidad de la misma.

PRÁCTICAS ESTRATÉGICAS EN EXPLOTACIONES AVÍCOLAS FUNDAMENTOS

Manuel Quiroz

Hoy en día, las empresas avícolas enfrentan retos particulares para satisfacer las demandas del mercado consumidor de alimentos, cada vez más exigente sobre los productos que adquiere. Enfrentar estos retos es una tarea de por si complicada, considerando que el proceso productivo de alimentos (en este caso a nivel primario o de granja) implica la presencia de peligros que pueden tener consecuencias de gran impacto a la salud, economía y medioambiente.

Entre los retos más relevantes por enfrentar se encuentran los siguientes:

1. Desarrollar formas de producción a gran escala de materia prima (pollo, pavo, huevo etc.) para la elaboración de alimentos saludables, demostrando un proceso acorde con estándares de calidad-sanidad-inocuidad establecidos a nivel nacional e internacional como una forma de prevenir peligros (principalmente enfermedades transmitidas por alimentos o ETA´s) durante la comercialización.
2. La necesidad de alcanzar parámetros de producción competitivos que se reflejen en el precio del producto final.
3. Demostrar un proceso productivo acorde con el cuidado al medio ambiente, la comunidad y el propio personal de las empresas.

De acuerdo con los expertos, esta tendencia hacia lo bueno, bonito y barato, hace necesaria la evolución de las diferentes prácticas hasta ahora utilizadas en las explotaciones avícolas.

Y cuando hablamos de prácticas, nos referimos literalmente a las prácticas o actividades cotidianas que se realizan dentro de los diferentes ciclos productivos para alcanzar los objetivos o metas de producción.

Técnicamente, las diferentes prácticas o actividades que el personal ejecuta, son componentes de un procedimiento o proceso, y la producción avícola debe ser entendida como un conjunto de procesos que requieren de una sincronización adecuada y un estándar de operación para evitar variaciones que puedan alterar las características del producto terminado (ave huevo, etc.).

En la actualidad el desarrollo de procesos en la industria, (particularmente en la industria alimentaria) es un tema que ha recibido gran impulso tanto en el sector primario (granjas, incubadoras, plantas de alimento, etc.) como a nivel de planta de proceso (rastros, plantas de empaque, etc.), ya que la organización que se logra, permite alcanzar los estándares de calidad-sanidad-inocuidad requeridos para un comercio globalizado, elevando la eficiencia de sus procesos, generando valores agregados reconocidos (certificación ISO, HACCP, etc.) y contribuyendo al desarrollo de empresas eficientes que pueden competir incluso con las reconocidas como líderes.

Una de las prácticas que más ha requerido evolucionar, es la bioseguridad. Esta evolución del concepto se hace necesaria conforme se ha confirmado que el 80% de los peligros asociados a la producción de alimentos tienen su origen en fallas de tipo generalmente operativo y que estas fallas impactan a la sanidad de la explotación con la consecuente entrada de patógenos que ocasionan enfermedades (manifestación del peligro).

EVOLUCIÓN DEL CONCEPTO Y PRÁCTICA DE LA BIOSEGURIDAD

Adquisición de insumos y ejecución de acciones sanitarias aisladas en función de limitar la presencia y circulación de los agentes patógenos propios de las aves

Acciones para el **MANEJO DE LOS PELIGROS** propios de cada proceso, IDENTIFICANDOLOS, CONTROLÁNDOLOS y VERIFICANDO LAS ACCIONES en función de los parámetros de productividad que se requiere alcanzar

La siguiente tabla muestra algunos ejemplos de fallas operativas, su origen y el peligro asociado. Como podemos observar la magnitud de manifestación del peligro depende de su asociación a una o más fallas operativas.

EJEMPLOS DE FALLAS OPERATIVAS Y LOS PELIGRO ASOCIADOS

Falla	Origen de la falla	Peligro asociado
1. Mantenimiento deficiente de casetas	• Falta de tiempo para el descanso de casetas • Falta de tiempo para el mantenimiento de una caseta • Fallas en limpieza y desinfección • Nula coordinación entre el personal de limpieza y operativo • Falta de equipo e insumos para el mantenimiento • Falta de capacitación al personal • Fallas en el control de proveedores de insumos	• Deficiencias en suministros de agua, ventilación, luz, alimento, etc. • Se favorecen ambientes propicios para el desarrollo de microbios • Desarrollo de plagas vectores de microbios • Enfermedades asociadas a mala ventilación y deficiencias en el control de temperatura, humedad, etc. • Intoxicación de animales por presencia de hongos en alimento • Desarrollo deficiente de las parvadas
2. Fallas en la comunicación y o programación de actividades (tardía, mal dirigida, poco clara)	• Ruta de comunicación larga o compleja • Problemas con acceso a la información • Falta de un plan de revisión (check-list) • Falta de registros • Falta de evaluación de procesos y desempeño laboral • Falta de procedimientos y guías o instructivos	
5. Fallas operativas, administrativas y falta de visión preventiva	• Falta de organigrama • Personal insuficiente • Exceso de responsabilidades a una misma persona • Falta de seguridad laboral • Recepción a destiempo de insumos, equipos o aves • Aplicación a destiempo de medicina preventiva • Almacén mal surtido • Fallas de inventario y medicamentos caducados • Departamento de compras poco ágil • Compras por precio y diferentes a las requeridas • Desarrollo de reglas que no se cumplen • Falta de plan de contingencia y acciones correctivas • Nula o mínima investigación	

Recientemente en diferentes países y para los diferentes segmentos de producción se han publicado una serie de normas recomendadas para la reingeniería de los procesos para la producción de alimentos (por ejemplo, Manuales de buenas prácticas, Manuales HACCP, Normas ISO, Normas AIB, Normas Eurep- GAP, etc.). Estas normas buscan una mayor integración de las prácticas, particularmente las relacionadas con la bioseguridad (prácticas administrativas, operativas, de capacitación, bioseguridad etc.), pero desafortunadamente ha sido difícil integrarlas al sector pecuario, básicamente por la falta de claridad sobre sus beneficios, la falta de comprensión sobre como integrarlas y/o porque en la mayoría de las veces la evolución de los procesos parece implicar una reestructuración de la misma empresa con la finalidad de adecuarse a los diferentes requerimientos planteados por las nuevas normas, teniendo que absorber los costos que esto implica. Sin embargo tenemos en la actualidad casos de países donde su implementación á sido exitosa, como Chile, por mencionar alguno.

La experiencia de las empresas chilenas ha de mostrado que para la implementación de estas nuevas formas de producción es de gran relevancia contar con personal suficientemente documentado y entrenado sobre los principios y aplicación de estas normas y reglamentos de impacto en el sector.

La gestión para el desarrollo e implementación de estas normas y el personal encargado de gestionarlas serán valiosos en la medida en que contribuyan con el análisis de los procesos para modificar, incluir y/o desarrollar los que agreguen algún valor y faciliten el alcance de los objetivos de la empresa y evitar aquellos que no aporten al sistema productivo.

Dado el avance en que se encuentran estos conceptos, creo importante repasar algunos principios y términos con el objetivo de documentar al principiante, medico, encargados de granja etc., sobre la razón y alcance del manejo de la bioseguridad mediante procesos e instructivos acordes con sistemas como las Buenas Prácticas de Producción Primaria o Buenas Practicas Pecuarias.

Para comenzar, es importante destacar que estos conceptos parten del sistema conocido como HACCP, del cual podemos puntualizar lo siguiente:

- El HACCP es un sistema desarrollado para las empresas productoras de alimento que toman el producto primario y lo procesan hasta llevarlo al producto final, por lo tanto no es estrictamente un sistema aplicable al sector productivo pecuario (primario), aunque sus principios si lo sean.
- Su objetivo principal es la producción de alimentos seguros (inocuos)
- El HACCP opera bajo los siguientes principios:
    - Evalúa los peligros
    - Identifica los puntos críticos de control (PCC) diferenciándolos de los puntos de control (PC)
    - Establece límites de tolerancia para los PCC´s
    - Monitorea PCC´s
    - Toma las acciones correctivas
    - Establece un sistema de registros
    - Verifica que el sistema este funcionando

De estos principios, uno de los más relevantes para la producción pecuaria es el segundo, que corresponde a la diferenciación de puntos de control.

Lo que nos dice el HACCP a este respecto es lo siguiente:

✓ Si es mas probable que SE DETECTE UN PELIGRO cuando el sistema no esta operando (por ejemplo falla en el sistema de calefacción, presencia de plagas, fallas en el suministro de agua

etc.), o en un punto fuera del proceso (p.ej. vigilancia perimetral) SE DEFINE entonces como punto de control (PC)

✓ Si es mas probable que se DETECTE UN PELIGRO hasta que el sistema está operando (brote de una enfermedad), entonces este paso debería considerarse como un Punto de Control Crítico (PCC)

ESTRICTAMENTE, LOS **PC** (PELIGROS EVIDENTES) SE CONTROLAN MEDIANTE PROGRAMAS PREVENTIVOS, MIENTRAS QUE LOS **PCC** SE CONTROLAN MEDIANTE UN PROGRAMA EMERGENTE.

El razonamiento es **"NO PUEDO CONTROLAR LOS PUNTOS FINOS DE MI PROCESO, SI ANTES NO CONTROLO LO BÁSICO Y EVIDENTE"**

Y este razonamiento nos lleva a lo que se conoce como **PROGRAMAS DE PRERREQUISITOS** (programas previos a la implementación del HACCP, desarrollados para puntos de control), algunos de los cuales son los siguientes

- Definición de la Misión y Visión de la Empresa
- Manual de Calidad
- Prácticas de Sanitización
- Buenas Prácticas de Manufactura (GMP´s)
- Control de plagas
- Control de químicos
- Retiro y rastreabilidad del producto
- Quejas del consumidor relacionadas con la seguridad del alimento

Como en el sector productivo pecuario no se produce lo que se considera propiamente alimento, sino solo la materia prima que se procesará posteriormente en las plantas, entonces no es posible aplicar un HACCP (que por definición se aplica solo a procesos de elaboración de alimentos), pero si sus principios y prerrequisitos, y uno de los mas importantes son las Buenas Prácticas de Producción Primaria o Buenas Prácticas Pecuarias, equivalentes a las GMP`s.

Esto significa que, el primer paso para un negocio seguro en el sector de producción primaria (granjas, incubadoras etc.) es trabajar sobre los puntos básicos de control (**prácticas estratégicas)** que varían en cada empresa pero pueden incluir equipos, materiales, instalaciones, sanidad, capacitación, orden, documentación, administración, etc., asegurando la productividad del sistema y posteriormente se puede ya trabajar en programas específicos (desarrollo de procedimientos emergentes para puntos críticos) tanto en granja como durante la transformación del producto en alimento.

En esencia las **Buenas Prácticas de Producción Primaria** son un conjunto de técnicas y procedimientos desarrollados con la finalidad de reducir, controlar y/o evitar los riesgos predecibles durante la producción y el transporte del producto

Estas prácticas promueven:

- Procesos definidos
- Verificación de procesos
- Sanidad
- Orden
- Documentación

En todas las áreas y actividades de una explotación, para lo cual se requiere iniciar una serie de ajustes que permitan a una empresa trabajar con esquemas como el que se ejemplifica en la siguiente figura:

Los objetivos (valor agregado) de implementar un sistema de buenas prácticas de producción primaria son:

1. Alcanzar los parámetros productivos proyectados
2. Evitar el rechazo del producto en el rastro y/o planta de proceso
3. Facilitar la obtención de los parámetros de calidad/sanidad/inocuidad en el producto durante el su transformación en alimento
4. Que los procesos no tengan un impacto negativo al medio ambiente
5. Competitividad de la empresa

En la medida en que logremos comprender esta información, será posible comprender y desarrollar procesos con una visión clara de rentabilidad y dar a nuestra empresa respuesta a las necesidades actuales de eficiencia, calidad y seguridad de la producción al momento de su transformación en alimento.

Considerando un ejemplo de procesos a implementar como lo es la instalación de un tapete sanitario (y que aparentemente solo consiste en diluir desinfectante en una charola y pisar cada vez que se entra y se sale), el proceso que se requiere incluye como primer paso la evaluación del desinfectante más efectivo, que dure, que se pueda reconocer fácilmente el momento en que se inactiva, la dilución adecuada, la cantidad de la solución recomendable, el recipiente mas adecuado, el tiempo de recambio, condiciones de uso, costo, etc.

En el segundo paso y una vez que se define el proceso, se debe generar un instructivo de trabajo, para que, independientemente de quien de mantenimiento a los tapetes, sepa cuando y como debe hacerlo. Este paso incluye también la capacitación de los probables encargados de dar mantenimiento al tapete, de tal manera que aseguramos que nuestro instructivo es simple y entendible y que el personal no tiene duda sobre el procedimiento.

El tercer paso consiste en verificar que el procedimiento realmente agrega valor al proceso productivo. En el caso del tapete significa que realmente está contribuyendo al mantenimiento de la sanidad. Este paso tiene que ver con la documentación del proceso (lo bueno y lo malo) para fomentar una mejora continua, con base en la experiencia adquirida

CONCLUSIÓN

Para la búsqueda de estos objetivos es importante considerar los siguientes principios sobre la relación entre bioseguridad y los procesos en una explotación que trata de ser productiva.

Primer principio: Las condiciones (sanidad, orden, capacitación, etc.) en que se ejecutan los procesos en una explotación pecuaria, determinan la probabilidad de ocurrencia (riesgo) de los peligros que pueden presentarse. **PELIGRO Y RIESGO NO SON LO MISMO**

Segundo principio. Cada empresa cuenta con dimensiones particulares (tamaño de ato, tecnificación, organización, etc) y la combinación de estas hace diferente y única a una explotación en relación a cualquier otra, aun y cuando pertenezcan a la misma empresa, por lo que es posible que el peligro y el nivel de riesgo asociado al peligro hagan necesaria la implementación de prácticas tan particulares como la explotación, por lo que se debe considerar a los manuales hasta ahora existentes solo como una guía.

Tercer principio. La bioseguridad es un concepto, y como tal no se puede traducir en procedimientos independientes a los procesos de producción, ya que su aplicación depende, se incluye y se regula al través de los mismos. O sea que no se puede apartar ni ver la bioseguridad como ente independiente a la producción.

Cuarto Principio. Todas las prácticas desarrolladas e implementadas en una explotación tienen impacto sobre la sanidad de la misma por lo que en la realidad no existe un programa de bioseguridad, sino un nivel de bioseguridad que se obtiene al través del diseño de prácticas que contemplan los peligros PREDECIBLES asociados a los diferentes procesos, el grado de riesgo asociado a cada peligro y las acciones para controlarlos (POE, POES, etc.). Estas se conocen generalmente como BUENAS PRÁCTICAS DE PRODUCCIÓN PRIMARIA O BUENAS PRÁCTICAS PECUARIAS.

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EFECTO DE LAS OCRATOXINAS Y LAS AFLATOXINAS SOBRE LAS VARIABLES PRODUCTIVAS EN POLLOS DE ENGORDA VACUNADOS CONTRA LA COCCIDIOSIS AVIAR

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La industria avícola es afectada constantemente por la presencia de *Eimerias* sp las cuales provocan pérdidas económicas importantes. Una medida para contrarrestar el efecto negativo de estos protozoarios es el uso de vacunas, sin embargo en ocasiones estas no dan la protección esperada, e incluso aparentemente la vacunación provoca la la enfermedad, a pesar de que la vacuna cuenta con buenas prácticas de mano factura. Una posible explicación es la presencia de micotoxinas en el alimento en concentraciones consideradas como no dañinas. En éste estudio se evaluó el efecto de las aflatoxinas y las ocratoxinas en pollos de engorda vacunados contra la coccidiosis aviar, a través de alteración en las variables productivas y la química sanguínea. Se utilizaron 90 aves de un día de edad, estirpe Ross para aplicar 8 tratamientos por un período de 28 de edad. Las aves fueron vacunadas al día de edad por aspersión. Al día 21 las aves fueron desafiadas con de *Eimeria* sp aisladas de campo y al día 28 se realizó la toma de muestras y el sacrificio. El peso, el consumo e índice de conversión de las aves se vio afectado por la presencia de las micotoxinas ($p<0.05$), es importante destacar que las aves vacunadas y que consumieron aflatoxinas u ocratoxinas tuvieron un comportamiento similar a las aves que únicamente fueron desafiadas. La evaluación del hematocrito y proteínas se vieron afectadas por la presencia de las micotoxinas y en las aves que fueron desafiadas con y sin vacunación ($p<0.05$). Respecto a la concentración sérica de transaminasas y bilirrubinas, solo se incrementó en aquellos tratamientos que consumieron algún tipo de micotoxinas ($p<0.05$).

Palabras claves: Aflatoxina B_1 | Ocratoxina| Pollo de engorda | Interacción | Coccidiosis | *Eimeria*

INCLUSIÓN DE ACEITE DE ATÚN COMO FUENTE DE ÁCIDOS GRASOS OMEGA TRES EN DIETAS PARA POLLO DE ENGORDA

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Resumen

Con el objeto de adicionar ácidos grasos omega tres (AGω3) en la carne de pollo, se adicionó aceite de atún (AAT) en la dieta de 192 pollos de engorda de la línea Ross x Ross de 1 a 49 días de edad, colocados en piso de cemento en un diseño completamente al azar en 4 tratamientos con 4 repeticiones de 12 pollos cada una, mitad hembras y machos. Se evaluaron 4 niveles de AAT a 0, 0.75, 1.0 y 1.25 %. A los 49 días, se muestrearon 6 machos y 6 hembras por tratamiento para pierna, muslo y pechuga. Se evaluó el perfil de lípidos de ácidos grasos poliinsaturados (AGP), monoinsaturados (AGM), saturados (AGS), omega-6 (AGω6) linoleico (LA) y araquidónico (AA); omega tres (AGω3) eicosapentaenoico (EPA), docosapentaenoico (DPA) y docosahexaenoico (DHA) como porcentaje de lípidos totales. Para su análisis estadístico se transformaron a la relación arco-seno, y se analizaron mediante un arreglo factorial 2 x 4, donde un factor fueron el sexo (hembra y macho) y el otro los niveles de AAT. Los resultados para pierna y muslo de AGP, AGM, AGS, LA y AA fueron similares ($P>0.05$) para sexos; y disminuyeron ($P<0.05$) al incrementar el % de AAT; para los AGω3 DHA los sexos fueron iguales ($P>0.05$) y se incrementó ($P<0.05$) con 1 y 1.25 % de AAT; DPA fue mayor ($P<0.05$) para las hembras sin embargo al aumentar el AAT disminuyó ($P<0.05$); para EPA los sexos fueron similares ($P>0.05$) y se incrementó al adicionar AAT ($P<0.05$). Para pechuga AGP, AGM, AGS y AGω6 en sexos fueron similares ($P>0.05$), al aumentar el AAT en la dieta los AGP se incrementaron ($P<0.05$) con 1 y 1.25 % en relación al testigo, AGM y AGS con 0.75 % de AAT disminuyeron ($P<0.05$); LA al adicionar AAT, aumento ($P<0.05$) con 1 y 1.25 % en relación al testigo; para AA al adicionar AAT disminuyó ($P<0.05$) con 0.75 %; DHA y EPA no tuvieron diferencia entre sexos ($P>0.05$), sin embargo al adicionar el AAT, se incrementaron ($P<0.05$) con 1 y 1.25 % en relación al testigo. Se concluye que el mejor nivel de inclusión del AAT es con 1.25 %.

Palabras clave: Omega tres, aceite de atún, pollos de engorda, ácidos grasos.

Introducción

El crecimiento en la producción de pollo de engorda en México en los últimos años, es debido a la demanda (2.5 millones de toneladas fueron producidas en 2005) por su menor precio que otras carnes para los mexicanos (www.una.org.mx) y consumida por la mayoría de la población; por lo que adicionarle en el alimento de las aves ingredientes que aporten AGω-3 de origen marino le da un valor agregado al producto para prevenir causas de origen nutricional en la población (Baucellis, *et al.*, 2000; Cherian, *et al.,* 1996) que pueden producir enfermedades cardiovasculares y cáncer de cólon (Ayerza, *et al.,* 2002; Van Elswyk, 1992), pues la disminución del colesterol de los productos avícolas a tenido poco éxito para el consumidor (Ávila, *et al*. 1997). Estudios sobre la grasa de la dieta, se han centrado en la manipulación de ácidos grasos específicos, por ejemplo los AGω-3 eicosapentaenoico (EPA), decosahexaenoico (DHA) de productos marinos (Carrillo, *et al.,* 1999;

Castro 2002) son compuestos antitrombóticos que contribuyen en la disminución de agregación de plaquetas y subsecuentemente la formación de placas arteroescleróticas (Ayerza, *et al.,* 2002; Baucellis, 2000). La presente investigación se realizó con finalidad evaluar la adición del aceite de atún en la dieta de los pollos y su enriquecimiento con omega tres, así como conocer el perfil lipídico de la carne de pollo.

Material y Método

Se utilizaron 192 pollos de la línea Ross x Ross de 1 a 49 días de edad, colocados en piso de cemento en un diseño completamente al azar en 4 tratamientos con 4 repeticiones de 12 pollos cada una, mitad hembras y machos. Las dietas se elaboraron a base de sorgo y pasta de soya con 4 niveles de aceite de atún (AAT) a 0, 0.75, 1.0 y 1.25 %, que a expensas de aceite de soya se fueron adicionando hasta un nivel de 3 %. Las dietas fueron elaboradas para iniciación (0 a 21 días) y finalización (21 a 49 días). A los 49 días, se sacrificaron 10 aves por tratamiento (5 machos y 5 hembras) obteniendo las muestras de pierna, muslo y pechuga, las cuales fueron almacenadas en un congelador a -20°C, hasta su análisis. La extracción de lípidos se realizó de acuerdo al método de Folch (1957), el extracto lipídico fue metilado de acuerdo al método de AOAC (2000).

Los datos obtenidos en el laboratorio para el perfil lipídico, se muestran como porcentaje de lípidos totales y para su análisis estadístico se transformaron a la relación arco-seno, mediante un arreglo factorial 2 x 4, donde un factor fueron el sexo (hembra y macho) y el otro los niveles de AAT, utilizando el paquete estadístico SAS (Statystical Analysis System V.9.1) donde se obtuvo el análisis de varianza, las medias y cuando existió diferencia significativa entre ellas ($P<0.05$) se realizó la prueba de tukey (Steel and Torrie, 1988).

Cuadro 1. Dietas experimentales de iniciación (1-21 días en pollos de engorda con niveles de aceite de atún

Ingrediente	**AAT 0%**	**AAT 0.75%**	**AAT 1%**	**AAT 1.25%**
Sorgo	60.84	60.84	60.84	60.84
Pasta de Soya	31.74	31.74	31.74	31.74
Aceite de soya	**3**	**2.25**	**2**	**1.75**
Aceite de atún	**0**	**0.75**	**1**	**1.25**
Ortofosfato de Ca	2.24	2.24	2.24	2.24
Carbonato de Ca	0.92	0.92	0.92	0.92
Otros	1.26	1.26	1.26	1.26
Total	100	100	100	100
Análisis calculado*				
EM(Kcal/Kg)	3001	3007	3006	3005
PC %	21	21	21	21
Lisina %	1.17	1.17	1.17	1.17
Met- Cis %	0.84	0.84	0.84	0.84
Treonina %	0.78	0.78	0.78	0.78
Arginina %	1.211	1.211	1.211	1.211
Trip. %	0.266	0.266	0.266	0.266
Calcio total	1	1	1	1
Fosforo disponible	0.45	0.45	0.45	0.45

*Requerimientos NRC 1994

Cuadro 2. Dietas experimentales de finalización (22-49 días) en pollos de engorda con aceite de atún

Ingrediente	AAT 0%	AAT 0.75%	AAT 1%	AAT 1.25%
Sorgo milo 9	63.74	63.74	63.74	63.74
Soya	**29.19**	**29.19**	**29.19**	**29.19**
Aceite de soya	3	2.25	2	1.75
Aceite de atún	0	0.75	1	1.25
Ortofosfato de Ca	1.6	1.6	1.6	1.6
$CaCO_3$	1.04	1.04	1.04	1.04
Otros	1.08	1.08	1.08	1.08
Total	100	100	100	100
Análisis calculado*				
EM(Kcal/Kg)	3043	3004	3039	3038
PC %	20	20	20	20
Grasa cruda%	5.017	5.017	5.017	5.017
Lisina %	1	1	1	1
Met- Cis %	0.75	0.75	0.75	0.75
Treonina %	0.7	0.7	0.7	0.7
Arginina %	1.138	1.138	1.138	1.138
Calcio total	0.9	0.9	0.9	0.9
Fosforo disponible	0.35	0.35	0.35	0.35

*Requerimientos NRC 1994

RESULTADOS

Los resultados obtenidos al adicionar diferentes niveles de AAT, para los ácidos grasos polinsaturados, se encuentran en el Cuadro 3.

Cuadro 3. Ácidos grasos poliinsaturados* en pierna, muslo y pechuga en pollos alimentados con dietas a diferentes niveles de aceite de atún

PIERNA Y MUSLO	AAT 0% Promedio ± EEM	AAT 0.75% Promedio ± EEM	AAT 1.0% Promedio ± EEM	AAT 1.25% Promedio ± EEM	Promedio ± EEM
HEMBRA	29.93±2.35 [a]	13.0±2.03 [b]	15.54±2.96 [b]	18.49±3.82[b]	14.24±1.98
MACHO	22.20±1.89 [ab]	17.60±1.10 [b]	16.28±0.7 [b]	20.05±1.15 [ab]	19.03±0.79
PROMEDIO	26.06±1.92 [a]	15.30±1.33 [b]	15.91±1.90 [b]	19.27±1.90 [b]	
Pechuga					
HEMBRA	6.31±0.79 [c]	4.26±0.75 [b]	9.50±1.45 [a]	7.81±1.45[c]	6.97±0.69
MACHO	4.63±0.68 [c]	2.81±0.23 [b]	8.91±1.11[a]	9.33±1.90[a]	6.27±0.81
PROMEDIO	5.47±0.56 [b]	3.53±0.44 [b]	9.21±0.87 [a]	8.49±1.12 [a]	

a, b, c./Medias con diferente literal son estadísticamente significativas (P<0.05)
EEM. Error estándar de la media.
Porcentaje del total de ácidos grasos
AAT. Aceite de atún.

Para pierna y muslo, en los tratamientos, las hembras disminuyeron (P<0.05) los AGP; y para los efectos principales, los AGP fueron similares (P>0.05) para machos y hembras, al incrementar el AAT en la dieta, disminuyeron (P<0.05) en relación al testigo.

Al incluir AAT en la dieta, en pechuga no hubo diferencia (P>0.05) entre sexos y el nivel de AGP fue mayor (P<0.05) para 1 y 1.25% en relación al testigo.

Para los ácidos grasos monoinsaturados, los resultados se muestran en el Cuadro 4.

Cuadro 4. Ácidos grasos monoinsaturados* en pierna, muslo y pechuga en pollos alimentados con dietas a diferentes niveles aceite de atún

Pierna y Muslo	AAT 0% Promedio ± EEM	AAT 0.75% Promedio ± EEM	AAT 1.0% Promedio ± EEM	AAT 1.25% Promedio ± EEM	Promedio ± EEM
HEMBRA	35.22±1.74 [a]	14.30±1.70 [b]	16.33±1.51[b]	20.10±3.92 [b]	21.49±2.18
MACHO	22.86±1.65 [b]	18.57±0.56 [b]	17.89±1.24 [b]	22.22±1.09 [b]	20.39±0.74
PROMEDIO	29.04±2.35 [a]	16.44±1.10 [b]	17.11±0.96 [b]	21.16±1.95 [b]	
PECHUGA					
HEMBRA	30.75±3.65 [a]	13.17±1.87 [c]	20.43±2.10 [c]	18.94±3.79 [b]	20.82±1.99
MACHO	20.61±1.81[b]	10.33±0.95 [b]	20.68±1.42 [c]	23.27±3.31[a]	18.48±1.45
PROMEDIO	25.68±2.55 [a]	11.75±1.09 [b]	20.55±1.19 [a]	20.86±2.53 [a]	

a, b, c./Medias con diferente literal son estadísticamente significativas (P<0.05)
EEM. Error estándar de la media.
*Porcentaje del total de ácidos grasos
AAT. Aceite de atún.

Para pierna y muslo, entre tratamientos, las hembras disminuyeron (P<0.05) los niveles de AGM y para efectos principales no hubo diferencia (P>0.05) entre machos y hembras, pero disminuyeron (P<0.05) en relación al testigo similar a AGP. En pechuga, no existió diferencia (P>0.05) para sexos, pero con 0.75 % de AAT hubo diferencia (P<0.05) con los demás tratamientos. El efecto de la adición de aceite de atún sobre los ácidos grasos saturados, se encuentra en el Cuadro 5.

Cuadro 5. Ácidos grasos saturados* en pierna, muslo y pechuga en pollos con dietas a diferentes niveles de aceite de atún

PIERNA Y MUSLO	AAT 0% Promedio ± EEM	AAT 0.75% Promedio ± EEM	AAT 1.0% Promedio ± EEM	AAT 1.25% Promedio ± EEM	Promedio ± EEM
HEMBRA	23.29±1.91[a]	10.54±1.39 [b]	12.37±1.54 [b]	15.27±3.00 [b]	15.37±1.47
MACHO	17.28±1.55 [ab]	13.53±0.54 [b]	13.48±1.09 [b]	15.97±0.36 [ab]	15.06±0.59
PROMEDIO	20.29±1.53 [a]	12.03±0.86 [b]	12.93±0.91 [b]	15.62±1.43 [b]	
PECHUGA					
HEMBRA	22.51±2.19 [a]	9.73±1.61[b]	14.01±2.174 [c]	15.21±3.13 [c]	15.36±1.50
MACHO	14.69±1.42 [c]	6.62±0.85 [b]	16.27±1.30 [a]	18.44±2.26 [a]	13.77±1.24
PROMEDIO	18.60±1.79 [a]	8.17±1.00 [b]	15.14±1.25 [a]	16.64±1.97 [a]	

a, b, c./Medias con diferente literal son estadísticamente significativas (P<0.05)
EEM. Error estándar de la media.
* Porcentaje del total de ácidos grasos.
AAT/ aceite de atún

Para pierna y muslo los AGS tampoco tuvieron diferencia (P>0.05) entre sexos y disminuyeron (P<0.05) en relación al testigo al aumentar el nivel de AAT.Para pechuga, no hubo diferencia (P>0.05) entre sexos, pero con 0.75% disminuyeron (P<0.05) en relación a los demás tratamientos. Para los omega-6 los resultados se muestran en el Cuadro 6.

Cuadro 6. Ácidos grasos omega-6* en pierna, muslo y pechuga en pollos con dietas a diferentes niveles de aceite de atún

OMEGA-6 PIER Y MUSLO	SEXO	AAT 0% Promedio ± EEM	AAT 0.75% Promedio ± EEM	AAT 1.0% Promedio ± EEM	AAT 1.25% Promedio ± EEM	Promedio ± EEM
LA	HEMBRAS	24.12±1.97 [a]	9.84±1.57 [b]	11.18±2.12 [b]	13.22±2.62 [b]	14.59±1.61
	MACHOS	17.15±1.85 [ab]	13.84±0.90 [b]	12.04±0.61 [b]	14.57±0.91 [b]	14.40±0.68
	PROMEDIO	20.63±1.72 [a]	11.84±1.08 [b]	11.61±1.05 [b]	13.89±1.33 [b]	
AA	HEMBRAS	1.65±0.14 [a]	0.59±0.10 [c]	0.92±0.18 [bc]	0.91±0.20 [bc]	1.02±0.11
	MACHOS	1.21± 0.12 [ab]	0.81±0.06 [bc]	0.82±0.03 [bc]	0.92±0.07 [bc]	0.94±0.05
	PROMEDIO	1.43±0.11 [a]	0.70±0.06 [b]	0.87±0.08 [b]	0.92±0.10 [b]	
PECHUGA						
LA	HEMBRAS	0.23±0.04 [b]	0.53±0.09 [b]	1.68±0.24 [a]	1.26±0.28 [a]	0.90±0.16
	MACHOS	0.31±0.08 [b]	0.35±0.30 [b]	1.23±0.17 [a]	1.44±0.34 [a]	0.80±0.14
	PROMEDIO	0.27±0.04 [b]	0.44±0.05 [b]	1.45±0.16 [a]	1.35±0.20 [a]	
AA	HEMBRAS	2.24±0.25 [a]	0.97±0.21 [b]	1.75±0.33 [c]	1.54±0.24 [c]	1.62±0.16
	MACHOS	1.92± 0.39 [a]	0.59±0.05 [b]	1.77±0.18 [a]	1.67±0.29 [c]	1.46±0.17
	PROMEDIO	2.10±0.21 [a]	0.78±0.12 [b]	1.76±0.16 [a]	1.60±0.17 [a]	

a, b, c./Medias con diferente literal son estadísticamente significativas a (P<0.05)
EEM. Error estándar de la media
Porcentaje del total de ácidos grasos.
AAT/ aceite de atún.

En pierna y muslo, LA y AA no tuvieron diferencia entre sexos (P>0.05), sin embargo al adicionar AAT, disminuyeron (P<0.05) en relación al testigo.
En pechuga, LA no mostró diferencia (P>0.05) entre sexos, al adicionar AAT, pero al ir incrementando el AAT, aumento (P<0.05) con 1 y 1.25 % en relación al testigo; para AA existió diferencia (P>0.05) entre sexos, sin embargo al adicionar AAT disminuyó (P<0.05) con 0.75% en relación al resto de los tratamientos. Para los ácidos omega tres los resultados se presentan en el Cuadro 7.

Para pierna y muslo, los AGω3, DHA no tuvo diferencia entre sexos (P>0.05) para los efectos principales, sin embargo con 1 y 1.25 % de AAT, se incrementó (P<0.05) el nivel de DHA en relación al testigo; para DPA, el nivel del AGω-3 fue mayor (P<0.05) para las hembras sin embargo al aumentar el AAT disminuyó (P<0.05); para EPA, no hay diferencias (P>0.05) entre sexos y se incrementó al adicionar AAT (P<0.05). En Pechuga, los AGω3, DHA y EPA no tuvieron diferencia entre sexos (P>0.05), sin embargo al adicionar el AAT, se incrementaron (P<0.05) con 1 y 1.25 % en relación al testigo.

Cuadro 7. Ácidos grasos omega tres* en pierna y muslo en pollos alimentados con diferentes niveles de AAT

OMEGA 3 PIERNA Y MUSLO	SEXO	AAT 0% Promedio ± EEM	AAT 0.75% Promedio ± EEM	AAT 1.0% Promedio ± EEM	AAT 1.25% Promedio ± EEM	Promedio ± EEM
DHA	HEMBRAS	0.026±0.02 [b]	0.69±0.04 [ab]	0.90±0.23 [ab]	1.23±0.35 [a]	0.77±0.12
	MACHOS	0.19±0.01 [b]	0.48±0.07 [b]	0.91±0.05 [ab]	1.30±0.08 [a]	0.72±0.10
	PROMEDIO	0.22±0.01 [c]	0.58±0.05 [bc]	0.90±0.11 [ab]	1.27±0.17 [a]	
DPA	HEMBRAS	0.026±0.005 [a]	0.017±0.00 [c]	0.016±0.004 [c]	0.012±0.00 [c]	0.019±0.002 [a]
	MACHOS	0.022± 0.0 [c]	0.009±0.006 [b]	0.012±0.001 [b]	+	0.012±0.001 [b]
	PROMEDIO	0.025±0.004 [a]	0.011±0.0018 [b]	0.013±0.0013 [b]	0.012±0.00 [b]	
EPA	HEMBRAS	0.17±0.007 [c]	0.53±0.09 [bc]	0.87±0.16 [ab]	1.29±0.28 [a]	0.71±0.12
	MACHOS	0.10±0.017 [c]	0.62±0.040 [bc]	1.11±0.22 [ab]	1.31±0.06 [a]	0.78±0.12
	PROMEDIO	0.13±0.013 [c]	0.57±0.050 [b]	0.99±0.13 [a]	1.30±0.13 [a]	
PECHUGA						
DHA	HEMBRAS	0.40±0.037 [b]	1.05±0.16 [b]	2.92±0.60 [a]	2.78±0.27 [a]	1.79±0.29
	MACHOS	0.54±0.131 [b]	0.65±0.06 [b]	2.54±0.34 [a]	2.99±0.63 [a]	1.61±0.29
	PROMEDIO	0.47±0.07 [b]	0.85±0.10 [b]	2.73±0.33 [a]	2.87±0.29 [a]	
EPA	HEMBRAS	0.023±0.04 [b]	0.53±0.09 [b]	1.68±0.24 [a]	1.26±0.28 [a]	0.90±0.16
	MACHOS	0.31±0.08 [b]	1.68±0.24 [b]	1.23±0.17 [a]	1.44±0.34 [a]	0.80±0.14
	PROMEDIO	0.27±0.04 [b]	0.44±0.05 [b]	1.45±0.16 [a]	1.35±0.20 [a]	

a, b, c./Medias con diferente literal son estadísticamente significativas (P<0.05)
EEM. Error estándar de la media.
* Porcentaje del total de ácidos grasos
+no se observó presencia de DPA

Discusión

Los resultados para pierna y muslo así como en la pechuga para AGP, AGM, AGS, LA y AA fueron similares (P>0.05) para sexos; y disminuyeron (P<0.05) al incrementar el % de AAT, a diferencia de los AGω3 DHA y EPA que se incrementaron (P<0.05) con 1 y 1.25 % de AAT, DPA aumento (P<0.05) en las hembras sin embargo adicionar más AAT disminuyó (P<0.05), estos resultados concuerdan con Cherian, 1996 que incremento los omega tres en el huevo; Por otra parte los ácidos grasos omega seis contenidos en algunos productos de origen animal y vegetal (aceites y grasa) disminuyen su concentración al adicionar fuentes de ácidos grasos omega tres en la dieta y para este experimento al aumentar los niveles de ácidos grasos omega tres, los ácidos grasos omega seis disminuyeron.
Trabajos realizados con AAT en dietas para gallinas de postura, indican que los AGϖ3 en el huevo se incrementan al aumentar el AAT en la dieta pues tiene un alto contenido de EPA y DHA, lo que concuerda con Castillo et al. (2000) que incremento linealmente la deposición de los dos ácidos grasos, González y Leeson (2001) indican el AGω3 que más se deposita en la yema de huevo es el DHA, y en este estudio también en la carne de pollo.

La reducción en el contenido de los AGϖ6 fue proporcional al incremento de aceite de atún, inverso a los AGϖ3; Herber y Van Elswyk, (1996) y Baucellis *et al*. (2000), indican que esto puede deberse a la competencia entre los omega tres y seis. Se cree que los AGϖ3 son preferentemente incorporados en las membranas biológicas a expensas de los AGϖ6 y ambos están implicados en la producción de eicosanoides como las prostaglandinas, tromboxanos y leucotrienos. Sin embargo, los eicosanoides derivados de la familia de AGϖ6, tienen efectos opuestos a los derivados de la familia de AGϖ3 y existe una competencia entre las enzimas involucradas en la desaturación y elongación del LA a ALA. Por lo tanto un balance adecuado es importante para mantener la salud.

Los ácidos grasos EPA y DHA son los que mayores efectos benéficos aportan a la salud, su consumo recomendado es de 650 mg/día (en una dieta de 2000 kcal diarias) (Simopoulos,2000).

Los resultados de este experimento, indican que el aceite de atún adiciona ácidos grasos omega tres (eicosapentanoico y docosaexanoico) a la carne. Se concluye que el mejor nivel de inclusión del AAT es con 1.25 %.

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EFECTO DEL USO PERMANENTE DE UN PROGRAMA ANTICOCCIDIAL EN LOS PARÁMETROS PRODUCTIVOS DE POLLO DE ENGORDA DESAFIADO CON *Eimeria* SPP

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RESUMEN

Se empleó un diseño completamente al azar con dos tratamientos: A. Dieta sin coccidiostato y B. Dieta con el programa permanente, cada uno con 4 repeticiones de 30 pollos por corral. Durante los 49 días, que duró la prueba cada semana se registraron los parámetros productivos, mientras que la pigmentación cutánea y el número de ooquistes por gramo de heces (opgh) fueron evaluados a partir del día 21 y a esta misma edad cada ave fue inoculada por vía oral con 60,400 ooquistes esporulados de *Eimeria* spp. Las variables productivas fueron evaluadas mediante un análisis de observaciones repetidas y la prueba de T de Student. Los resultados promedio obtenidos a los 49 días de edad no mostraron diferencia significativa ($P>0.05$) en la ganancia de peso (A: 2904g, B: 2963g), consumo de alimento (A: 6228g, B: 6033g), conversión alimenticia (A: 2.14 kg, B: 2.04kg) entre ambos tratamientos. En las aves sin coccidiostato la pigmentación cutánea fue menor en el grupo A ($P<0.05$) (A: 11.23, B: 15.4) y se eliminaron una mayor ($P<0.05$) cantidad de opgh con respecto al tratamiento B. Esto sugiere que el uso continuo del programa anticoccidial administrado en esta prueba mostró resultados favorables de pigmentación y eliminación de opgh (A: 25,352, B: 9,227) con respecto al grupo sin coccidiostato; sin embargo, estos no fueron los esperados en aves tratadas con fármacos anticoccidiales. Lo anterior puede relacionarse a disminución de la efectividad de un compuesto o ambos por la adición permanente en la dieta durante varios años. Lo mismo puede suceder en granjas avícolas donde no se realizan pruebas de eficacia anticoccidial previo a la planeación de los programas o después de varios años de uso continuo y pueden pasan desapercibidos por semanas o parvadas antes de afectar en forma evidente la pigmentación u otros parámetros productivos menos susceptibles.

Palabras clave: *coccidiosis aviar, pigmentación cutánea, pollo de engorda, Eimeria.*

INTRODUCCIÓN

La coccidiosis aviar (CA) es la enfermedad parasitaria más común e importante a nivel mundial, porque genera enormes pérdidas económicas debido a su prevención y tratamiento [1, 2].

Con el descubrimiento y desarrollo de los productos ionóforos, el control de la coccidiosis en pollo de engorda se mejoró. Sin embargo, incluso el producto mas efectivo no puede eliminar todas las coccidias, por lo que el riesgo de que se presente la enfermedad siempre estará latente [3].

Por otro lado, el periodo de uso de estos fármacos inevitablemente favorece la selección y desarrollo de poblaciones de coccidias resistentes, por lo que causan serias limitaciones y la efectividad de estos productos disminuye consideradamente [1,4]. Debido a que en ocasiones se mantiene en una granja la misma formulación anticoccidiana durante varias parvadas o años sin evaluar su efectividad, en este estudio se evaluó un programa anticoccidiano dual permanente de nicarbazina en el alimento iniciador y monensina en la finalización y su efecto en aves desafiadas con *Eimeria* spp.

OBJETIVO

Identificar y analizar el efecto de un programa anticoccidiano dual permanente (nicarbazina + monensina) sobre los parámetros productivos, pigmentación cutánea, grado de severidad de las lesiones intestinales y número de ooquistes en heces en pollo de engorda desafiado con *Eimeria* spp.

MATERIAL Y MÉTODOS

Instalaciones. Las aves fueron alojadas aleatoriamente en dos tratamientos con cuatro replicas cada uno: A. Dieta sin coccidiostato y B: Dieta con el programa permanente de coccidiostatos. Los pollos se distribuyeron en corrales dentro del centro de Enseñanza, Investigación y Extensión en Producción Avícola (CEIEPAv) de la FMZV de la UNAM.

Animales de experimentación. Se utilizaron 240 pollitos mixtos, provenientes de una incubadora comercial de la estirpe Ross 308, de 1 día de edad. Las aves fueron criadas de manera comercial bajo sistemas convencionales de manejo hasta el final del experimento.

Alimento. Se utilizó una dieta a base de sorgo + pasta de soya. Los niveles de nutrientes cubrieron las necesidades recomendadas por el manual de la estirpe. El alimento y el agua se ofrecieron a libre acceso durante toda la prueba. En el grupo tratado, el alimento iniciador se les proporcionó desde la

llegada hasta los 21 días de edad y se le agregaron 125ppm de nicarbazina, mientras que el alimento finalizador se dio del día 22 al 49 de edad con 100ppm de monensina sódica.

Pigmento. Todas las aves recibieron en la dieta de finalización 80 ppm de xantofilas amarillas. A partir de la tercera semana se realizó semanalmente la lectura del pigmento cutáneo en la zona aptérica costal izquierda, con el colorímetro de reflectancia Minolta CR400 (Minolta Co Osaka Japan). En 10 pollos por cada réplica de ambos tratamientos.

Inóculo de desafio. Se elaboró con cepas vacúnales de *E. acervulina* (46%), *E. maxima* (18%), *E. mivati* (14%), *E. tenella* (22%). La dosis infectante por pollo fue de 60,400 ooquistes esporulados por ave y fueron administrados por vía oral por medio de una cánula esofágica a los 21días de edad.

Número de ooquistes por gramo de heces. Se tomaron muestras de heces frescas de 5 pollos por réplica, en cada réplica se mezcló el contenido y 2 g se conservaron en relación 1:2.5 en una solución de dicromato de potasio al 2.5% para su posterior examen cuantitativo mediante la técnica de McMaster. El numero de ooquistes por gramo de heces fue calculado= # de ooquiste en la cámara X 100 / 2 X 2.5 [5,6].

Severidad de las lesiones intestinales. Las lesiones intestinales asociadas a la infecciones con *Eimeria* fueron evaluadas semanalmente a partir de los 14 días de un pollo por corral de acuerdo a la escala de Johnson y Reid [7].

Análisis estadístico. Los pesos y el pigmento cutáneo fueron sometidos a un análisis estadístico conforme al diseño experimental empleado y las diferencias entre las medias, de los tratamientos se evaluaron con la prueba de observaciones repetidas en el tiempo. Los resultados del número de ooquistes en heces fueron transformados a arco seno. El grado de severidad de las lesiones intestinales se analizó con la prueba no paramétrica de Kruskall Wallis y se utilizó la prueba U de Mann - Witney para determinar las diferencias entre las medianas de los tratamientos [8]. Todas las pruebas se evaluaron con una significancia de $P<0.05$.

RESULTADOS

Se observó que durante las primeras 6 semanas no hubo diferencia en la ganancia de peso entre los animales tratados con coccidiostato y sin coccidiostato ($P>0.05$), mientras que en la semana siete existió una tendencia a ganar menor peso en los pollos sin coccidiostato (521g) con respecto a los

que si se les adicionó el coccidiostato (551g) (P<0.05); sin embargo al comparar los dos tratamientos las ganancias de peso durante esta semana fueron similares (415g vs. 424g) (P>0.05).

A la semana 3 y 4 no se observó diferencia entre los tratamientos en pigmentación cutánea y a partir de la quinta semana las aves sin coccidiostato tuvieron menor pigmentación cutánea (6.62) con respecto aquellas que si recibieron el coccidiostato de uso permanente (9.83) (P<0.05) (Cuadro 1). Esta diferencia se mantuvo constante hasta las séptima a pesar de que los animales sin coccidiostato consumieron mas pigmento (4064 mg) con respecto a los animales que tuvieron el coccidiostato (3933mg).

En lo que respecta al conteo de ooquistes por gramo de heces se observo que las aves que tenían el programa anticoccidiano continuo mostraron menor cantidad de ooquistes por gramo de heces semanales (P<0.05) esto refleja un menor daño tisular al intestino y por ende una mayor pigmentación en este grupo. También se observa que a la séptima semana los conteos de ooquistes por gramo de heces fueron muy similares esto puede ser debido a que las aves que no tenían coccidiostato mostraron una inmunidad a las coccidias (Cuadro 2).

La severidad de las lesiones macroscópicas intestinales se mantuvieron sin diferencia entre ambos grupo y en un grado 1 + y se presentaron solo en algunas aves, solo un ave del tratamiento sin coccidiostato mostró lesiones mas severas (2+).

DISCUSIÓN Y CONCLUSIONES

No se observó una diferencia importante en las variables productivas, entre los dos grupos; es decir, el beneficio de manejar un programa anticoccidiano, es bajo cuando este es permanente y las coccidias nativas han desarrollado resistencia a estos fármacos [9]. El pigmento fue la variable mas afectada en el grupo sin coccidiostato y a pesar de que estas consumieron mayor cantidad de alimento, pigmentaron menos que las que recibieron coccidiostato. Sin embargo ambos grupos presentaron niveles bajos de pigmentación [10,11]. Es posible que a nivel de campo esté sucediendo algo similar cuando un brote de coccidiosis es subclínico o leve y pasa desapercibido debido a que no afecta directamente el peso corporal, pero si se afecta el pigmento, principalmente al final del ciclo, lo que incrementa el costo de producción y la calidad del producto final.

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Cuadro 1

Pigmentación cutánea en pollo de engorda con un programa anticocidiano permanente y desafiado con *Eimeria* spp.

Edad (semanas)	Tratamiento sin coccidiostato*	Tratamiento con coccidiostato*
3	-0.77a ± 0.34	-1.06a ± 0.16
4	3.19a ± 1.92	5.21a ± 1.22
5	6.62b ± 1.85	9.83 a ± 1.01
6	9.07ab ± 3.39	11.52 a ± 1.72
7	11.23b ± 4.18	15.39 a ± 2.43

Nota: Letras distintas entre tratamientos y dentro de una misma semana de edad denotan diferencia estadística significativa (P<0.05).

Cuadro 2

Número de ooquistes por gramo de heces en pollo de engorda con un programa anticoccidiano permanente y desafiado con *Eimeria* spp

Edad (semanas)	Tratamiento sin coccidiostato*	Tratamiento con coccidiostato*
3	51825a ± 34680	10675b ± 18084
4	50400a ± 33266	24912.5b ± 16472
5	18712.5a ± 26468	9525b ± 18618
6	5112.5a ± 1440	537.5b ± 493
7	712.5a ± 1263	487.5a ± 292

Nota: Letras distintas entre medias de los tratamientos dentro de una misma semana de edad denotan diferencia estadística significativa (P<0.05).

RESPUESTA ANTIBIÓTICA IN VITRO EN DOS CEPAS DE *Mycoplasma synoviae* AISLADAS EN GALLINAS DE POSTURA CLINICAMENTE SANAS Y ENFERMAS

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Los micoplasmas aviares son de gran importancia para la industria avícola debido a que representan una fuente de pérdidas económicas importante ya sea como patógeno único o como parte de un complejo respiratorio. Hablando particularmente de *Mycoplasma synoviae* su importancia se ha relacionado principalmente con las perdidas asociadas a problemas locomotores y retraso en el crecimiento, en el caso particular de las gallinas de postura comercial se ha vinculado con decremento en la producción de huevo. Aunque estos problemas se manifiestan, es más frecuente que la infección por *Mycoplasma synoviae* se presente sin signos clínicos ni lesiones, como una enfermedad benigna de las vías respiratorias altas, o como una condición respiratoria grave en asociación con otros agentes. El objetivo de este trabajo es mostrar la respuesta antibiótica *in vitro* de dos cepas de *Mycoplasma synoviae* aisladas en una misma granja de crianza de gallina de postura comercial provenientes de aves clínicamente sanas y enfermas. Los parámetros que se consideraron para determinar que un ave era clínicamente sana fueron: la ausencia de signos respiratorios y la ausencia de lesiones articulares, en el caso de las aves enfermas estas debían tener signos respiratorios y lesiones articulares. Las muestras para el aislamiento de *Mycoplasma synoviae* fueron hisopos traqueales provenientes de 10 aves sanas y 10 aves enfermas los cuales fueron remitidos al laboratorio para realizar el aislamiento. Los resultados fueron los siguientes: aves sanas 10/10 tubos positivos a *Mycoplasma synoviae*, aves enfermas 9/10 positivos a *Mycoplasma synoviae*, se selecciono un tubo de las aves sanas y uno de las enfermas para realizar una prueba de RAPD, dicha prueba consiste en la clonación de las cepas de *Mycoplasma synoviae* aisladas, con la finalidad de someter su DNA a una amplificación con iniciadores aleatorios, el patrón electroforético de los productos de amplificación de las cepas fue comparado contra el patrón electroforético del DNA de una cepa vacunal y el resultado fue igual a la cepa vacunal, cabe señalar que estas aves no fueron vacunadas. Estas cepas clonadas fueron utilizadas para pruebas de concentración mínima inhibitoria, los resultados están expresados en µg/ml y fueron los siguientes: para lincomicina aves sanas 1.25, aves enfermas 2.5, eritromicina aves sanas >2.5, aves enfermas >2.5, tiamulina aves sanas 0.312, aves enfermas 0.625, enrofloxacina aves sanas >2.5, aves enfermas 2.5, tilosina aves sanas 0.039, aves enfermas 0.039, doxiciclina aves sanas 0.625, aves enfermas 1.25. Como podemos observar la cepa de *Mycoplasma synoviae* aislada de las aves enfermas requiere una mayor cantidad de µg/ml de cada uno de los antibióticos utilizados para inhibir su crecimiento a excepción de la tilosina en donde la cantidad requerida es la misma que para las aves sanas. En este caso aunque son pruebas in vitro es importante considerar los resultados cuando se pretende establecer un programa de medicación.

Palabras clave: *Mycoplasma synoviae*, gallinas de postura, antibióticos, concentración mínima inhibitoria, RAPD

SURGIMIENTO Y DESARROLLO DE LA TECNOLOGÍA DE LA PIGMENTACIÓN EN LA AVICULTURA MEXICANA EN BASE AL USO DE CEMPASUCHIL[1]

Juan Manuel Cervantes Sánchez[2] y Juan José Saldaña González[3]

Resumen

El objetivo de la investigación fue hacer una reconstrucción historiográfica del desarrollo en México en la década de 1960 de la tecnología de la pigmentación del pollo de engorda y de la yema del huevo a partir de la extracción de carotenoides de la flor de cempasúchil *Tagetes erecta.* Para tal efecto fue necesario utilizar la metodología se la historia social de la ciencia latinoamericana. En esta investigación fueron analizados principalmente los factores internos que propiciaron el desarrollo de esta tecnología. Estos fueron: la presencia del cempasúchil en la cultura mexicana, la disposición de las instituciones mexicanas por el estudio de las plantas nativas, el establecimiento de la avicultura industrial por medio de la introducción en México del paquete tecnológico del pollo de engorda y de la gallina de postura, la preferencia del público mexicano por el pollo y huevo pigmentado. Así mismo se analizaron brevemente algunos factores externos, como por ejemplo la influencia de la fundación Rockefeller y de los químicos españoles que desarrollaron la química orgánica en México. Teniendo estos antecedentes un grupo de investigadores mexicanos que trabajaban para la fundación Rockefeller México comenzaron a buscar algunas alternativas para pigmentar los productos avícolas, entre las que se encontraba el cempasúchil (*Tagetes erecta*). Fue así que se desarrolló la tecnología que trascendió las fronteras mexicanas e hizo su aparición en diferentes eventos académicos de Estados Unidos. La tecnología de la pigmentación también llamó la atención de los empresarios, mismos que años más tarde desarrollaron esta tecnología a nivel industrial.

Palabras claves: Cempasuchil, *Tagetes erecta,* pigmentación, aves, Fundación Rockefeller

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EVALUACIÓN DE UNA VACUNA ORAL CONTRA INFLUENZA AVIAR Y *Campylobacter jejuni* EXPRESADA EN *Cicotiana benthamiana*

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Introducción

Actualmente, *Campylobacter jejuni* es una de las principales causas de contaminación bacteriana en los alimentos de origen avícola. (1) En pollo de engorda comercial, la infección es comensal, encontrando a *C. jejuni* en mayor concentración en mucosa de criptas del ciego y en menor grado en intestino delgado. (2, 3) En humanos la infección esta relacionada al consumo de productos avícolas contaminados, además de incluir otros productos de origen animal, como leche no pasteurizada y carne de bovinos. (4, 5) *Campylobacter spp* (*Campylobacter jejuni, C. coli y C. lari*) es microaerofilica y sensible a la exposición al aire, desecación, pH bajos y al almacenaje prolongado (6)

Actualmente la importancia de la infección con *C. jejuni* a aumentado debido a la aparición de cepas resistentes a antibióticos en pacientes humanos. (6) Aunado a que la infección en humanos la infección con C. jejuni se relaciona con enfermedades autoinmunes como el síndrome Guillian-Barré o artropatías. (7, 8) *Campylobacter spp* se propaga por ruta fecal-oral y coloniza la mucosa de criptas de ciego y mucosa del intestino delgado. Dosis menores a 40 unidades formadoras de colonias son capaces de colonizar a un pollito de 1 dia de edad, aunque la dosis infectante puede variar según la cepa de C. jejuni. La transmisión es principalmente horizontal, siendo las aves silvestres, animales de granja y otros posibles vectores su reservorio. (1) Una de las metas actuales es el desarrollo de estrategias contra la infección y contaminación con C. jejuni a través de la cadena de producción de los productos de origen avícola, evitando perdidas económicas y daño al consumidor. Uno de nuestros esfuerzos se enfoca en la expresión de antígenos de *C. jejuni* en sistemas tanto procariotes como eucariotes para su empleo como vacuna contra la colonización bacteriana.

Influenza aviar (IA) es conocida por ser una enfermedad sistémica que produce síndromes desde infecciones asintomáticas, signos respiratorios, baja de postura, hasta mortalidad cercana al 100 %. (9) El virus de IA es clasificado en la familia de los Orthomyxoviridae genero influenzavirus tipo A. (10, 11) IA es considerada un problema global y su prevención y control requiere de la cooperación internacional. (9) En la superficie del virus de IA se encuentran dos tipos de proyecciones glicoproteicas, trímeros con forma de vara denominados hemaglutininas (HA) y tetrámeros con forma de hongo denominados neuraminidasa (NA). (10) El genoma viral del virus de IA esta compuesto por ocho segmentos cadena sencilla, sentido negativo RNA, que codifica para diez proteínas, ocho proteínas constitutivas del virus (HA, NA, NP, M1, M2, PB1, PB2, y PA), y dos no constitutivas del virus (NS1 y NS2) observadas en el citoplasma de la célula infectada. (12) La unión del virus de IA se realiza por medio de las HA, además de que estas juegan un papel importante en la virus neutralización medida por anticuerpos. NA contribuye como receptor celular y en procesos enzimáticos que intervienen en la liberación del virus de estos receptores, así como también en la neutralización viral mediada por anticuerpos. (13) El virus de IA es sensible al calor, pH extremos, solventes orgánicos y detergentes. (14,15) IA es estable en el ambiente protegido por materia orgánica, puede ser almacenado a -70ºCy resistir la liofilización. (16) Los virus de Influenza (VI) se organizan en géneros o tipos, de acuerdo a reacciones serológicas de proteínas como NP o M1, (17) la clasificación resulta en tres tipos, A, B, y C, donde IA pertenece al tipo A, los tipos B y C no han sido aislados en pollos. (18) Una subclasificación del virus de IA basada en reacciones serológicas de HA y NA, describe 15 HA y 9 NA reconocidas, (17) la mayoría de las combinaciones de HA y NA se ha observado en los virus de IA aislados en pollos y aves salvajes. (18) La vacunación y medidas de bioseguridad son la principal estrategia para el control de IA. En el presente, las vacunas no pueden proteger contra muchos serotipos, y esto es debido a que los pollos son susceptibles a cualquier serotipo de los 15 HA conocidos. (19) Las variaciones antigénicas en HA y NA entre los serotipos son muy comunes, debido a la presión por vacunación y a la recombinación. (20) Actualmente, la investigación con IA ha guiado sus esfuerzos hacia el desarrollo de una vacuna basada en un pequeño ectodominio de la proteína matriz 2 de VI (M2) denominado M2e, el cual es una proteína de membrana (homotetrámero) que funciona como un canal de iones. Este M2e es una proteína de 23 aminoácidos, altamente conservada en todos los VI tipo A, este se encuentra presente en pocas cantidades en las partículas virales pero en abundancia en las células infectadas. (21, 22, 23) Investigaciones indican que mediante el uso de M2e, es posible reducir la mortalidad y signología clínica en infecciones de VI de alta patogenicidad (21, 22, 24, 25). La expresión de M2e en un sistema eucariota que permita su empleo como vacuna es el propósito de la presente investigación.

A diferencia de los virus con nucleocápsides de mayor complejidad como los pertenecientes a las familias *Poxviridae* y *Retroviridae*, la mayoría de los virus cuentan con proteínas estructurales con dos simetrías básicas, helicoidal o icosahédrica, dando forma de vara o esférica al virus respectivamente. (26) Estas proteínas poseen capacidad de organizarse por si solas en estructuras macromoleculares (cápsides) llamadas también partículas parecidas a virus o VLP por sus siglas en inglés, (26) las cuales se pueden encontrar, en un rango de tamaño de 22 a 150 nm, similares al tamaño observado en virus al ser expresadas de manera individual en diferentes organismos, (21, 22, 27-31) estas proteínas no son infectivas ya que carecen de un genoma viral y no representan ningún riesgo a diferencia de las vacunas atenuadas o virus inactivados. (17, 18, 21, 25, 26, 32) Debido a su naturaleza antigénica, así como por su tamaño, el cual en algunos casos, se encuentra cercano a 40 nm, corresponde a un tamaño optimo para la captación de antígenos por parte de las células dendríticas (33), los VLP's son una opción para la vacunación al servir como una plataforma para epitopos de agentes patógenos expresados en su superficie, en lugar de solo administrar ectodomínios solubles como vacunas, el éxito de estos como vacunas dependerá de un completo entendimiento de la tolerancia inmunológica y una respuesta inmune ante un estimulo. (21, 26, 32, 34-37) Actualmente trabajos demuestran que el empleo de VLP's como vacunas tanto en animales como en humanos, ya sea por vía oral o inyectada resultan en una respuesta inmune sistémica y/o en mucosas. (21,31, 32, 34-40) Otra aplicación de los VLP's es su empleo como transporte de moléculas terapéuticas como vectores virales empleados como terapia génica y en vacunas ADN, explotando el tropismo natural de estas partículas. (26) Dentro de los VLP's expresados en diversos sistemas se encuentran la proteína pequeña de envoltura del virus de hepatitis B (HBsAg), núcleo antigénico del virus de hepatitis B (HBcAg), proteína mayor de la capside L1 del virus del papiloma humano (HPV-L1), proteína mayor truncada de la capside hepatitis E (HEV), HA, NA y matriz proteica del virus de influenza, Núcleo E1-E2 (HCV) del virus de hepatitis C, cápside (VP0,1,3) de poliovirus, envoltura Pr55gag del virus de la inmunodeficiencia adquirida humana (VIH), glicoproteína (GP) y matriz (VP40) del virus del Ebola, cápside del virus Norwalk, VP2, VP6 y VP7 del rotavirus y el conformado por las proteínas S, E y M del SARS coronavirus. (32) Existen varios tipos de VLP's, en los cuales es posible mediante la combinación de las secuencias codificantes para estos epitopos (21, 26, 32, 34-37) o por su combinación química la creación de la VLP's quimeras. (26, 32) Los VLP' quimeras consisten de una plataforma proteica unida a epitopos de uno o varios virus, con el núcleo estructural del VLP, (41) la cual puede presenta proteínas de patógenos, epitopos específicos de células T, proteínas de anclaje y entrada de virus a células permisibles, proteínas de las células hospederas, (42) como el caso del virus del VIH, el cual presenta moléculas como CD55, CD59 y CD46 que le confieren la capacidad de evadir lisis por el sistema del complemento. (43) Entre los virus quimera reportados se encuentran el HBcAg,

Woodchuck núcleo proteico hepatitis B (WHBc), HBsAg, HPV-L1, papilomavirus bovino (BPV), Yeast Ty, VIH, virus de inmunodeficiencia de simio-VIH (SHIV-gag), hepatitis B de patos (DHBV) y HEV. (32) En algunos casos es posible el empleo de VLP's en combinación con adyuvantes como los oligodesoxinucleotidos cortos con motivos de CpG, (CpG's DNA) para la estimulación de las células dendríticas (DC), por medio de los receptores Toll-like 9 (26, 44). Los CpG's DNA se encuentran en ADN bacteriano y consisten de un dinucleotido CpG no metilado central flanqueado por dos 5' purinas y dos 3' pirimidinas. Estos CpG's DNA han demostrado ser un adyuvante útil en combinación con antígenos proteicos, aumentando una respuesta inmune humoral y celular ante su aplicación, (45) ya que de manera directa activan linfocitos B (LB), células NK (NK), macrófagos y células dendríticas y aumentan la producción de citocinas como IFN γ, interleucina-6 (IL-6), IL-12 y factor de necrosis tumoral α. (45) Otros adyuvantes orales como toxinas bacterianas, pueden ser empleadas, ya que se conoce que promueven la generación de células inmunes de memoria sistémicas y en tejido linfoide asociado a mucosas. (31,46) La expresión de VLP's han sido reportado en diferentes sistemas heterólogos procariotes y eucariotes como E.coli, S. thyphimurium, S. cereviseae, S. pombe, P. pastoris, S. frugiperda (Sf-9), T.ni (Hi-5), X. laevis, y células de mamífero COS-1, CHO, HepG2, HeLa, BHK, y plantas como tabaco, tomate, papa, maíz y lechuga. (26, 32) Debido a su fácil producción y costo, el empleo de levaduras se ha popularizado para la expresión de moléculas útiles para la industria, a pesar de su limitada capacidad de glucosilacion, en el caso de Escherichia coli la falta de glucosilación limita su uso en algunos casos. Los sistemas de cultivos celulares de mamífero, a pesar de permitir las modificaciones apropiadas y un correcto ensamblado de las proteínas, al ser sistemas menos manejables y de mayor costo, su empleo no es una opción a gran escala. (32)

Desde hace aproximadamente 10 años, la ingeniería genética ha permitido el uso de las plantas como biofábricas o biorreactores mediante el empleo de técnicas con el objetivo de generar plantas con características como la resistencia a insectos, virus, hongos y herbicidas; así como la expresión de proteínas de valor comercial para la industria como anticuerpos, biofarmacéuticos y vacunas. (26, 31). El uso de la genética aplicada a plantas es cada vez mas extendido debido a las ventajas potenciales para generar compuestos. (26, 30) En primer lugar, los sistemas vegetales son más económicos que la infraestructura industrial que se basa en el uso de sistemas de fermentación o en biorreactores. En segundo lugar, ya está disponible la tecnología para cosechar y procesar plantas y sus productos a escala industrial. En tercer lugar, el requisito de la purificación del compuesto puede ser eliminado cuando el tejido de la planta que contiene la proteína recombinante se utiliza como alimento (como en el caso de las vacunas comestibles). En cuarto lugar, se puede dirigir a las proteínas recombinantes a determinados compartimientos intracelulares, o expresarlos directamente

en esos compartimientos (como por ejemplo el cloroplasto). En quinto lugar, se puede producir la proteína recombinante en plantas a escala industrial. Finalmente, los riesgos a la salud que se presentan por posible contaminación del producto recombinante con patógenos humanos son mínimos. Inclusive el cruce de líneas de plantas transgénicas permite la expresión de múltiples proteínas en la misma planta. (26) Hay dos áreas en donde esta tecnología está teniendo un impacto importante, en la producción de anticuerpos y en la producción de compuestos biofarmaceúticos incluyendo antígenos. (30, 31, 47)

Si uno asume que se requiere de mas de diez miligramos de una vacuna de subunidad normal para su administración oral, (1,000 veces mas de lo requerido en una vía inyectada), se podría considerar que se requerirían cientos de gramos de una planta para lograr una respuesta inmune, sin embargo es posible aumentar esta concentración procesando el materia vegetal, así como tecnologías que permitirán aumentar la expresión de estas proteínas en las plantas. La expresión de antígenos en plantas, permite a su vez obtenerlo encapsulado de manera natural en el tejido de la planta, este previene la degradación del antígeno al ser administrado de manera oral, permitiendo que este sea liberado gradualmente en el tracto digestivo. Esta protección hace que también que se reduzca la cantidad de proteína requerida para su administración oral.

La expresión en plantas, tiene como otro beneficio, el almacenamiento de estos antígenos en las plantas por un tiempo prolongado, sin daño a estas proteínas, como en semillas, (30) lo cual elimina gastos de almacenamiento en refrigeración.

Actualmente se reportan varios métodos para la producción de proteínas exógenos en plantas, transformación del genoma nuclear, transformación del cloroplasto, e infección viral transitoria, de los cuales solo transformación nuclear y sistemas virales han sido empleados para la expresión de VLPs, no así con transformación de cloroplastos. (26, 31)

La transferencia de ADN, dependa de lo que se busca mejorar en la planta, así como la especie a la cual pertenece está. (26) Los métodos directos van de la transfección del genoma de organelos, por electroporación, métodos químicos, micropuntura de láser de células aisladas. Uno de los mas comunes es el bombardeo de partículas o biobalística, empleada en plantas en las que no es posible el empleo de *Agrobacterium tumefaciens* como método de transferencia de ADN. En este método, pequeñas partículas de oro, tungsteno o platino son recubiertas con ADN desnudo y después son disparadas por pistolas génicas en el tejido. (26)

En el caso de *A. tumefaciens*, un método indirecto, se emplea la capacidad de esta bacteria para transferir ADN exógeno a las células vegetales. (48) Las plantas que de manera natural son afectadas por *A. tumefaciens* incluyen dicotiledonarias, gymnoespermas y algunas monocotidelonarias (26). *Agrobacterium* es una bacteria presente en la tierra, que al detectar una planta con una herida, se desplaza guiada por las azucares y los compuestos fenólicos exudados por la herida, se adhiere y comienza a transforma las células de la planta al inducirse la transcripción de genes de virulencia presentes en un plásmido especifico denominado Tumor-inductor (Ti-ADN) plásmido, el cual contiene el ADN de transferencia (T-ADN) que junto con proteínas bacterianas (VirD1, VirD2, VirE2) inducen la transcripción, procesamiento de T-ADN, su exportación e integración al genoma de la planta. El T-ADN codifica para una hormona de planta que induce la producción de enzimas que estimulan el crecimiento de tumores y enzimas metabólicas de sustratos específicos para *Agrobacterium*. Como resultado se genera un microambiente ideal para esta bacteria. (48) Para la transferencia de ADN con *Agrobaterium*, se requiere que el gen de interés sea insertado entre los bordes naturales del T-ADN presente en el plásmido original de la bacteria ó Ti-ADN, El cual se delimita por dos bordes con dos secuencias de guía imperfectas de 25 pares de bases de longitud y son estas *cis*-elementos requeridos por el T-ADN de la bacteria para su inserción al genoma del núcleo de las células de las plantas. (26, 49) La inserción aleatoria observada en este método, sugiere un mecanismo de recombinación no-homologa, el cual consiste de la reparación de doble hebra de ADN roto independiente de la secuencia, el cual aparenta ser el medio de inserción del T-ADN en los sitios de reparación del ADN genómico. (48) Desde los primeros experimentos para la elaboración de plantas transgénicas mediante el empleo de *Agrobacterium* en 1983, (26) se han logrado grandes avances en el entendimiento del proceso de inserción del T-AND.

La transformación del genoma de organelos, como el cloroplasto, ofrece ventajas para la expresión de antígenos, incluyendo alta expresión debido a la gran cantidad de cloroplastos presentes en las hojas de las plantas y la gran cantidad de copias de genoma de cloroplastos presente en ellos, disminuye el silenciamiento de los genes y reduce la contaminación de las poblaciones de plantas locales, al no estar presente el ADN exógeno en el polen de las plantas. (30, 31) La transformación del cloroplasto se encuentra limitada a pocas especies, de las cuales la más estudiada es el tabaco. El empleo de estas plantas no es recomendado para la administración oral, otra desventaja es que la expresión en estos organelos no permite la glucosilación de las proteínas, lo cual su empleo puede ser enfocado para proteínas bacterianas o proteínas eucarioticas no-glucosiladas. (30)

El empleo de virus activos de plantas provee una alternativa para la transformación para la expresión de proteínas recombinantes en plantas. En este caso, el antígeno es expresado por un genoma de virus de plantas modificado, y el genoma de las plantas productoras no es afectado. El virus modificado es inoculado en el tejido de las plantas, típicamente hojas, infectando a las células de la planta y permitiendo la expresión de las proteínas recombinantes en el citoplasma de las células vegetales, por lo que al igual que la expresión en cloroplastos, estas proteínas no son glucosiladas (30) El empleo de estos tiene ventajas como la producción de proteína recombinante en un tiempo de 3 a 14 días pos-infección, según el tipo de sistema usado. (26) Su empleo permite la fácil producción de una gran cantidad de diferentes construcciones para probar, aunado a que es fácil su inoculación, permitiendo su empleo en gran escala. (30)

Es importante considerar la apropiada glucosilación proteica, una correcta conformación y ensamblaje así como optimización de codones para la selección de un sistema de expresión adecuado a lo requerido. (32) En el caso de plantas, para alcanzar altos niveles de expresión se consideran varias estrategias, como la optimización de genes, promotores fuertes para un tejido especifico, secuencias lideres no traducibles y 3' fuertes, señalización sub-celular, fusión a transportadores estables, entrecruzamiento de plantas obtener homozigosis, entrecruzamiento de líneas transgénicas con alta expresión, así como transformación de plásmidos, cabe destacar que en la expresión dentro de cloroplastos, no permite la glucosilacion de las proteínas expresadas, (30) al igual que en otros sistemas procariotes.

Uno de los VLP's con los que se han experimentado y tenido éxito es HBcAg, el cual ha sido probado como VLP's quimera y ha sido utilizada como vacuna oral. (34, 26). Estos trabajos han demostrado que su empleo es capaz de inducir una respuesta inmune al ser administrado por separado por vía oral e intraperitoneal ante los virus contra los cuales se expresan sus epitopos, entre ellos M2e del virus de influenza expresado en HBcAg. (21, 25) Se reporta la expresión de HBcAg en plantas (34) con un nivel de expresión en un rango de 10 a 50 µg/g de hoja liofilizada, y de 24 µg/g a 2 mg/g de hoja fresca. (26)

Diversos experimentos señalan que la posición en la cual son insertados los epitopos dentro de la secuencia de HBcAg, determina la inmunogenicidad del VLP. (36, 50) La posición central de los epitopos reduce la respuesta contra el HBcAg y a la vez permite una mejor respuesta a los epitopos foráneos insertados en el VLP. (50) La fusión del epitopo al amino terminal del HBcAg, a través de una secuencia de unión con HBcAg, resulta en una respuesta alta contra el epitopo insertado sin alterar la inmunogenicidad propia del VLP, mientras que la fusión al carboxilo terminal de HBcAg,

permite al epitopo ser accesible a la superficie, pero no resulta en una inmunogenicidad tan alta como la observada en las otras dos posiciones. (35) La inserción en la parte interna entre el 75 aa's y 81 aa's, resulta en una mayor inmunogenicidad y antigenicidad contra el epitopo insertado. (35, 50) Se reporta que dentro de la parte interna de la secuencia de HBcAg, es posible acomodar secuencias de hasta 45 aa's. La fusión con los C–terminal o N –terminal de HBcAg permite la unión de secuencias mayores, según reportes de 90, 144 y hasta 720 aa's, (36) sin embargo no se alcanzan los mismos niveles inmunogénicos y antigénicos como se observa en la parte interna de HBcAg. (35, 50) No solo se debe de considerar la capacidad de inserción de HBcAg para su empleo, si no también la secuencia primaria del epitopo insertado, ya que se reporta que insertando dos secuencias pequeñas en HBcAg, una de ellas aparentemente desestabiliza el VLP y resulta en una degradación proteolítica de HBcAg. (35)

El virus de hepatitis B expresa dos polipéptidos en el gen codificante para su núcleo. Al iniciar la traducción en el primer codón de iniciación, el resultado es una proteína denomina HBeAg, en el segundo codón de iniciación se codifica una proteína de 183 aa's de un peso de 21 kDa, con un tamaño de 30 a 32 nm, (35) el cual corresponde a HBcAg. (50) HBeAg y HBcAg se encuentran ligados en su estructura primaria. (51) La elevada inmunogenicidad de HBcAg se debe probablemente a su naturaleza polimerica, a la presencia en su estructura primaria de potentes epitopos para linfocitos T Helper (LT-h) y a la habilidad de activación directa a linfocitos B (LB). (51) Los monómeros de HBc, conforman dos tamaños de VLPs, integrados por 180 ó 240 subunidades con un arreglo T=3 o T= 4 simetria icosahedrica. Su expresión se reporta en tanto en sistemas procariotes como en sistemas eucariotes. (51)

Material y métodos

En el presente trabajo se planto expresar el VLP quimera HBcAg en un sistema eucariote, *Nicotiana benthamiana*. La transformación de las plantas se logro mediante la técnica de agroinfiltración para la expresión transitoria del HBcAg en hojas de plantas, mediante el empleo de un vector binario (49) pHBV33 proporcionado por el CINVESTAV Irapuato, al cual se le inserto la secuencia codificante para M2e, CD154 (CD40) y 0113 proporcionados por la universidad de Arkansas. El sitio de inserción fue en la parte interna del vector binario mediante enzimas de restricción. Para la expresión de estas proteínas se decidió hacer las siguientes combinaciones: Plasmido 1 conteniendo M2e, plásmidos 2 conteniendo M2e + CD 154 y plasmido 3 conteniendo M2e + 0113. En el caso de dos epitopos combinados, se empleo una secuencia de separación entre las dos secuencias a ser expresadas en la superficie de HBcAg. Se empleo la cepa *E. coli* cepa DH5α para la clonación del vector binario y para la transformación de las plantas se empleo la cepa *A. tumefaciens* LB4404. Las

plantas se agroinfiltraron con una solución conteniendo *A. tumefaciens* técnica de acuerdo a Huang et. Las densidades opticas variaron de 0.02 a 0.04. Las hojas fueron colectadas a los 2 y 3 días pos-inoculación y posteriormente fueron liofilizadas. Las muestras fueron pesadas y diluidas en buffer ELISA en una relación de 1/10. Se realizo la prueba de ELISA con un control positivo M2e con una concentración de 500 µg/ml.

Resultados preliminares

Los resultados preliminares al realizar pruebas de ELISA demostraron la presencia del epitopo M2e expresado en la superficie de las tres quimeras diseñadas con VLP HBcAg.

Discusión y conclusiones

Los resultados indican que la expresión de los plásmidos 1, 2 y 3, fue elevada en los tres casos según los resultados preliminares de ELISA.

Se concluye que es posible la realización de pruebas en aves para la evaluación de la inmunogenicidad de los VLP's HBcAg expresados en *Nicotiana benthamiana*.

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EVALUACIÓN DE UN DESINFECTANTE COCCIDICIDA SOBRE LA VIABILIDAD DE OOQUISTES DE *Eimeria* SPP Y LOS PARÁMETROS PRODUCTIVOS EN POLLO DE ENGORDA

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RESUMEN

La coccidiosis aviar es la parasitosis mas común y de mayor costo para la industria avícola a nivel mundial. Las pérdidas económicas que ocasiona se relacionan principalmente a su prevención y tratamiento; así como, a la mala absorción, menor pigmentación cutánea, baja productividad y mayor mortalidad. Las condiciones propias de la producción avícola intensiva, aunadas a una gran resistencia de la *Eimeria* al medio ambiente y a los desinfectantes comunes han favorecido su presentación y permanencia en las granjas avícolas. Debido a lo anterior, han surgido nuevos productos desinfectantes y con ello, la necesidad de conocer su desempeño tanto *in vitro* como en campo. El objetivo de este trabajo fue evaluar el efecto de dos desinfectantes (uno general y otro específico) sobre la viabilidad de *Eimeria* spp *in vitro* y en los parámetros productivos de pollo de engorda. Los resultados obtenidos *in vitro* mostraron daño en la pared y un menor número de ooquistes en el grupo tratado con el desinfectante específico ($P<0.05$). Las aves criadas en la sección tratada con este mismo desinfectante no mostraron diferencia ($P>0.05$) con respecto al desinfectante general en la severidad de lesiones macroscópicas intestinales, conteos de ooquistes por gramo de heces, peso corporal, conversión alimenticia y consumo de alimento, excepto en la pigmentación cutánea ($p<0.05$). Lo anterior muestra una acción eficaz del producto sobre *Eimeria* spp, que se relaciona a un menor desafío, y por lo tanto, a una mejor salud intestinal y capacidad de absorción de pigmento.

Palabras clave: Desinfectantes, *Eimeria,* pigmentación cutánea, pollo de engorda.

INTRODUCCIÒN

La coccidiosis aviar es una enfermedad parasitaria común entre las especies domesticas, que ocasiona grandes gastos a nivel nacional e internacional, siendo esta enfermedad de las principales causas de productividad y pigmentación deficiente, actualmente se sabe que los desinfectantes comunes no llegan a afectar a los ooquistes, de allí el surgimiento de nuevos desinfectantes más potentes y específicos contra coccidias y la necesidad de evaluar su desempeño tanto *in vitro* como en campo.

MATERIAL Y METODOS

Prueba in vitro:

Este experimento se llevó a cabo en el área de parasitología del laboratorio de diagnóstico del Departamento de Producción Animal: Aves (DPA: Aves), de la FMVZ de la UNAM. México. D.F.

***Eimeria* spp**. Se utilizaron cepas vacunales de las especies *Eimeria tenella* (24%), *E. maxima* (19%), *E. mivati* (17%), and *E. acervulina* (40%) (Coccivac B, Shering-Ploug S.A. de C.V México). El inóculo se tituló en cámara de Neubauer.

Desinfectantes. Se usaron dos productos desinfectantes. El desinfectante especifico contra *Eimeria* spp. que se compone principalmente de o-cresol, 4-choloro-alpha-phenyl, propan-1-ol; clorocresol; acido salicílico; y sal sódica de alcano sulfonato, mientras que el producto que servirá como comparativo será un desinfectante elaborado a base de ácidos orgánicos y ácido ascórbico, además se ocupará agua destilada para el grupo testigo.

Diseño experimental: Se colocaron 3 grupos de 6 cajas de Petry o réplicas cada uno, en cada una se depositaron 1.6 ml con 488,000 ooquistes esporulados por ml y un volumen controlado de 1.62ml de desinfectante, de acuerdo al área de superficie por caja de Petry.

Los tratamientos fueron organizados de la siguiente manera:

Grupo A: inóculo de *Eimeria* + desinfectante específico contra coccidias.

Grupo B: inóculo de *Eimeria* + desinfectante a base de ácidos orgánicos.

Grupo C: inóculo de *Eimeria* + agua destilada estéril

Se dejó actuar cada producto durante 4 horas, y posteriormente se realizó el conteo de los ooquistes con la cámara de Neubauer, cuantificando únicamente las coccidias con la pared integra. Los conteos se realizaron para detectar diferencias entre grupos tratados. El grupo no tratado con desinfectantes se incluyó para descartar que la disminución se pudiera deber a que las muestras se mantuvieron a temperatura ambiente por 4 horas antes de su conteo.

Prueba *in vivo*

Se realizó en el Centro de Enseñanza, Investigación y Extensión en Producción Avícola (C.E.I.E.P.Av.) de la FMVZ de la UNAM, el cual se localiza en la calle de Salvador Díaz Mirón No 89 en la Colonia Santiago Zapotitlán de la Delegación Tláhuac, Distrito Federal a una altura de 2250 msnm. Las aves fueron colocadas en corrales dispuestos en una caseta experimental dividida en dos secciones.

Aves. Se utilizaron 480 pollitos Ross de 1 día de edad con un peso promedio de 49 ± 0.6g, mitad hembras y mitad machos, provenientes de una planta incubadora comercial. A su llegada, los pollitos fueron asignados aleatoriamente a ambos tratamientos y fueron criadas de manera comercial, bajo sistemas convencionales de manejo. Los requerimientos nutricionales fueron

cubiertos de acuerdo con los requerimientos de la estirpe y se les proporcionó agua y alimento a libre acceso durante toda la prueba.

Diseño experimental: Se realizó la limpieza de todas las instalaciones y equipo de rutina y posteriormente administraron el inóculo de *Eimeria* por medio de un aspersor con gota gruesa en todos los corrales. Al término de esto, se asignó a una sección el tratamiento con el producto "a probar" y a la otra la desinfección con el producto general. Estos productos también se utilizaron en los tapetes sanitarios respectivos de cada sección durante el tiempo que duró la prueba. Ambas secciones se cerraron por 3 días para que actuara el desinfectante

Desinfectantes. El desinfectante especifico contra *Eimeria* spp. que se compone principalmente de o-cresol, 4-choloro-alpha-phenyl, propan-1-ol; clorocresol; acido salicílico; y sal sódica de alcano sulfonato, mientras que el producto que servirá como comparativo será un desinfectante elaborado a base de ácidos orgánicos y ácido ascórbico que se utilizó 10 veces mas concentrado a lo recomendado para su uso en las instalaciones en la etiqueta del producto.

***Eimeria* spp**. Se utilizaron cepas vacunales de las especies *Eimeria tenella* (24%), *E. maxima* (19%), *E. mivati* (17%), and *E. acervulina* (40%) (Coccivac B, Shering-Ploug S.A. de C.V México). El inóculo se tituló en cámara de Neubauer y 3,840,000 ooquistes esporulados fueron administrados por cada corral en ambos tratamientos.

Conteo de ooquistes. Se realizó el conteo de ooquistes de acuerdo con la técnica de McMaster a partir de muestras de heces (Long y Rowell 1958), que fueron semanalmente colectadas y conservadas con dicromato de potasio al 2.5% para su análisis cuantitativo posterior (12 horas después).

Severidad de lesiones macroscópicas en intestino. Se realizaron cada semana inmediatamente después de sacrificar por dislocación cervical 1 ave por réplica de cada tratamiento. Las lesiones fueron evaluadas de acuerdo a la escala de Johnson y Reid (1970), en una escala de 0 a 4+, donde 0 corresponde a la ausencia de lesiones y 4+ al grado más severo o la muerte del ave.

Pigmento cutáneo. La deposición de xantofilas amarillas en la piel fue evaluada al día 49 de edad de 20 pollos por grupo, a partir de la zona apterita pectoral derecha con un colorímetro de reflectancia CR-400 (Minolta Co. Osaka Japan).

Análisis estadístico. Los valores de pigmento cutáneo y el número de ooquistes en heces se evaluaron conforme al diseño experimental empleado y la comparación entre las medias se realizó con la prueba de T de student. Para la comparación de las lesiones se utilizó la prueba de Kruskall - Wallis y las diferencias existentes entre medianas de cada grupo se analizaron con la prueba U de Mann-Whitney. Para todas las pruebas se fijó un nivel de significancia $P<0.05$.

RESULTADOS

En relación al conteo de ooquistes viables a las 4 horas postratamiento el grupo A (desinfectante específico) presentó en promedio un número estadísticamente menor ($P<0.05$) de ooquistes/ml (240,000 ± 20,428 DS) en comparación con el resto de los dos grupos. Aunque el grupo B también mostró una reducción en el número de ooquistes/ml (342,400 ± 21,395 DS), esta fue solo diferente con respecto al grupo C (473,600 ± 27,897 DS). Cabe señalar que en el grupo tratado con el desinfectante coccidicida se observaron con mayor frecuencia esporoblastos o esporozoitos libres, ooquistes con la membrana dañada (Figura 1) y granulaciones o precipitados alrededor de la membrana externa que en el grupo B o C (Figura 2).

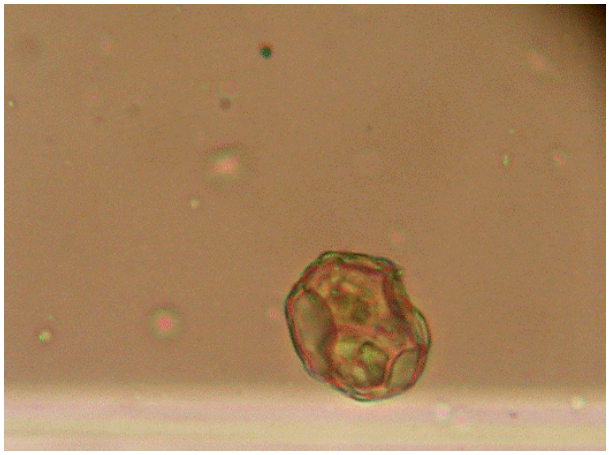

Figura 1. Ooquiste con la pared dañada en el grupo tratado con el desinfectante coccidicida.

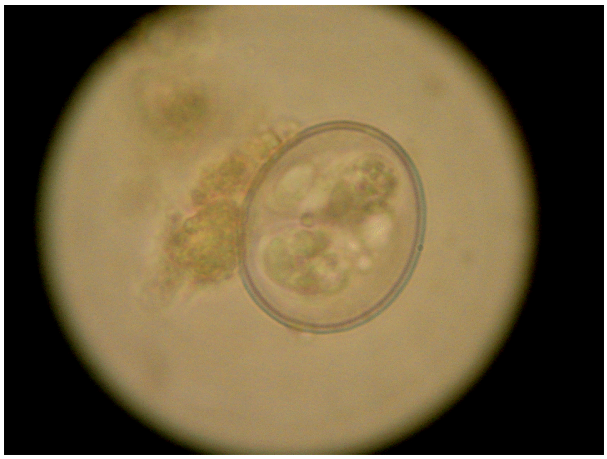

Figura 2: Ooquiste normal en el grupo testigo.

Con respecto a la prueba *in vivo* solo se encontró diferencia entre ambos grupos en el grado de pigmentación en piel, siendo superior ($P<0.05$) en el grupo criado en la sección tratada con el desinfectante especifico con valores de 27.69 ± 4.89^{a} y 23.56 ± 3.42^{b} respectivamente (Cuadro 1).

Cuadro 1
Variables productivas a los 49 días de edad en pollos de engorda criados en casetas tratadas con dos desinfectantes

	Desinfectante específico (Media ± desviación estándar)	Desinfectante general (Media ± desviación estándar)
Ganancia de peso (g)	2,929±72.9[a]	2,911±67.8[a]
Consumo de alimento (g)	5,431±191.5[a]	5,482±174.7[a]
Conversión alimenticia (kg:kg)	1.854±1[a]	1.884±1[a]
Mortalidad %	10.5±5.7[a]	10.2±4.3[a]
Pigmentación de la piel	27.69±4.89[a]	23.56±3.42[b]

[ab] Valores con diferente literal dentro de la misma fila son estadísticamente distintos (P<0.05)

DISCUSIÓN y CONCLUSIONES

Lo anterior sugiere una acción eficiente del producto sobre *Eimeria* spp *in vitro* que concuerda con un menor desafío y por lo tanto a una mejor integridad intestinal y capacidad de absorción de pigmento. La diferencia entre el grupo b y el testigo en la prueba *in vitro* pudo deberse a que el desinfectante elaborado a base de ácidos orgánicos y ácido ascórbico se utilizó 10 veces mas concentrado de lo recomendado para su uso en las instalaciones en la etiqueta del producto. Aunque no se observaron diferencias entre el resto de las variables posiblemente debido al desafío moderado, la diferencia en pigmento es significativa debido a que la buena pigmentación es muy apreciada y juega un papel muy importante en la comercialización del producto final.

Con base en los resultados obtenidos bajo las condiciones experimentales empleadas se concluye que el desinfectante coccidicida mostró un efecto negativo en la cantidad total de ooquistes, lo cual se relacionó con un mayor daño de la pared; así como que el uso del desinfectante coccidicida en las instalaciones previo a la crianza y en el tapete sanitario durante toda la prueba mejoró la pigmentación cutánea del pollo de engorda de manera significativa.

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LA HOMEOPATÍA; EN UNA EXPERIENCIA EN AVES DE ABASTO

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Resumen
La Homeopatía es una medicina desarrollada para los seres humanos, sin embargo las posibilidades que tiene hacia el paciente, al conseguir la curación de los enfermos sin producir efectos indeseables. Ha hecho que desde el inicio de está se halla buscado por parte de los veterinarios la manera de aplicar esta corriente medica en los animales, existiendo al presente documentos Veterinarios Homeopáticos que refieren la terapéutica correspondiente a la clínica de los mamíferos, en la cual la individualidad clínica puede ser hasta cierto grado respetada al prescribir el tratamiento homeopático.

La avicultura al ser una actividad de poblaciones plantea el problema: ¿cómo respetar la individualidad clínica o personalización terapéutica en un lote?

La prueba llevada a cabo en un lote de aves de abasto con el remedio *Pulsatilla* a la 6 y 30x expuso la incapacidad de las aves a mostrar signos específicos a este, sin embargo puso de manifiesto la capacidad de los remedios homeopáticos para mejorar el funcionamiento del organismo de los pollos de abasto y de esta manera mejorar la producción de ellas, sin correr el riesgo de dejar residuos tóxicos, lográndose esto con un costo inferior a cualquier aditivo en uso.

Introducción

La Homeopatía es una corriente medica creada explícitamente para el humano por el Dr. Samuel Hanhemann, siendo esta la razón de poseer características tan precisas en su método, siendo estas características inherentes al nivel de desarrollo evolutivo propio del hombre.

No obstante esto, desde el origen de la Homeopatía, se ha buscado la manera para soslayar las limitantes evolutivas existentes entre los animales y el hombre, y permitir la aplicación de este método medico a estos. Ya que la Homeopatía ha mostrado ventajas que son difíciles de soslayar, tanto para las circunstancias que caracterizaron el desarrollo económico de la sociedad humana en un pasado, no tan lejano y mucho más importante en el escenario presente.

Siendo algunas de estas ventajas: el costo, el nulo efecto contaminante y ser una corriente holìstica.

O sea el costo de adquisición de los medicamentos, la designación específica de estos en esta corriente es REMEDIO (1), es menor a los Alopáticos.

- La contaminación, que puedan generar las substancias que se administra e ingieren los animales, en el presente son de fundamental importancia, ya que en la actualidad se sabe del riesgo que los residuos medicamentosos significan para la salud humana, al permanecer tanto en el organismo

animal y sus productos, pudiendo pasar por la ingesta de los mismos, al organismo humano. La otra posibilidad de este riesgo se daría en relación a la contaminación que puede darse a nivel ambiental, esto por medio de los desechos animales.

La ventaje en razón de este factor que tiene la Homeopatía, es que en este método el efecto terapéutico esta en razón al valor energético o sea la potencia (ver adelante) de los remedios y no en la proporción del contenido material del sustrato medicamentoso (2).

- El elemento holistico, esta dado en el hecho de que para esta corriente medica, los signos y síntomas que manifiesta el enfermo así como su historia. Son parte de un todo llamado enfermedad (3).

Antecedentes de la Homeopatía (4).

Esta fue desarrollada por el Dr. Cristiano Federico Samuel Hahnemann, medico alemán (1755-1842), el cual a partir de su propia experiencia y la de colegas que lo antecedieron, así como la de contemporáneos. Concluyo en la inutilidad del método reconocido y usado en su época. Y gracias al hecho de haber tenido contacto con el documento escrito por el Dr. Cullier químico, se dio cuenta de que la *Quina,* sustancia estudiada por el Dr. Culler se correspondía en el cuadro clínico por él referido, con el que el Dr. Hahnemann había observado en pacientes que sufrían de "fiebres recurrentes".

A partir de este conocimiento, Hahnemann inicia el estudio de numerosas substancias que en esa época eran parte de los recursos médicos, lo primero que hizo fue administrarlas a individuos sanos, bajo un estricto control. A este procedimiento le llamo "Experimentación Pura", siendo él uno de los primeros experimentadores, este procedimiento le permitió conocer los signos y síntomas específicos de la sustancia ha prueba.

Ya para el año de 1796, se encuentro en posesión de una cantidad importante de información teórica y practica, que le permite publicar el ensayo "Sobre un nuevo principio de descubrir las virtudes de las substancias medicinales", en 1810 el Organón de la Medicina Racional, documento de presentación de la corriente medica que él designo como HOMEOPATIA, a este documento le siguieron numerosos títulos, resultado del trabajo que el mencionado medico desarrollo hasta su muerte. Los cuales han servido a sus seguidores para continuar con la investigación homeopática, más aun para buscar la explicación de las interrogantes científicas, que desde sus inicios la Homeopatía genero.

Hahnemann y la salud animal (5, 6).

La interpretación que prevalecía en el mundo occidental, con respecto a las causas de las enfermedades que afectaban a los animales, era las mismas que se daban en el caso de los humanos, por lo cual se les administraban los mismos tratamientos ejemplo de esto eran los enemas, sangrías, vomitivos, laxantes, dietaciòn extrema etc. teniendo los mismos resultados o sea la muy difícil recuperación del paciente y con frecuencia la muerte del mismo.

Hahnemann se intereso también por la salud de los animales y en particular, por la de los caballos, hay que recordar que en su época el medio de locomoción humana, era provisto por los equinos. Y así en el año de 1796 declara lo siguiente "si las leyes de la medicina que yo reconozco y proclamo

son ciertas y naturales, ellas deben poder ser aplicadas en los animales también como en el hombre", sin embargo él mismo desestima a la experimentación pura en los animales, bajo las especificaciones establecidas para el ser humano. Recomendando que cuando se llevara a cabo este procedimiento en animales, se extremara el recurso de observación, a fin de detectar el más mínimo cambio en la actitud del animal experimentador.

Particularidades de la Homeopatía (5, 6,7 y 8).

Los conceptos que ha continuación se exponen no son los únicos en soportar el Método Homeopático, sin embargo a consideración del autor del presente documento, son los indispensables para hacer comprensible a un lector no adentrado en esta corriente, el presente trabajo, siendo estos conceptos:

- La Ley de los Semejantes (*Similla Simillubus Curentur*)
- Experimentación Pura.
- Potencia y dinamodilución.

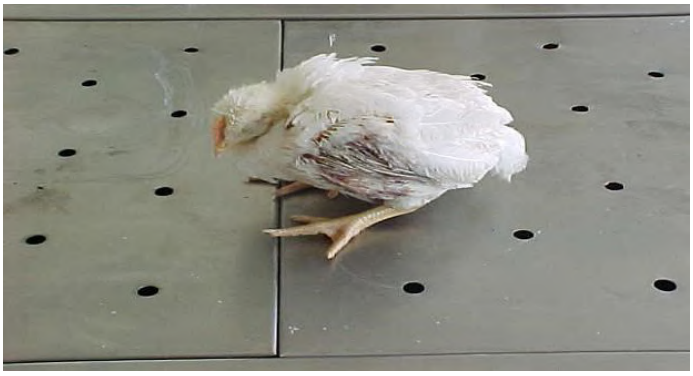
La teoría del Semejante, fue expuesta por primera vez por Hipócrates y ya como concepto Homeopático quiere decir: "la enfermedad solo puede ser curada en forma natural con el medicamento que durante la experimentación en el hombre sano, produce alteraciones o síntomas semejantes a los de la enfermedad", entendiéndose por curar, no solo la supresión de los signos y síntomas.

Experimentación Pura es el método mediante el cual se realiza la indagación controlada en individuos lo más sanos posible, sobre los efectos que estos manifiestan por la administración de una droga o sustancia en su estado más simple o puro, de las experimentaciones se desprendo signos y síntomas propios a cada remedio para ser recapitulados en las Materias Medicas Homeopática.

La potencia o dinamodilución se refiere al grado de dilución del medicamento, abarca desde la Tintura madre hasta las potencias más altas y su uso esta determinado por el grado de semejanza entre e1 medicamento experimentado (remedio) y los síntomas que presenta el enfermo, "es el grado de energía que alcanza una sustancia por medio de la dinamizaciòn"

Comprobación de la *Pulsatilla* en aves de abasto (8,9,10 y 11).

El trabajo que se documenta, se realizo en la Escuela de Medicina Veterinaria y Zootecnia de la Universidad Autónoma "Benito Juárez" de Oaxaca y consistió en administrar a una parvada de 45 aves de abasto el remedio *Pulsatilla*, con el propósito de comprobar la semejanza entre el cuadro clínico de Enfermedad Crónica Respiratoria Complicada (ECRC), que presentan las aves a causa de la infección por el *Micoplasma gallisepticum* principalmente y el efecto complicante dado por bacterias del genero *Echericha coli (E.coli)* y otras.



La parvada fue encasetada en la nave de la

EMVZ, esta es del tipo de Nave de Ambiente Natural (NAN), se les sometió al manejo establecido para este tipo de aves y naves y el seguimiento se llevo a cabo sobre dos vertientes; la primera la evidencia de signos inherentes al efecto EXPERIMENTADOR del remedio Homeopático (12 y 13).

Condiciones de la primera vertiente de la investigación (12 y 13).

Los grupos de estudio se establecieron a las 4 semanas de vida de la parvada, siendo 3 con un número de 15 integrantes pareados;

Primer grupo recibió *Pulsatilla* a la 6x (sexta potencia).
Segundo grupo recibió *Pulsatilla* a 30x (treinta potencia).
Tercero el control.

La administración del medicamento se inicio a la 4ª semana de edad y se prolongo hasta la 5ª semana de edad, la frecuencia de administración fue de 2 gotas (gotero estándar de uso homeopático) tres veces al día, directo al pico.

Condiciones de la segunda vertiente de la investigación (14).
Estas se obtuvieron en razón del seguimiento Zootécnico, el cual se soporta en parámetros establecidos, que son:

- Precocidad 53 – 56 días.
- Mortalidad 2%.
- I.C./parvada 2.0
- I.C./anualizado 1.95

La Hipótesis establecidas fue "La *Pulsatilla* es capas de desarrollar en pollos sanos de 4 semanas de edad el cuadro correspondiente a la Enfermedad Crónica Respiratoria Complicada".

La parvada se obtuvo de la Empresa Incuoax, cuya planta de incubación se encuentra establecida en Xoxocotlan, Oaxaca, el pollito 50 en la parvada inicial, pertenece a la Línea 500 Broiler Performance (15) , cuyo peso inicial promedio fue de 40 gr. El manejo desde la recepción, como ya se dijo fue el establecido para las explotaciones comerciales y fue el mismo hasta la conclusión de la 3ª semana de edad, el alimento fue de tipo industrializado y su administración se limito a la restricción preventiva al Síndrome Ascítico (SA) (16), el agua provino del pozo de la Escuela, el calor fue proporcionado por una criadora tipo campana de gas, de capacidad sobrada para las 50 aves y la ventilación se llevo a cabo con el manejo según el caso de las cortinas.

Las excepciones al manejo fueron; no se administro ningún tipo de aditivo, desinfectante o químico en el agua de bebida, con respecto al alimento se restringió en el horario nocturno, ya que nunca se proporciono luz adicional a las aves y a partir de la 3ª semana se dieto en el día, con respecto a sanidad no se aplico por ninguna vía vacuna alguna.

Las variables objeto de observación durante la administración de la *Pulsatilla* fueron:

- Presentación de signos inherentes a la *Pulsatilla*; tos, estornudos, moqueo, traqueobronquitis, inquietud, friolentas, disminución en el consumo de agua.
- Cambios en la conducta (actitud y comportamiento), hurañas como ejemplo (17).

- Modificación de los parámetros zootécnicos (14).

La observación de la parvada se realizo 3 veces al día, por el tiempo que duro la prueba, verificándose la actitud y el comportamiento de los pollos en prueba, contra el grupo control. La evaluación zootécnica como es lo usual, se llevo a cabo a la conclusión de la respectiva semana de vida y la información se concentró en el registro (ver registros).

Resultados.

La respuesta zootécnica de la parvada de la 1ª a la 3ª semana.

Sem.	Mort.	% Mort	Consumo Kg.	Consumo por ave	Peso	Ganancia de peso	Induce de conversión	Observaciones
1	0	0	8	0.160	127.84	87.84	1.82	
2	0	0	20.5	0.410	358.13	230.29	1.78	
3	5	2.5	31	0.660	763.40	405.27	1.62	La mortalidad se debió a SA, dietaciòn de 12.00 a 17 hrs.

Respuesta zootécnica de la parvada de la 4ª semana, hasta la conclusión.

Lote 1 6x.

Sem	Mort	Mort %	Consumo Kg	Consumo por ave	Peso	Ganancia de peso	Índice de conversión	Observaciones
4	0	0	13.33	0.888	0.619	0.466	1.9	Aves con bajo peso por SA
5	4	2.6	14.00	1.272	1.085	0.740	1.6	3 muertas por SA, 1 por necropsia.
6	2	1.8	10.00	1.111	1.825	0.611	1.8	2 muertas por SA
7	1	1.1	9.000	1.125	2.436	0.545	2.0	1 por necropsia

Lote 2 30x

Sem.	Mort.	Mort. %	Consumo kg	Consumo por ave	Peso	Ganancia de peso.	Índice de conversión	Observaciones
4	1	0.66	13.33	0.888	0.474	0.457	1.9	1 muerta por SA
5	1	0.71	14.00	1.076	0.931	0.611	1.8	1 muerta por SA
6	2	1.5	14.00	1.086	1.542	0.754	1.6	1 ave triada y 1 por necropsia
7	1	0.9	12	1.200	2.296	0.495	1.4	1 por necropsia

Lote 3 control

Sem.	Mort.	Mort. %	Consumo Kg	Consumo por ave	Peso	Ganancia de peso	Índice de conversión	Observaciones
4	0	0	13.33	0.888	0.350	0.479	1.9	
5	4	2.6	14.00	0.933	0.824	0.633	1.7	1 muerta por SA
6	3	2.0	14.00	1.272	1.462	0.741	1.9	3 muerta por SA
7	0	0	12.00	1.090	2.203	0.466	2.6	1 por necropsia

Análisis de los resultados zootécnicos.

Este se llevo a cabo por medio del análisis estadístico usando la Distribución "t"
FORMULA DE DISTRIBUCION t (18)

$$t1 = \frac{(\bar{X}1 - \bar{X}2) - (M1 - M2)\ 0}{\sqrt{\frac{S1^2}{n1} + \frac{S2^2}{n2}}}$$

Donde:
t1 = coeficiente de confiabilidad
$\bar{X}1$ = valor de medias
$\bar{X}2$ = valor de las medias
S1 = desviación estándar
S2 = desviación estándar
n1 = valor de la muestra
n2 = valor de la muestra
M1 y M2 = valor comparativo de la muestra

Se obtuvieron las medidas de tendencia central, de los resultados de producción obtenidos de los tres lotes de aves.
DESVIACIÓN ESTÁNDAR
Es útil como medida de variación en un conjunto de datos.
Fórmula de desviación estándar

$$S = \sqrt{\frac{\sum = (Xi - \bar{X})^2}{n - 1}}$$

Donde:
S = desviación estándar
Xi = valor de la muestra
$\bar{X}$ = valor de la media
n = valor de la muestra

Resultado (8).

Las aves, concretamente el pollo de abasto a través de esta prueba, se demuestra la ***INCAPACIDAD DE ESTAS PARA EXPRESAR SIGNOS ESPECIFICOS Y CARACTERÍSTICOS DEL MEDICAMENTO HOMEOPÁTICO ADMINISTRADO; SIGUIENDO LOS LINEAMIENTOS DE LA EXPERIMENTACIÓN PURA SEGÚN OBSERVACIÓN REALIZADA.***.

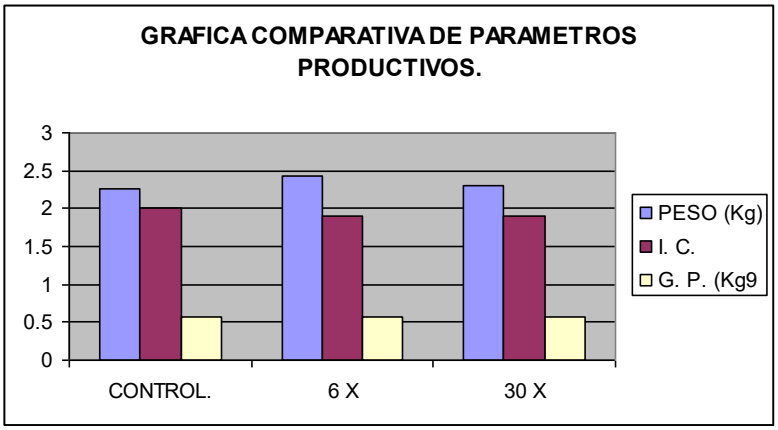
La primera limitación de la experimentación pura en la avicultura sería:

- Las aves no son capaces de exponer síntomas.

- La segunda es que no obstante que las aves tienen la posibilidad de expresar signos estos son, limitados, en su variedad e intensidad, la causa de esto es el desarrollo del Sistema Nervioso Central de las aves, se encuentra muy distante evolutivamente hablando con el del humano. Motivo por el cual al administrar un remedio homeopático a estos animales, no se observaron signos característicos. Esto explica el por que las Materias Medicas Homeopáticas Avícolas existentes, siempre son extrapolación de las Materia Medicas Humanas.

De la segunda vertiente de la investigación o sea la zootécnica, se tuvo los siguientes resultados a los parámetros establecidos:

Peso, Ganancia de peso e Índice de conversión.



Resultados promedios de los parámetros de los sublotes:

El peso promedio de las aves del sublote 3 es de 2.236 Kg
El peso promedio de las aves del sublote 2 es de 2.296 kg.
El peso promedio de las aves del sublote 1 es de 2.436 kg.

Cuadro 1 Comparación del peso final.

grupo	Peso/7 sem. kg	Diferencia grs.
Lote 1 6x	2.436	+ 173
Lote 2 30x	2.296	+ .033
Lote 3 control	2.263	

La Diferencia en la Ganancia de Peso lograda entre los lotes, carece de significancia

Cuadro 2 El índice de conversión tuvo los siguientes resultados.

Grupo	I.C. 7 sem grs.	Diferencias grs.
Lote 1 6x	1.91	- 0.08
Lote 2 30x	2.01	+0.02
Lote 3 control	1.99	

Donde el lote 1 tuvo mejor conversión por 0.08 y el .lote 2 tuvo desventaja de 0.02 en comparación al lote control.
Para el análisis estadístico se procedió a comparar el índice de conversión promedio de los lotes de la siguiente manera:

- Lote control vs. Lote 1
- Lote control vs. Lote 2

El resultado de la primera comparación fue de t1= 2.03138 y el valor critico fue de 0.094192 los resultados de la segunda comparación son: t1= 2.2431 y el valor critico 0.6671 con lo cual queda comprobada la hipótesis nula M1=M2
La mortalidad se excluyo, ya que desde el principio de la prueba esta se debió principalmente al efecto del Síndrome Ascítico y Muerte Súbita ambos problemas tienen su origen en los llamados **Desordenes Metabólicos,** también estuvo involucrado el **Buche Péndulos.** En ningún caso las perdidas se origino por daños del Aparato Respiratorio o por efecto de la comprobación de la *Pulsatilla* objetivo de la investigación.

La precocidad quedo igualada en la parvada por haberla liquidado en el mismo momento.

CONCLUCION DEL ANÁLISIS ESTADÍSTICO (8).

- La hipótesis nula fue aceptada lo que implica la nula significancia estadística entre las diferencias de los valores considerados.

CONCLUCIONES DE LA INVESTIGACIÓN (8).

- La *Pulsatilla* a la potencia 6x y 30x administrada directamente al pico en dosis de dos gotas por ave, tres veces al día, a partir de la 4ª semana de edad a la 5ª semana en el pollo de abasto no es capaz de reproducir el cuadro clínico de ECRC.
- De lo anterior se concluye, las aves no tienen la posibilidad de manifestar signos útiles o referentes para a partir de ellos desarrollar la Materia Médica Homeopática Avícola.
- La *Pulsatilla* administrada de la manera descrita, genera modificación en los parámetros zootécnicos establecidos para la avicultura, con un costo menor.
- La influencia de la *Pulsatilla* sobre los parámetros zootécnicos hace suponer que otros medicamentos puedan tener un efecto más importante, esto llevaría a la comprobación del grupo de medicamentos homeopáticos designados como constitucionales.

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CASO DE CAMPO EN POLLO DE ENGORDA. MENINGOENCEFALÍTIS BACTERIANA

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Resumen:
En una granja avícola ubicada en el estado de Morelos México se presento en pollitos de engorda de 5 días de edad un cuadro nervioso, observándose en las aves la presencia de tortícolis, tremor e incoordinación con una morbilidad del 3 % y una mortalidad acumulada durante la primer semana del 1.73 %. Las aves fueron vacunadas para prevenir la presencia de Enfermedad de Marek, a los 18.5 días de incubación, por vía *in ovo.* Al realizar la necropsia de las aves sacrificadas que presentaron los signos clínicos se observaron lesiones granulo matosas y edematosas en encéfalo y cerebro, los demás órganos internos no presentaron cambios patológicos aparentes. Se mandaron aves vivas con sintomatología nerviosa al laboratorio de diagnósticos para realizar pruebas encaminadas a descartar una contaminación bacteriana y micótica así como pruebas serológicas, virológicas y de histopatología, para descartar la infección por virus de Newcastle y Encéfalo mielitis Aviar. Los resultados del diagnostico indicaron la presencia de lesiones microscópicas y microscópicas en cerebro y encéfalo, dichas lesiones se identificaron como piogranulomas en meninges. Las lesiones microscópicas observadas por histopatología indicaron la presencia de meníngeo encefalitis bacteriana. Se procedió a realizar los cultivos requeridos para la identificación de la bacteria aislada, determinándose por pruebas bioquímicas la presencia de *Pseudomona aureoginosa* como el agente causal. Las pruebas de diagnostico serológicas y virológicas e histopatología determinaron la ausencia de virus causante de la Enfermedad de Newcastle y de Encéfalo mielitis aviar. Las pruebas para la determinación de una contaminación micótica fueron negativas. Dado los resultados obtenidos por medio del diagnostico de laboratorio se concluyo que el cuadro nervioso fue ocasionado por una contaminación bacteriana a nivel de cerebro y encéfalo que se debió a una falla en el proceso de bioseguridad implementado en la planta de Incubación, al permitir por algún descuido la contaminación bacteriana durante la preparación y aplicación de la vacuna de Marek in ovo a los 18.5 días de Incubación.

MICOPLASMOSIS OCULAR EN GALLINA DE POSTURA

Jiménez Ricardo[1], Camacho José Ángel[2], Juárez Mireya[3], Victor M Petrone
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La micoplasmosis aviar es una enfermedad de gran importancia particularmente cuando se habla de complejos respiratorios. La aparición de la enfermedad relacionada con *Mycoplasma gallisepticum* depende de la presencia concomitante de otros patógenos o factores debilitantes, ya que es frecuente que las infecciones sin complicaciones no causen signos clínicos o mortalidad, excepto en aves jóvenes. En este punto es importante señalar que los casos de queratoconjuntivis descritos en gallinas de postura asociados a *Mycoplasma gallisepticum* se han descrito durante la crianza y han estado vinculados a la presencia de agentes inmunodepresores como infección por el virus de Gumboro. El objetivo de este trabajo es realizar la descripción clínica y patológica de un caso de micoplasmosis ocular en gallinas de postura en producción. Este caso fue observado en una granja de postura comercial compuesta por dos secciones, cada sección con 6 casetas de 13,000 aves cada una el problema se presento a la semana 19 de edad después de la aplicación de una vacuna ocular contra el virus de Newcastle y se caracterizo por exoftalmia, conjuntivitis, blefaritis, lagrimeo, opacidad y congestión corneal uni o bilateral, aunado a la presencia de signos respiratorios, estos cambios fueron observados aproximadamente en el 3% de las aves al inicio del problema afectando posteriormente al 45% de las aves. Se remitieron al laboratorio para histopatología fragmentos de tráquea, pulmón, ojo, hígado, proventrículo, ovario, corazón, bazo, riñón, articulación, nervio y encéfalo. Las muestras fueron colectadas de aves recién sacrificadas a las 22, 27, 36 y 40 semanas; así mismo, fueron colectadas muestras de humor acuoso e hisopos de ojo para realizar aislamiento de *Mycoplasma* sp en aves de 40 semanas. En las muestras remitidas para histopatología las lesiones más relevantes fueron las observadas en ojo las cuales consistieron en queratoconjuntivitis y panuveítis fibrinoheterofilica y linfocitaria de moderada a severa, aunado a lesiones traqueales y pulmonares que se caracterizaron por traqueítis y neumonía fibrinoheterofilica en las etapas iniciales progresando a traqueítis y neumonía linfocitaria en las etapas finales. En las muestras de las aves de 22 semanas se encontraron lesiones compatibles con enfermedad de Marek en secciones de hígado, proventrículo y encéfalo. De los hisopos y humor acuoso remitidos para aislamiento se logro recuperar una cepa de *Mycoplasma gallisepticum* cabe señalar que las aves no habían sido vacunadas. Esa cepa de *Mycoplasma gallisepticum* fue clonada para pruebas de concentración mínima inhibitoria en donde la tiamulina, tilosina y doxiciclina fueron los antibióticos que se requirieron en menor cantidad para inhibir el crecimiento de esa cepa de *Mycoplasma gallisepticum*. En conclusión, la inmunodepresión causada por la infección del virus de Marek y la presencia constante de virus vacunales como Newcastle y Bronquitis, contribuyeron en la patogénesis de las lesiones oculares causadas por la infección de *Mycoplasma gallisepticum*.

Palabras clave: *Mycoplasma gallisepticum*, gallinas, queratoconjuntivitis, panuveitis, concentración mínima inhibitoria

EXPERIENCIAS DE CAMPO EN CRIANZA ABIERTA *VS* CRIANZA "BLACK OUT" EN AVES REPRODUCTORAS ROSS

Jiménez Ricardo
Asesor independiente

RESUMEN

El éxito en la producción de pollito ross de excelente calidad, depende de la combinación exitosa de varias técnicas de manejo interrelacionadas durante el periodo de crianza de las aves. Una de estas técnicas clave de manejo, esta relacionada con la duración del fotoperíodo y la intensidad de la luz que les proveemos a las aves durante su vida. La luz juega un papel determinante no solo por el hecho de que las aves necesitan luz para ver, encontrar comida, agua y el nido; si no, también porque es indispensable para el optimo desarrollo de su aparato reproductor. En este punto debemos mencionar que la diferencia entre la duración del fotoperíodo y su intensidad durante la crianza y la producción controlara y estimulara el desarrollo ovárico y testicular. De la respuesta a los incrementos en el fotoperíodo y la intensidad de la luz dependerá el lograr el perfil correcto en el peso corporal y uniformidad. Considerando las condiciones ambientales y el tipo de instalaciones con las que se cuente se han considerado tres posibles combinaciones de iluminación del ambiente tales como: crianza y postura en ambiente controlado, crianza oscurecida (black out) y postura abierta, crianza y postura abierta. El objetivo de este trabajo es mostrar los resultados obtenidos en dos parvadas manejadas con crianza oscurecida y abierta dentro y fuera de estación.

Palabras clave: Crianza abierta, crianza cerrada (black out), reproductoras, ross, fotoperíodo

EVALUACIÓN DE LA PROTECCIÓN CONFERIDA POR VACUNAS CONCENTRADAS CONTRA INFLUENZA AVIAR

Gabriel Gómez, Gabriela Ascención, Javier Calderón
Avícola ALCER, Lab. Boehringer Ingelheim

INTRODUCCIÓN

La influenza aviar es una enfermedad viral que afecta a un amplio rango de especies aviares, entre ellas a las aves domésticas. En estas últimas puede ocasionar infecciones inaparentes, ligeros cuadros respiratorios, hasta una severa enfermedad sistémica con cuadros de alta mortalidad. Esta enfermedad fue reconocida en Italia en 1878 y se le denominó como Peste Aviar (Fowl Plague). La Influenza Aviar fue identificada hace más de 100 años y actualmente está en todo el mundo. Es una enfermedad con un alto impacto económico para la industria avícola mexicana debido a las pérdidas asociadas a mayor mortalidad, menor peso corporal, incremento de la conversión alimenticia y aumento del costo de producción. En 1994 fue reconocida la presencia de Influenza Aviar (IA) de alta patogenicidad en parvadas comerciales en México. Actualmente el virus de baja patogenicidad está ampliamente difundido en el país. Además, es importante mencionar que la inestabilidad antigénica del virus de IA conduce a la imposibilidad de utilizarse como virus activo atenuado o modificado para la inmunización de aves. Por esta razón es importante contar con vacunas que nos brinden la protección necesaria para minimizar los impactos que ocasiona la infección de este virus. Desde hace más de 10 años se ha utilizado la vacuna emulsionada en aceite. Sin embargo, en algunas regiones del país se ha observado que no es suficiente la inmunidad conferida por una inmunización y se ha adoptado la estrategia de administrar dos aplicaciones a lo largo del ciclo de vida de las aves. Actualmente se cuenta con la tecnología que permite incluir dentro de una emulsión mayor masa antigénica, de esta forma se reduce la cantidad de emulsión que necesita ser administrada. Debido a lo anteriormente mencionado este tipo de vacunas pueden ser administradas incluso desde el día de edad por vía subcutánea. El presente trabajo tiene por objeto mostrar los resultados de la evaluación de la protección conferida por vacunas concentradas al ser aplicadas a los 10 y los 21 días de edad como complemento al programa de vacunación contra IA.

DEVELOPMENT AND EVALUATION OF A NOVEL BACTERIAL VACCINE VECTOR SYSTEM AGAINST VIRAL, BACTERIAL AND PROTOZOAL PATHOGENS

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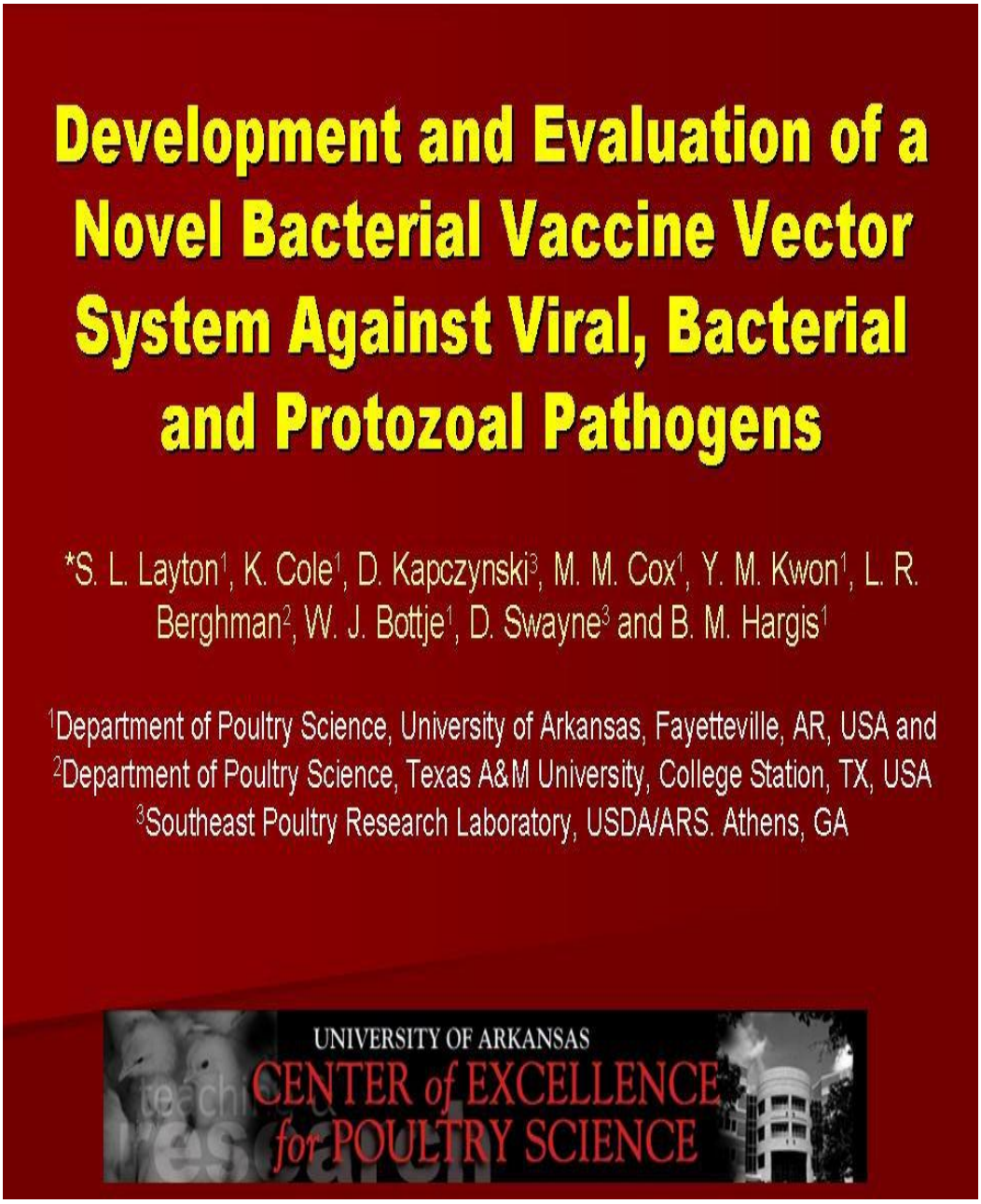
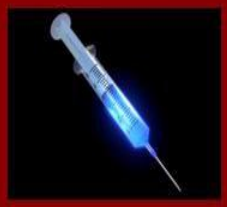
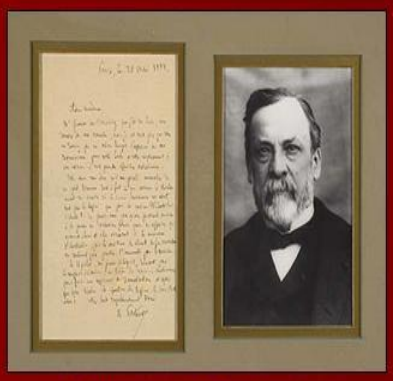
[2]Southeast Poultry Research Laboratory, USDA/ARS. Athens, GA



Vaccination

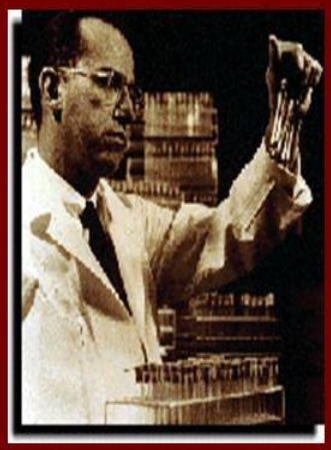
Important Medical Advancement





http://www.ehistorybuff.com/pastuerals.html

http://www.wisedude.com/inventions_discoveries/ll_



http://focosi.immunesig.org/preventionprimaryimmunovaccine.html

Vaccination (Traditional)

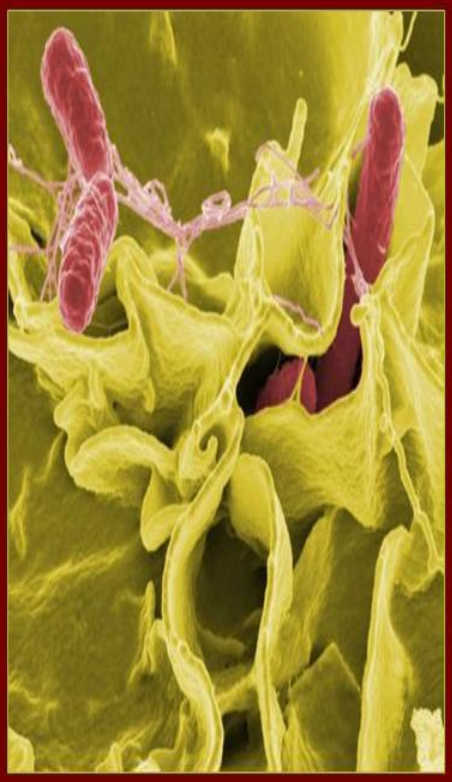

Image from National Foundation for Infectious Diseases

- Live Attenuated
  - Weakened primary pathogen
  - More favorable administration routes
  - Vaccine reaction

- Killed
  - Killed by chemical or mechanical inactivation
  - More cost prohibited (labor/more antigen needed for response)

Salmonella as a Vaccine Vector

- Low pathogenicity *Salmonella* isolates effectively present antigens to immune cells after mucosal penetration
- Recent work from our laboratory indicates that obligatory expression of cell surface epitopes can result in strong and persistent antibody responses

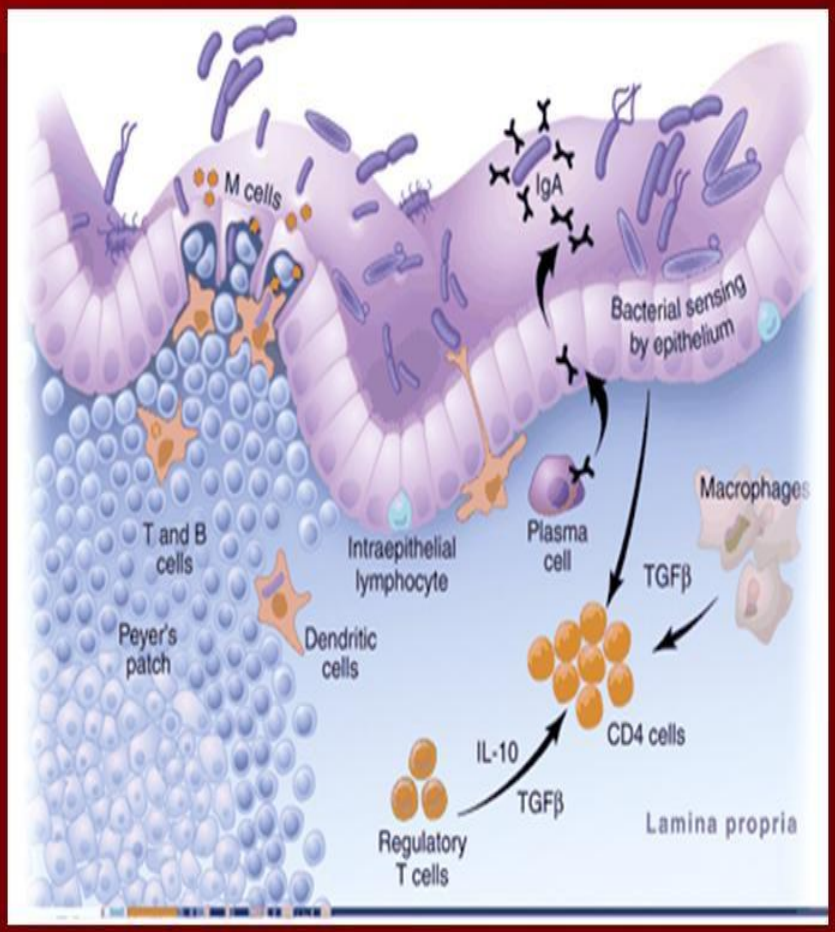


http://www.livescience.com/imageoftheday/siod_041201.html

Attenuated Salmonellae as Vectors

- Some isolates are invasive
- Genetically tractable
- Well characterized virulence mechanisms
- Provide cross-protection against related *Salmonella* serovars
- Strong mucosal immune response

Mucosal Immunity



Advantages of mucosal routes of immunization.

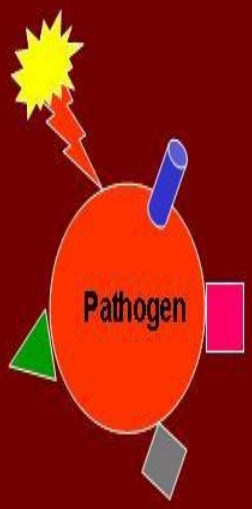
- Induces protective immunity at the site of infection
- Induces both systemic and mucosal immunity
- Effective in the presence of maternal antibodies
- No injection site reaction, no needles required
- Readily administered (i.e. oral vaccines combined with feed)

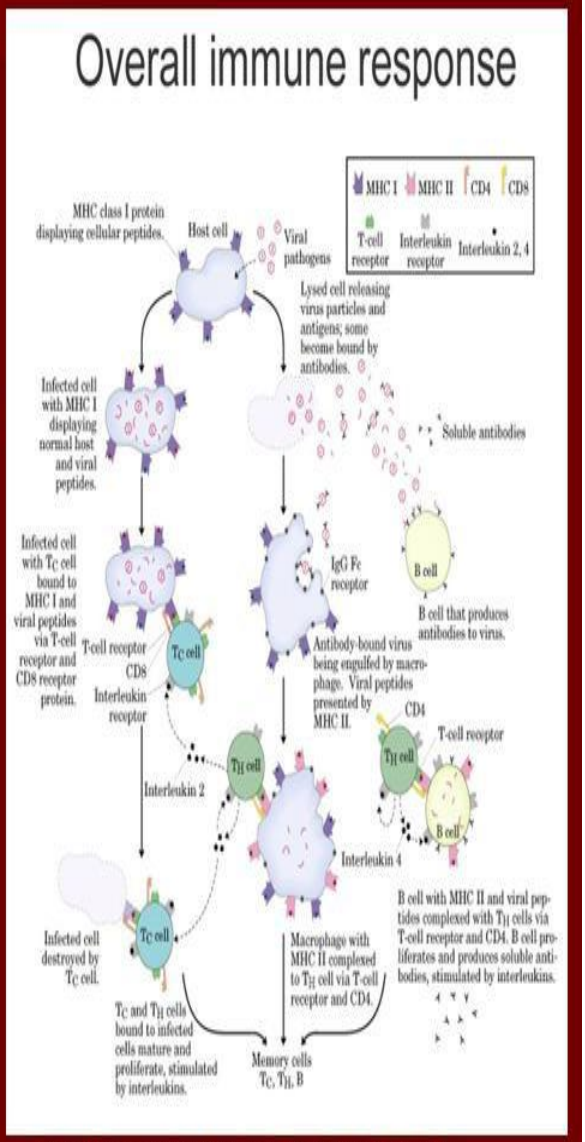
Even if mucosal immunization does not totally eliminate infection

- Mucosal antibody limits the degree of replication and shedding of the pathogen, thereby, reducing the pathogen load in the environment and consequently dramatically reducing the rate of herd infection and transmission of disease through the herd.

Epitope Selection

- Short Amino Acid Sequences Which are Highly Conserved
- Antigenic/Immunogenic
- Immunoprotective
- B-cell/ T-cell Response

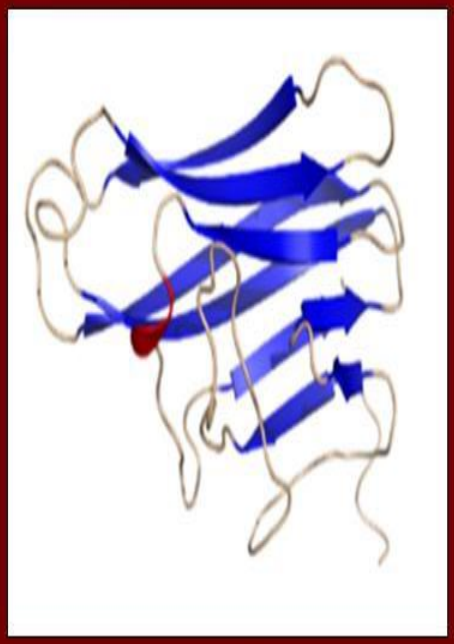




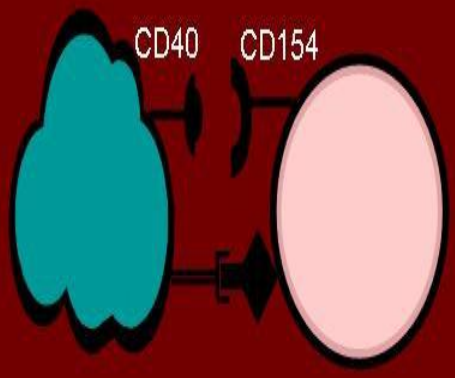
http://courses.cm.utexas.edu/jrobertus/ch339k/overheads-1/ch7_immune-res.jpg

CD154 (CD40L)

- Type II Glycoprotein
- Member of TNF Ligand Family
- Binds CD40 on B-cells and Activated T-cells



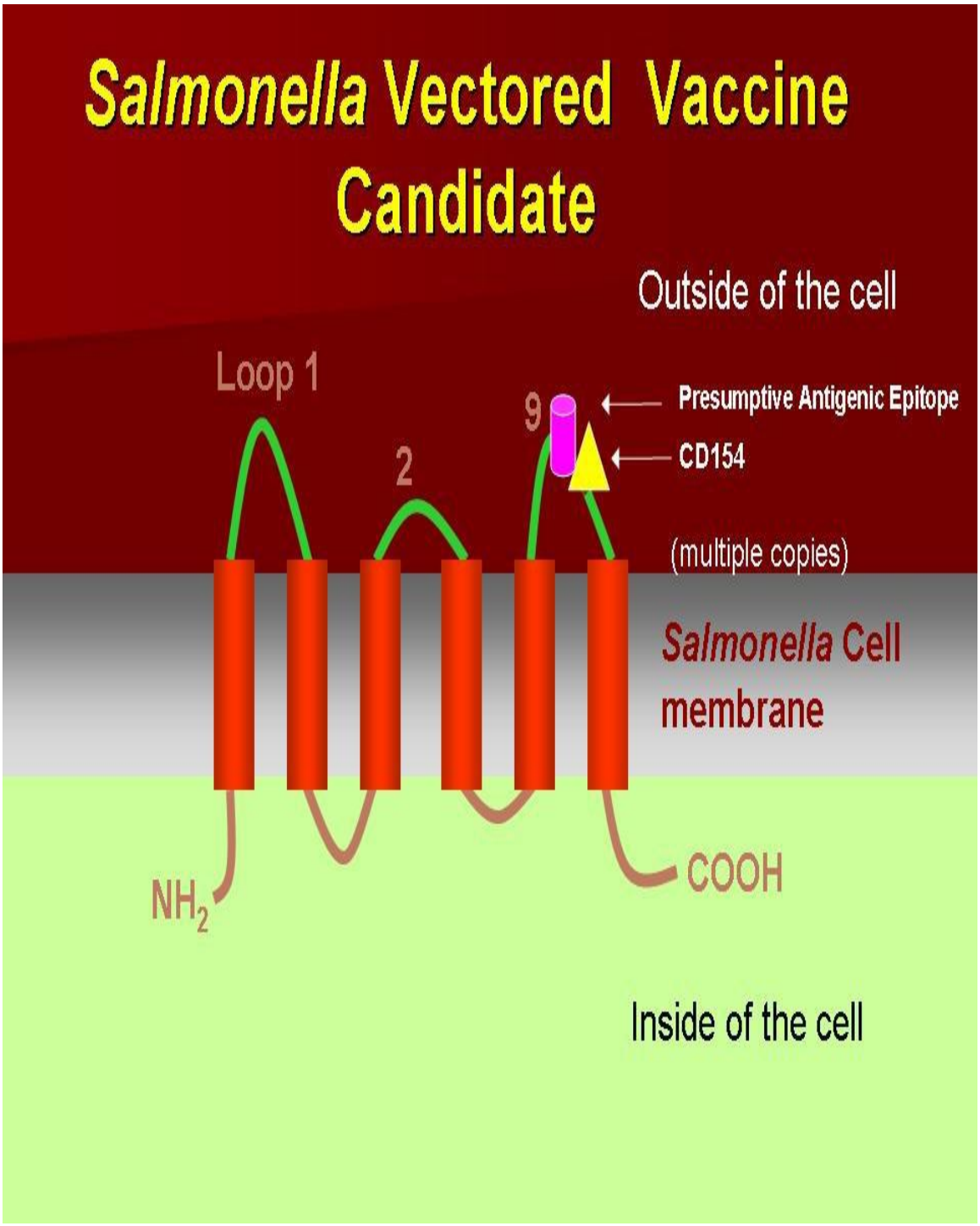
Crystal structure of human CD40L (CD154)

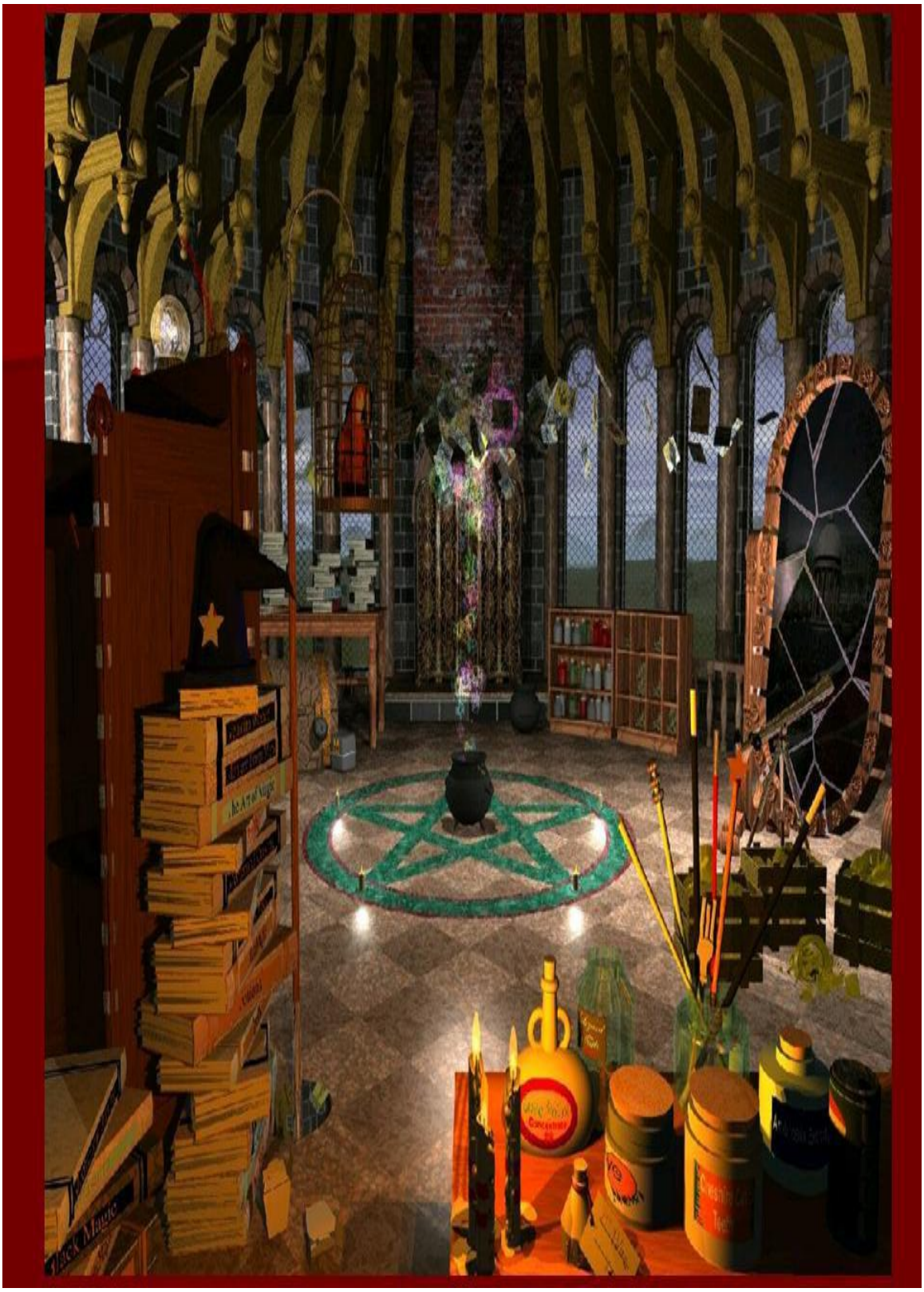


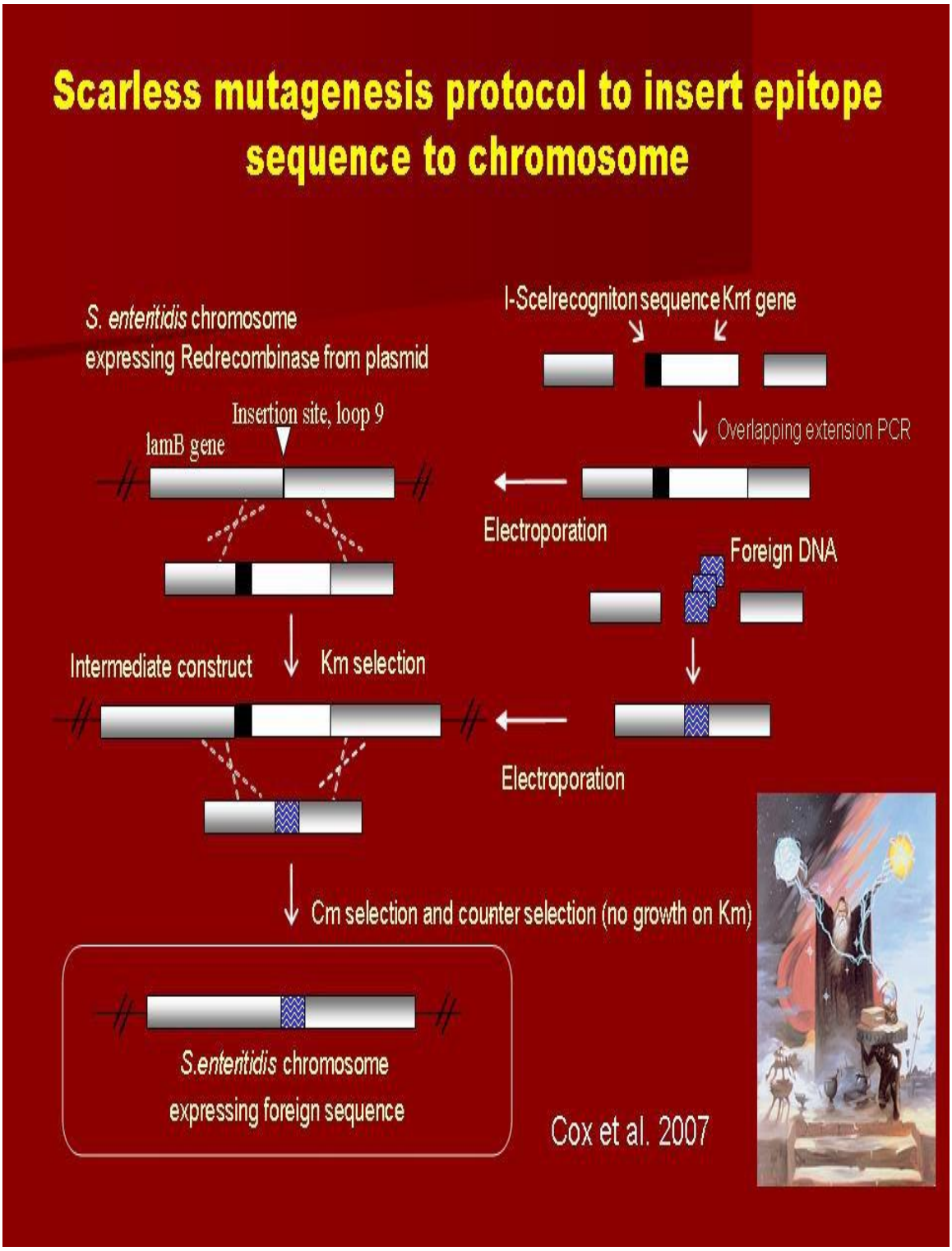
- **WAEKGYYTMS**

Biologically Active Binding Region

Vega et al. 2003


Salmonella Vectored Vaccine Candidate
Outside of the cell
Loop 1
9
2
Presumptive Antigenic Epitope
CD154
(multiple copies)
Salmonella Cell membrane
NH2
COOH
Inside of the cell




Scarless mutagenesis protocol to insert epitope sequence to chromosome
S. enteritidis chromosome expressing Redrecombinase from plasmid
Insertion site, loop 9
lamB gene
I-Scelrecogniton sequence Km gene
Overlapping extension PCR
Electroporation
Foreign DNA
Intermediate construct
Km selection
Electroporation
Cm selection and counter selection (no growth on Km)
S.enteritidis chromosome expressing foreign sequence
Cox et al. 2007

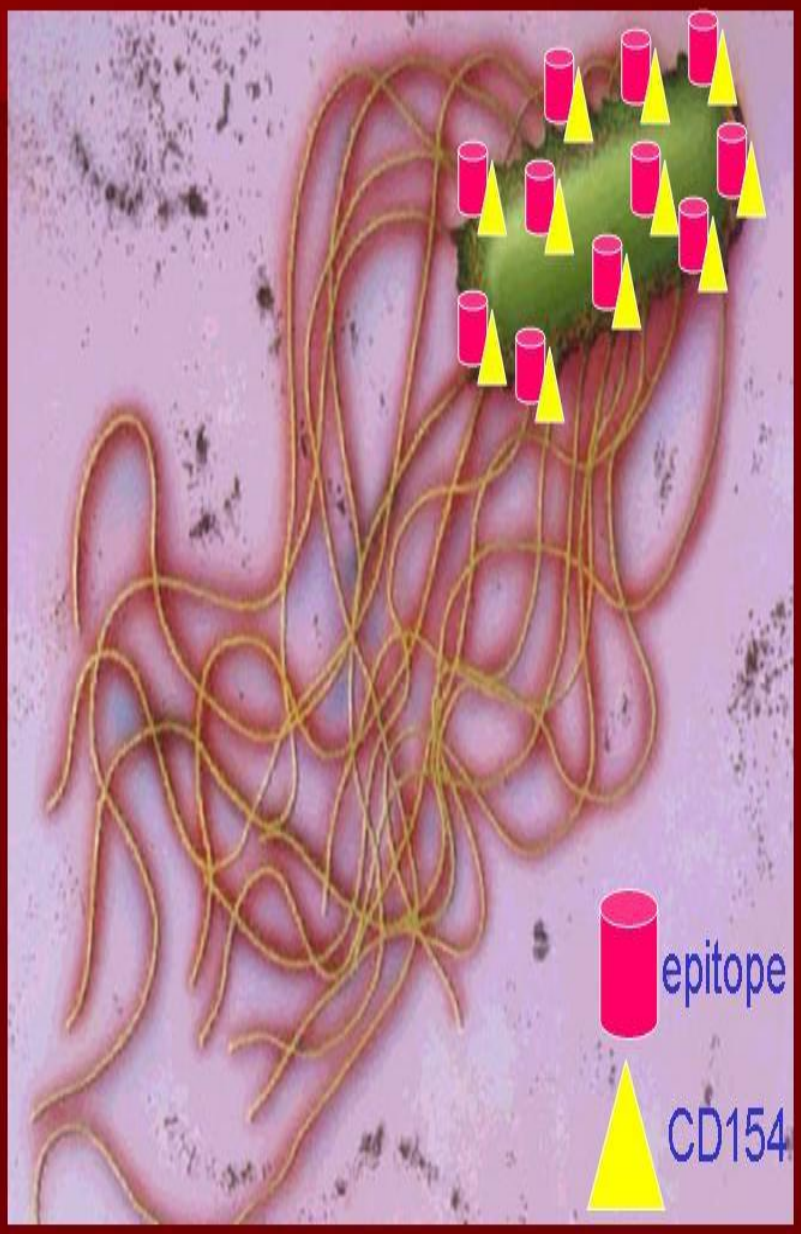
lamB Loop 9 Sequence
(partial)

AATGTACAAGTGGACGCCAATCATGAGCACCCTGCTGGAAGTCGGCTACGAC
AACGTGAAATCTCAGCAGACCGGCGATCGTAACAATCAATATAAAATCACCCT
GGCGCAACAGTGGCAGGCGGGCGACAGCATCTGGTCGCGTCCGGCTATCCG
TATTTTCGCCACCTACGCGAAATGGGATGAGAAATGGGGCTATATCAAAGACG
GCGATAACATTTCCCGTTATGCCGCAGCGACTAACTCCGGC ☺ ATTTCCACC
AACAGCCGTGGCGATAGCGATGAGTGGACCTTCGGCGCCCAGATGGAAATCTG
GTGGTAA

lamB Loop 9 Sequence with Insert
(partial)

AATGTACAAGTGGACGCCAATCATGAGCACCCTGCTGGAAGTCGGCTACGACAA
CGTGAAATCTCAGCAGACCGGCGATCGTAACAATCAATATAAAATCACCCTGGC
GCAACAGTGGCAGGCGGGCGACAGCATCTGGTCGCGTCCGGCTATCCGTATTT
TCGCCACCTACGCGAAATGGGATGAGAAATGGGGCTATATCAAAGACGGCGAT
AACATTTCCCGTTATGCCGCAGCGACTAACTCCGGCSSSARGFAKSSSWAEKGY
YTMSSSATTTCCACCAACAGCCGTGGCGATAGCGATGAGTGGACCTTCGGCGC
CCAGATGGAAATCTGGTGGTAA

Cell Surface Expression of Chosen Epitope and CD154



Confirmed by

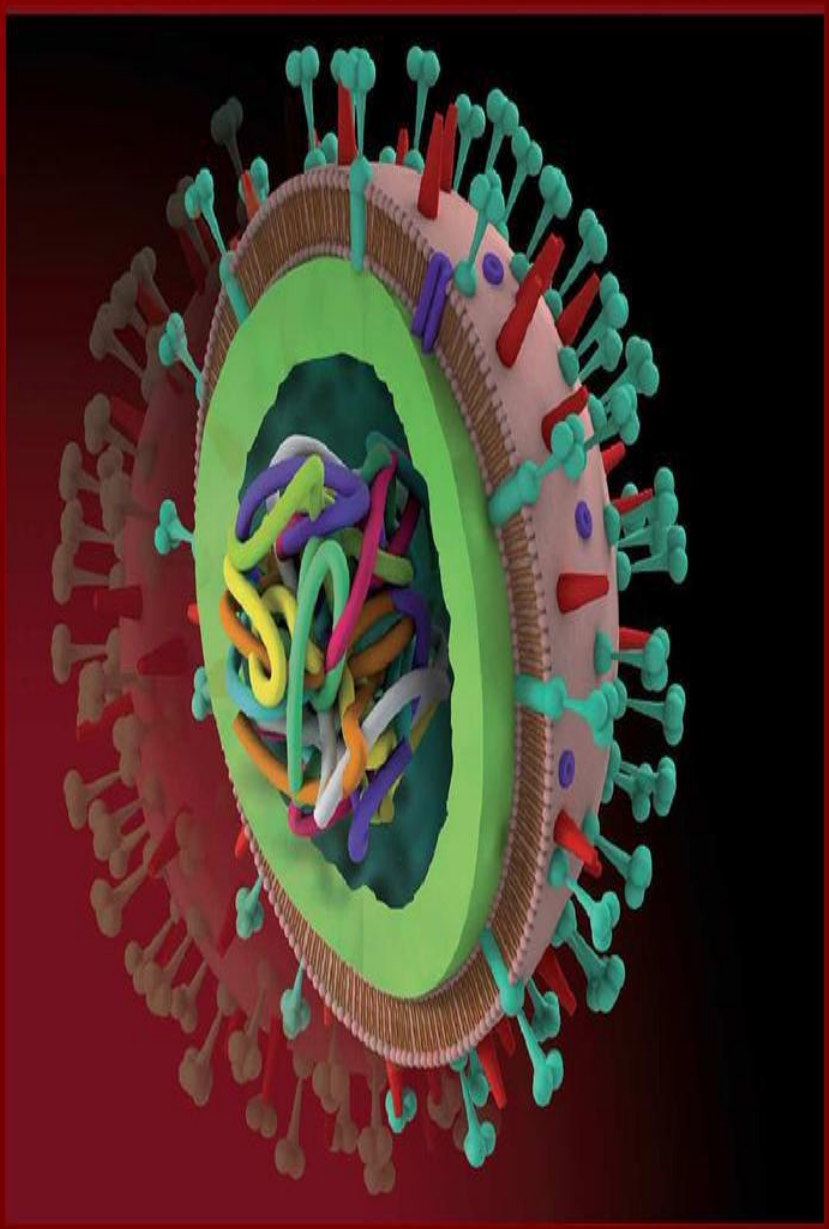
1. DNA sequencing
2. Plate agglutination

aroA Deletion (Δ*aroA*)

htrA Deletion (Δ*htrA*)

- *htrA* encodes a periplasmic serine protease that degrades aberrant proteins during extracytoplasmic stress conditions.
- *htrA* impairs host response to stress and its ability to survive in macrophages (increased susceptibility to hydrogen peroxide, H_2O_2).

Avian Influenza



Novel Vaccine Concept

"Non Immunogenic but Immunoprotective"

"Non Immunogenic but Immunoprotective"

- Potential antigenic epitopes that have been conserved (unchanged) over time.
  - Strategy is to hide these.
- Protective when presented to the immune system.
- Presented by live bacterial vector.

Influenza A Virus
M2e May Be Protective

Influenza

NEWS

A One-Size-Fits-All Flu Vaccine?

The threat of avian influenza has revived efforts to develop "universal" flu vaccines that protect against all human influenza strains. Although that goal remains elusive, vaccines that protect against seasonal flu variants could be closer

Modern medicine's main weapon against the influenza virus is woefully unsophisticated. Each year, companies have to make a new batch of flu vaccine because unlike, say, polio or chickenpox, flu strains change every year. The vaccine is grown in eggs, a process that takes up to 9 months, and people have to be vaccinated annually, which many don't bother to do. More troubling, if a pandemic strain of influenza came along, the virus could kill millions of people in the time it would take to prepare a matching vaccine.

What scientists dream of is a vaccine that can protect against any flu strain for years or even a lifetime. This so-called universal flu vaccine is still a long way off, if it's even possible. But many labs are dusting off past projects on broad flu vaccines, spurred by new funding and fears that H5N1, the deadly avian influenza that has swept across half the world, could acquire the ability to be transmitted from human to human. Until now, "flu has never been before high enough on the radar screen" for companies in particular to follow through with a strong push for a universal vaccine, says Gary Nabel, director of the Vaccine Research Center at the U.S. National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Maryland.

Doing so, however, means coming up with an alternative way to stimulate immunity to the virus. The tried-and-true technique for seasonal flu uses a killed virus vaccine that works mainly by triggering antibodies to hemagglutinin (HA), the glycoprotein on the virus's surface that it uses to bind to human cells. Hemagglutinin and neuraminidase (NA), another surface glycoprotein that helps newly made viruses exit cells, give strains their names (H5N1, for example). The sequences of HA and NA mutate easily, which is why each season's flu strain—although it may be the same in subtype, such as H3N2—"drifts" slightly from the previous year's, and the annual vaccine must be tailor-made.

To make a universal vaccine for influenza A, which includes the main seasonal flu strains and bird flu, as well as past pandemic strains, some scientists are hoping to use "conserved" flu proteins that don't mutate much year to year. (Influenza B, the other type, occurs only in humans and causes milder symptoms.) Some of the conserved protein vaccines in the works stimulate production of antibodies as do conventional flu vaccines, whereas others route certain immune system cells to battle the virus.

Weak spots. A universal flu vaccine would target "conserved" proteins, such as M2 or M1, an inner protein.

Other scientists are pursuing a slightly less ambitious goal: They are working on vaccines that match a particular HA, such as the H5 in H5N1, but that also protect against "drift" strains that typically emerge from year to year.

It's not yet clear whether any of these broad vaccines will ever work as well as a traditional, HA-matched vaccine. But they could help when the annual vaccine doesn't match the circulating strain exactly, and in a pandemic, they could reduce deaths until a matched vaccine is ready. "Anything that would dampen a pandemic would be useful," says virologist Robert Couch of Baylor College of Medicine in Houston, Texas.

One for all

One of the most hotly pursued strategies for a universal vaccine against influenza A is based on a flu protein called M2. This protein forms an ion channel crossing the membrane of a virus particle or infected cell, barely jutting out from the surface. It's an appealing target because the 23 amino acids that make up the ectodomain, or protruding part, of M2 (known as M2e) scarcely vary from one human flu strain to the next, even back to the 1918 Spanish flu.

Scientists first showed in the late 1980s that antibodies to M2 can slow flu infection in mice. In 1999, biochemist Walter Fiers's team at Ghent University in Belgium reported in *Nature Medicine* that it had reduced flu deaths in mice with a vaccine made of M2e fused to another protein, the core of the hepatitis B virus (HepB). (These proteins clumped into viralike particles bearing with M2e that stimulated more antibodies to M2e than did the protein by itself.) In its latest paper in *Vaccine* in January, Fiers's lab, now collaborating with the vaccine company Acambis in Cambridge, Massachusetts, has improved the candidate vaccine by attaching three copies of M2e to the HepB core, delivering it nasally—which boosts immune responses compared to injection—and adding an adjuvant, an ingredient that also increases the body's immune response.

Although M2e is typically conserved, there's a small chance that the protein could still evolve, enabling the virus to evade a vaccine. To assess that risk, Walter Gerhard's group at the Wistar Institute in Philadelphia, Pennsylvania, pushed the virus to mutate by exposing mice with weak immune systems to an H1N1 seasonal flu strain while giving them antibodies specific to M2e. As they reported last June in the *Journal of Virology*, M2e mutants appeared in some mice after 3 weeks, but there were only two types—fewer than might have been expected. "To us, that was reassuring," Gerhard says, because it should be possible to make an M2e vaccine to match the few anticipated variants.

Another major caveat is that although M2 vaccines may prevent deaths from flu, they may not keep people from getting sick, the way conventional vaccines normally do, notes Couch. That's because M2 antibodies seem to work by binding to infected cells and promoting their clearance, instead of blocking the virus (which

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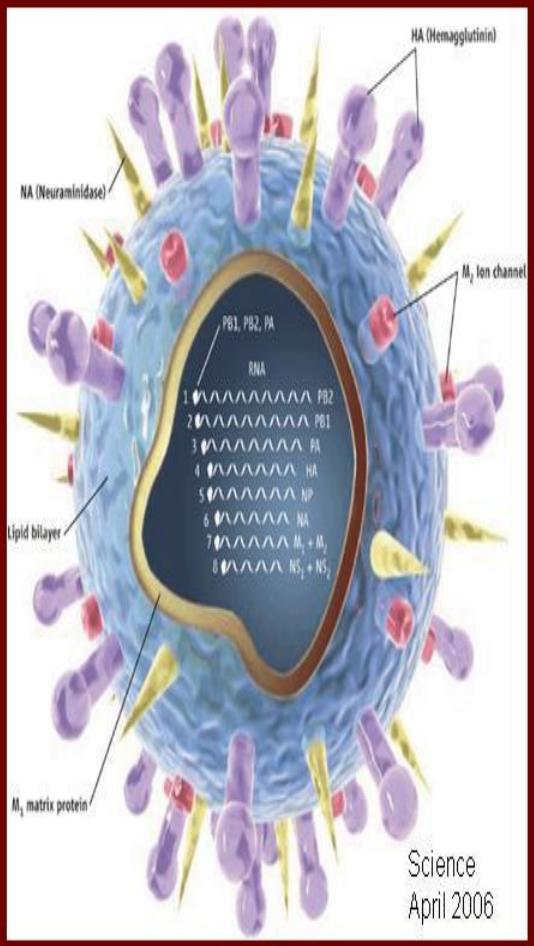
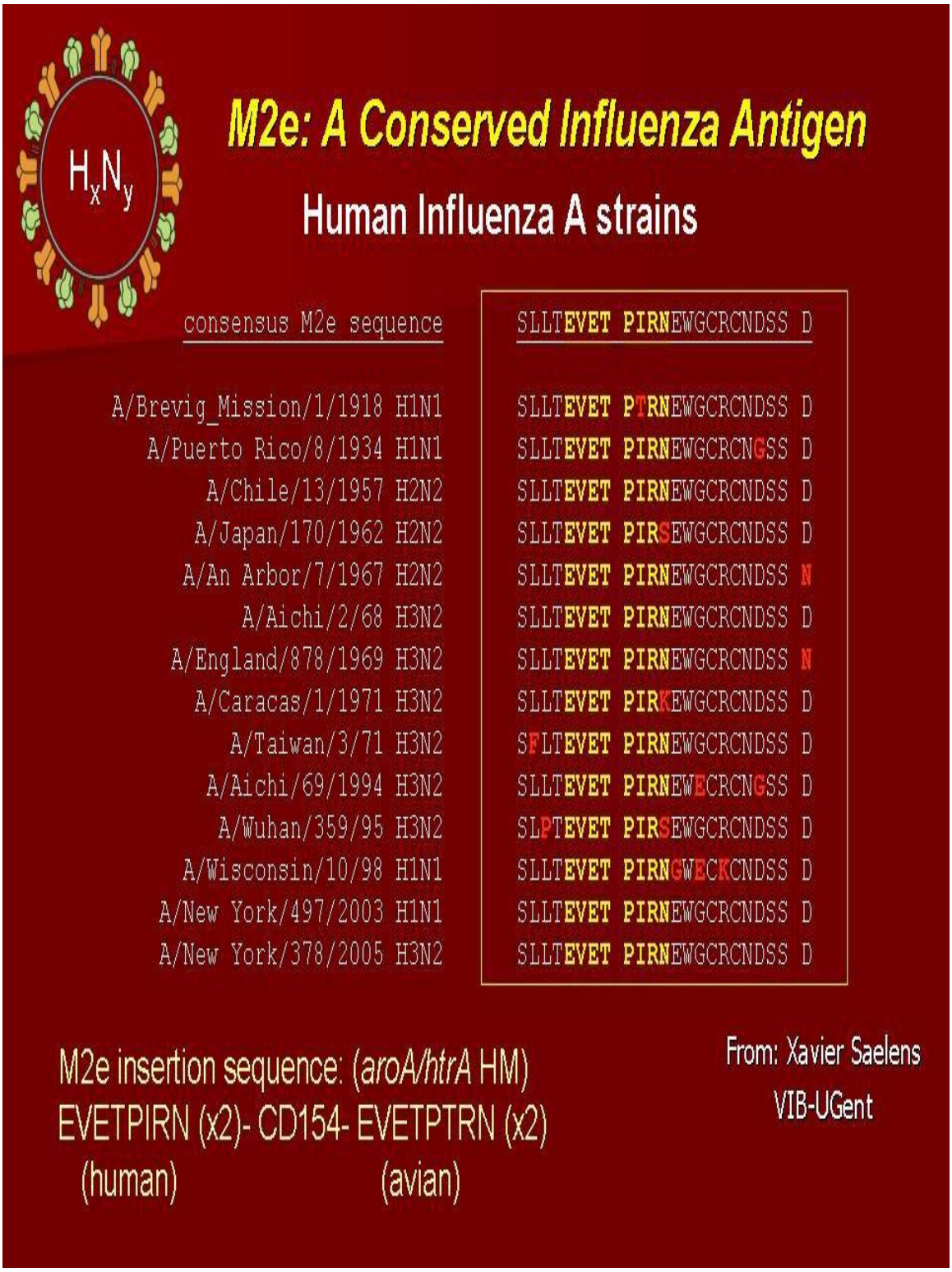
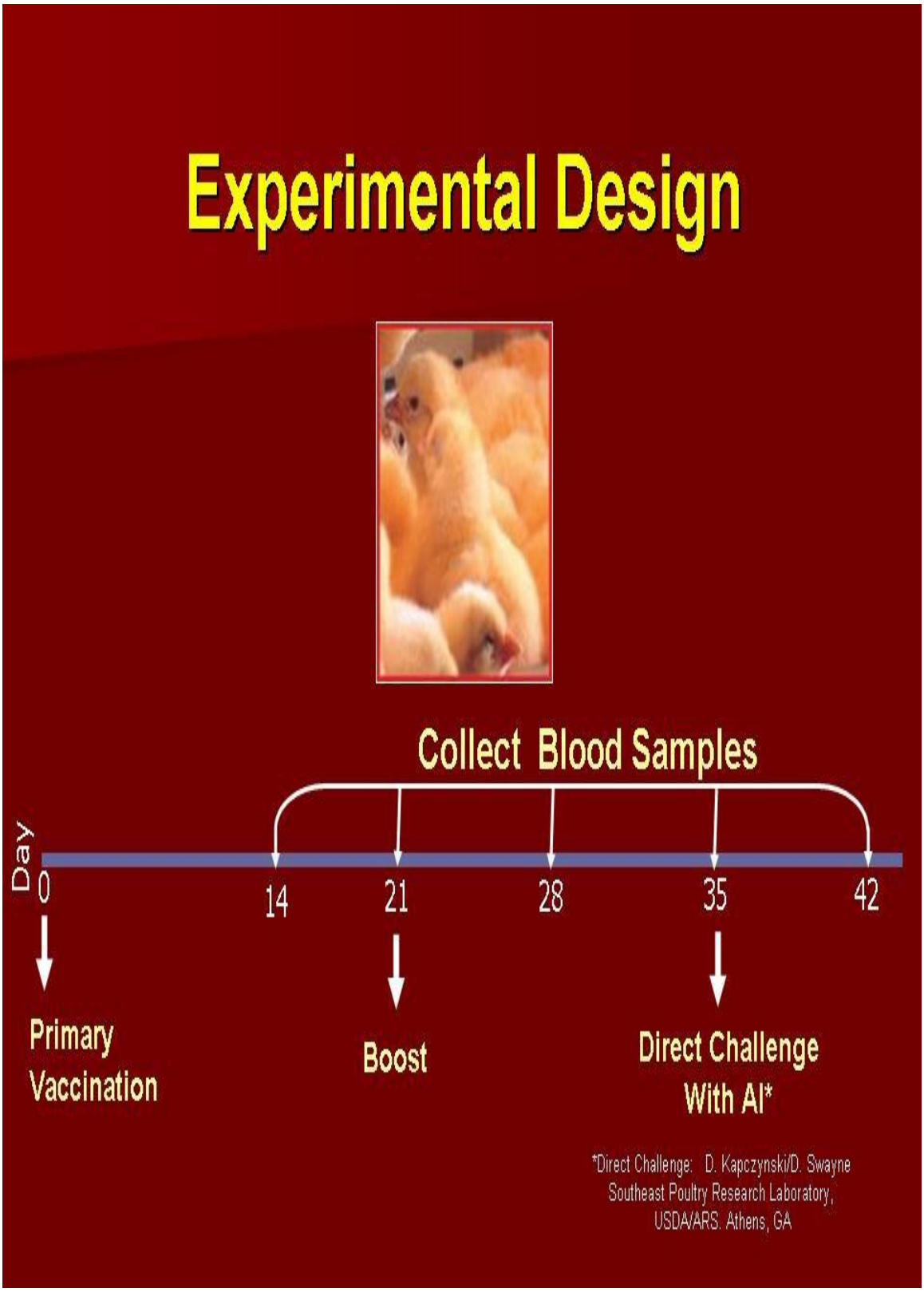
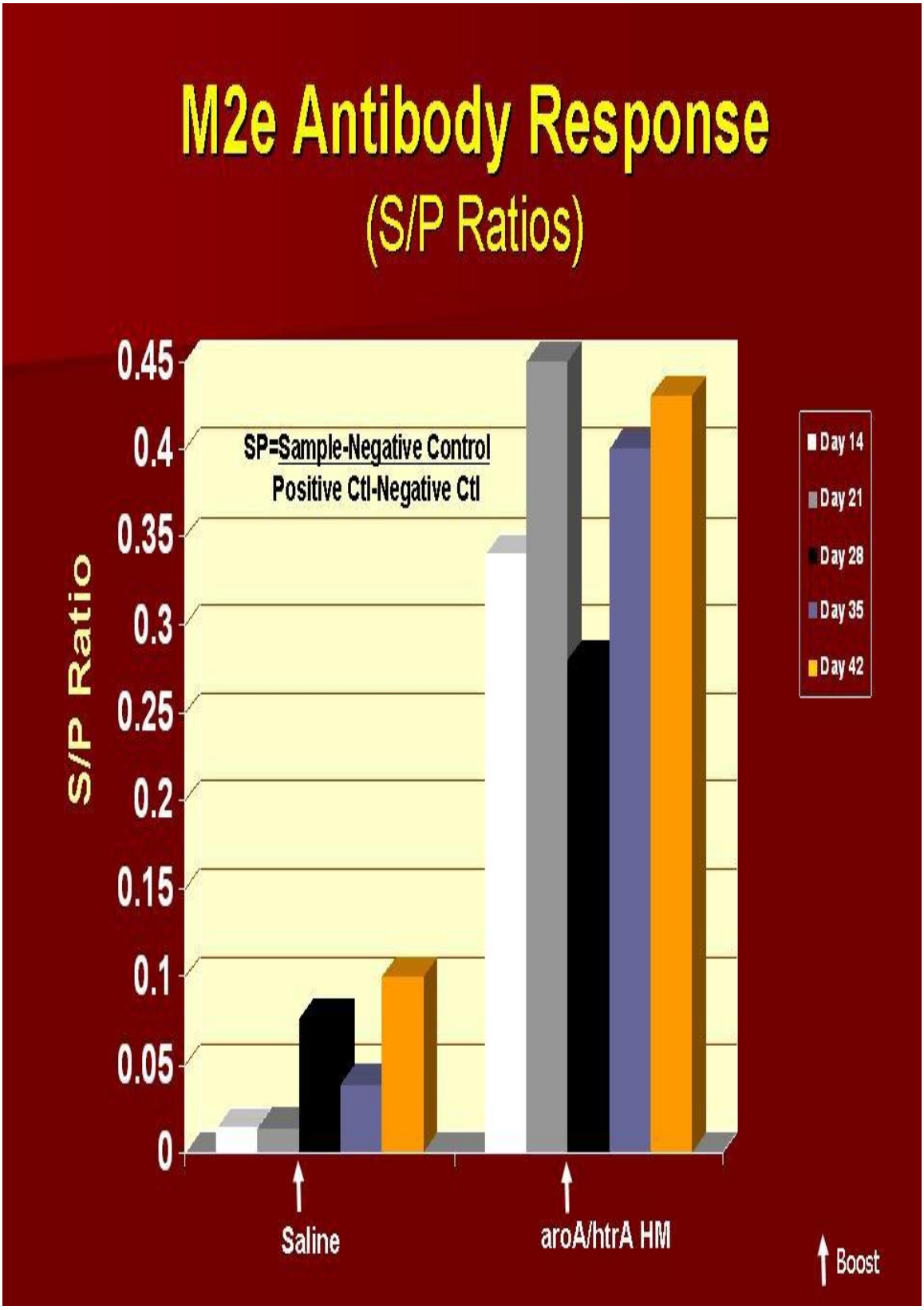



H_xN_y
M2e: A Conserved Influenza Antigen
Human Influenza A strains
consensus M2e sequence SLLTEVET PIRNEWGCRCNDSS D
A/Brevig_Mission/1/1918 H1N1 SLLTEVET PTRNEWGCRCNDSS D
A/Puerto Rico/8/1934 H1N1 SLLTEVET PIRNEWGCRCNGSS D
A/Chile/13/1957 H2N2 SLLTEVET PIRNEWGCRCNDSS D
A/Japan/170/1962 H2N2 SLLTEVET PIRSEWGCRCNDSS D
A/An Arbor/7/1967 H2N2 SLLTEVET PIRNEWGCRCNDSS N
A/Aichi/2/68 H3N2 SLLTEVET PIRNEWGCRCNDSS D
A/England/878/1969 H3N2 SLLTEVET PIRNEWGCRCNDSS N
A/Caracas/1/1971 H3N2 SLLTEVET PIRKEWGCRCNDSS D
A/Taiwan/3/71 H3N2 SFLTEVET PIRNEWGCRCNDSS D
A/Aichi/69/1994 H3N2 SLLTEVET PIRNEWECRCNGSS D
A/Wuhan/359/95 H3N2 SLPTEVET PIRSEWGCRCNDSS D
A/Wisconsin/10/98 H1N1 SLLTEVET PIRNGWECKCNDSS D
A/New York/497/2003 H1N1 SLLTEVET PIRNEWGCRCNDSS D
A/New York/378/2005 H3N2 SLLTEVET PIRNEWGCRCNDSS D
M2e insertion sequence: (aroA/htrA HM)
EVETPIRN (x2)- CD154- EVETPTRN (x2)
(human) (avian)
From: Xavier Saelens
VIB-UGent


Experimental Design
Collect Blood Samples
Day
0
14
21
28
35
42
Primary Vaccination
Boost
Direct Challenge With AI*
*Direct Challenge: D. Kapczynski/D. Swayne
Southeast Poultry Research Laboratory,
USDA/ARS. Athens, GA

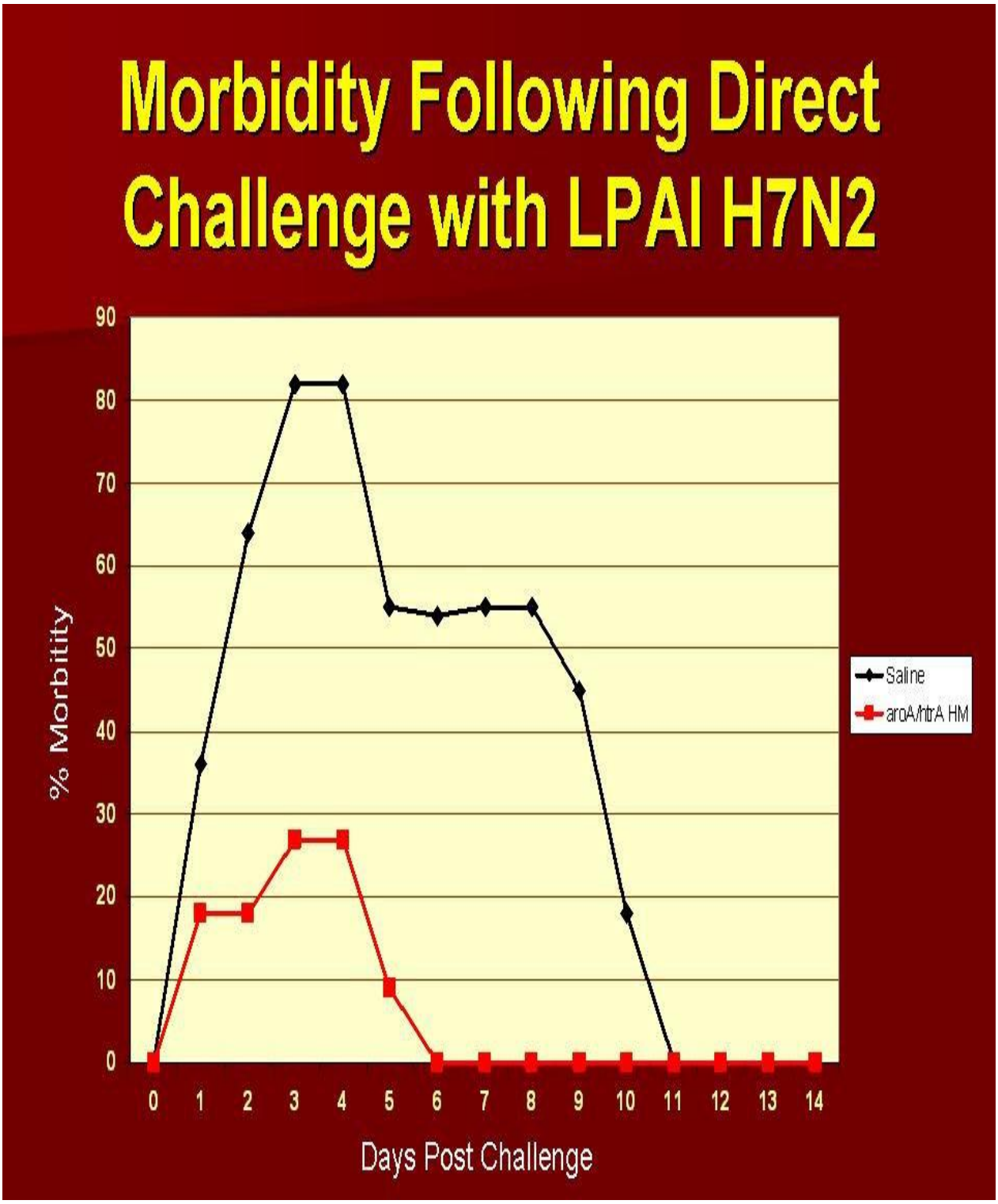

M2e Antibody Response
(S/P Ratios)
S/P Ratio
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
SP=Sample-Negative Control
Positive Ctl-Negative Ctl
Day 14
Day 21
Day 28
Day 35
Day 42
Saline
aroA/htrA HM
Boost

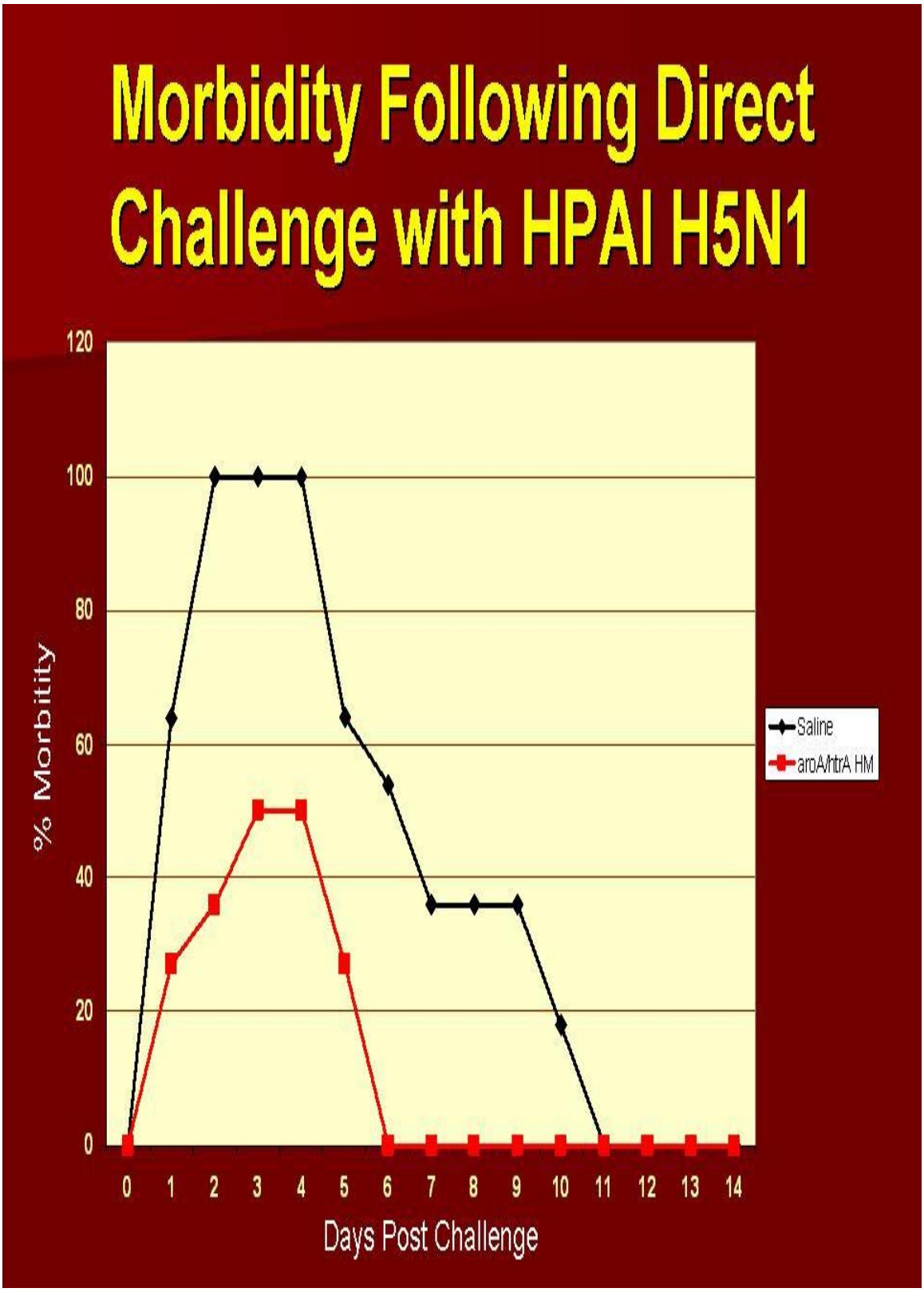
Virus Neutralization

Neutralizing Index

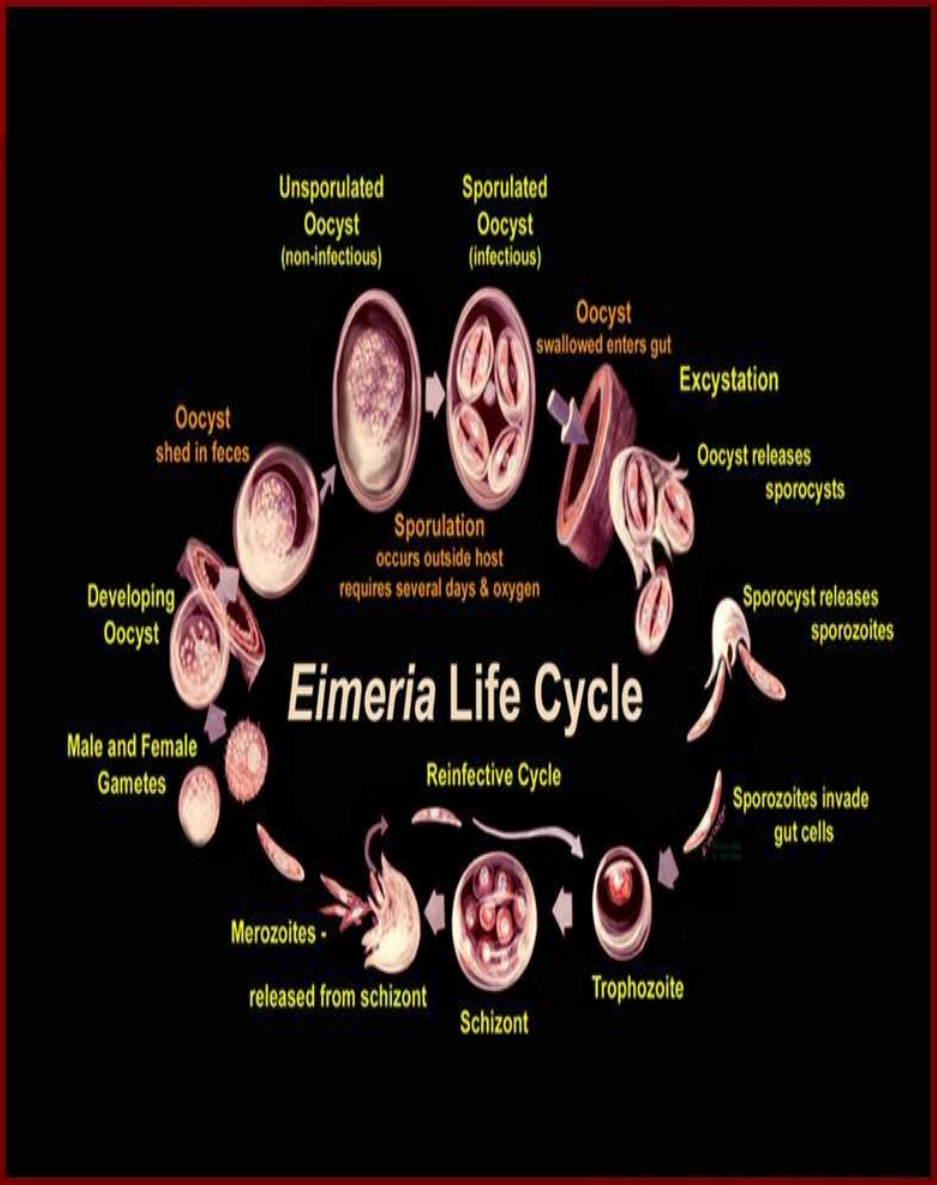
	Day 21	Day 28	Day 35	Day 42
Positive Control	7.8	8.8	8.8	8.8
aroA/htrA HM	5.8	6.3	6.3	6.3

- H9N2 (avian)
- >2 positive assay result
- ≥7 protective


Morbidity Following Direct Challenge with LPAI H7N2
% Morbitity
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days Post Challenge
Saline
arcA/mtrA HM


Morbidity Following Direct Challenge with HPAI H5N1
% Morbitity
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days Post Challenge
Saline
aroA/ntrA HM

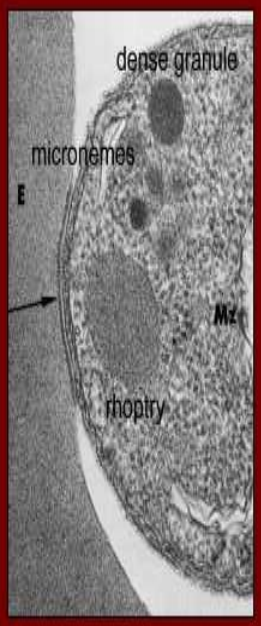
Coccidiosis



http://ars.usda.gov/Main/docs.htm?docid=11018

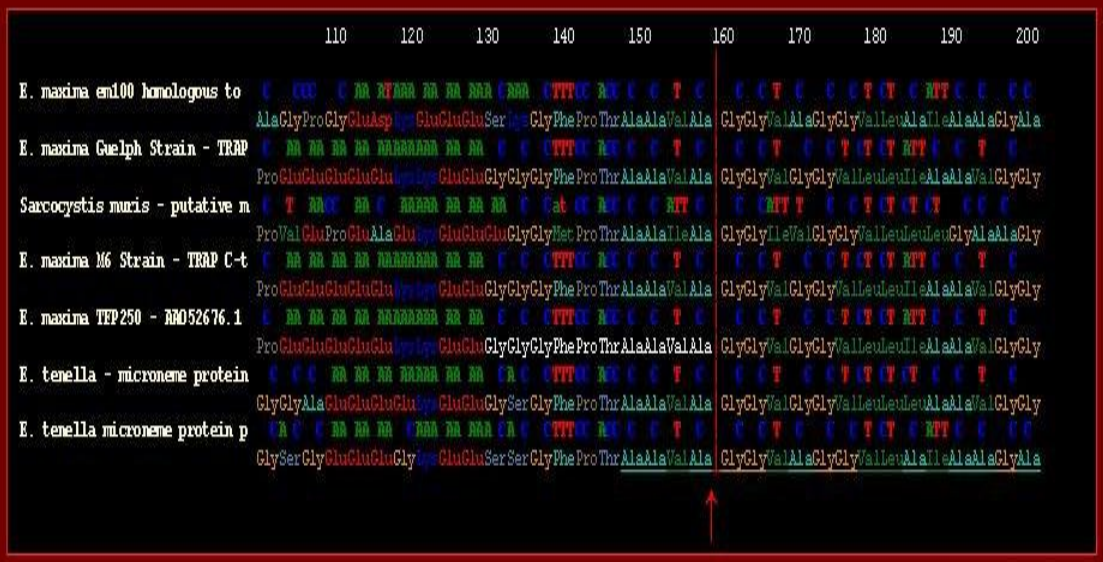
Eimeria (TRAP)

- TRAP (thrombospondin-related anonymous protein) associated with parasite gliding motility that is highly conserved within apicomplexan microneme proteins.

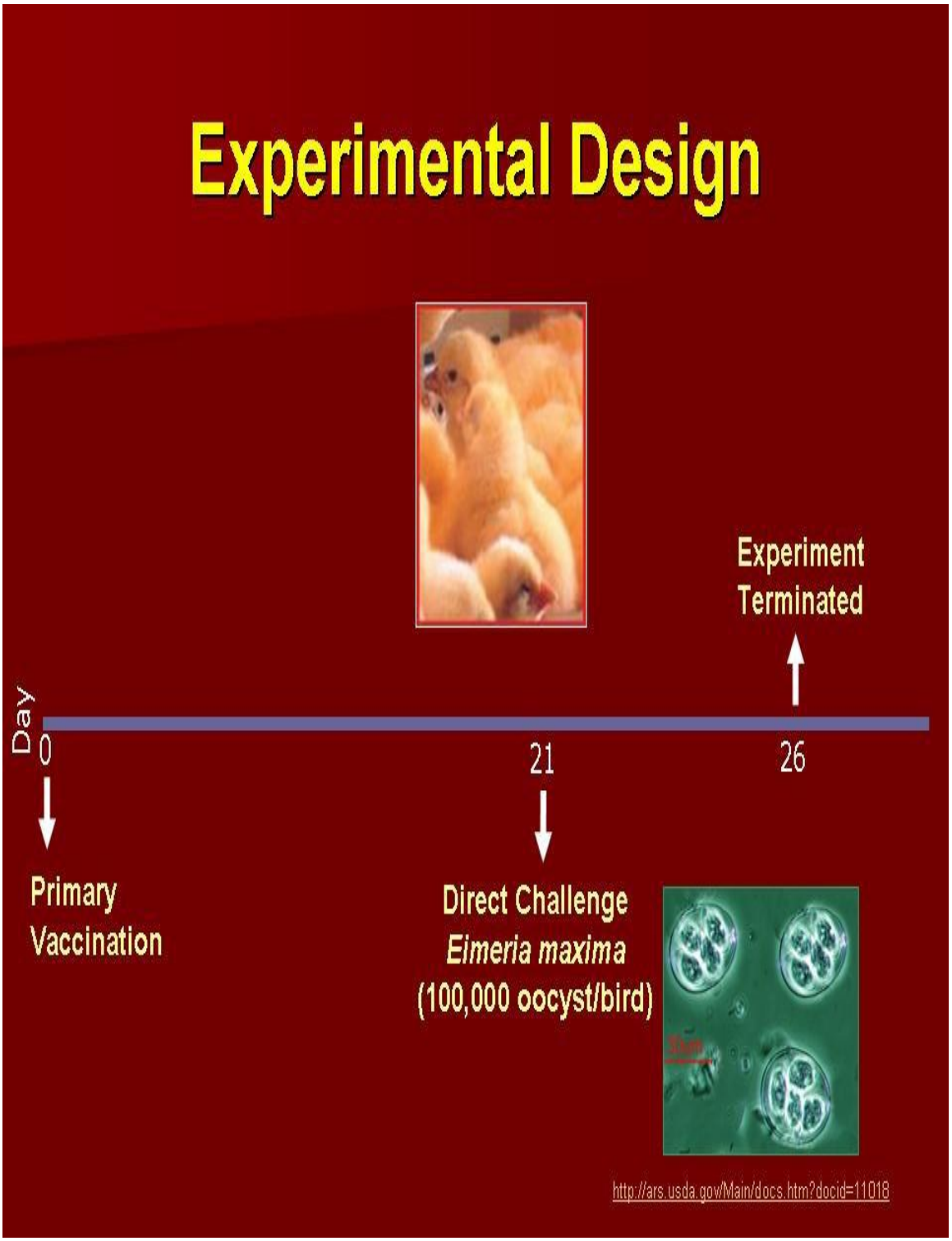


Aikawa et al (1978) J. Cell Biol. 77:72

Sequence Alignment at Cleavage Site of *Eimeria* Species

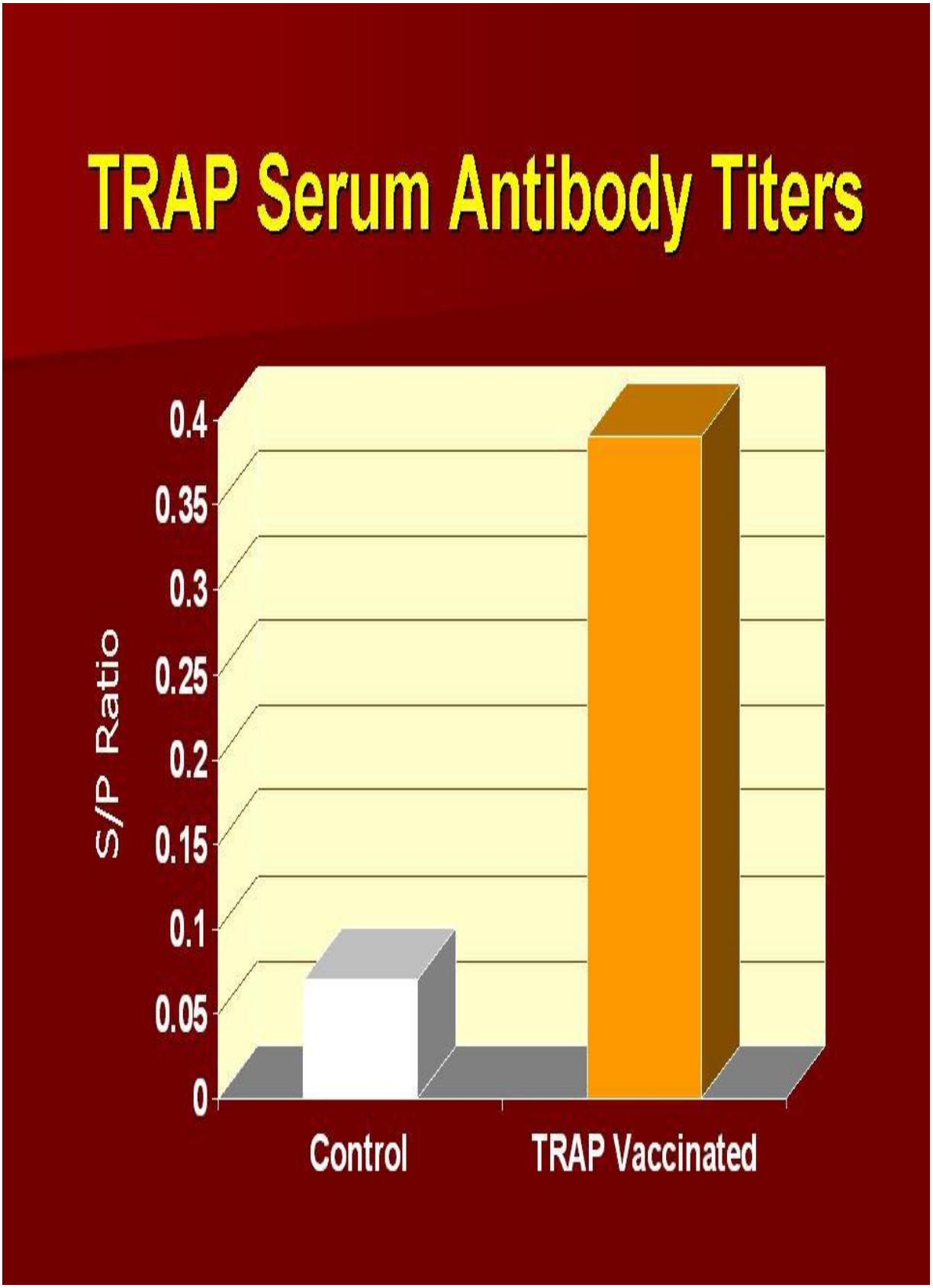


4 E. maxima strains
2 E. tenella strains


Experimental Design
Experiment Terminated
Day
0
21
26
Primary Vaccination
Direct Challenge
Eimeria maxima
(100,000 oocyst/bird)
http://ars.usda.gov/Main/docs.htm?docid=11018

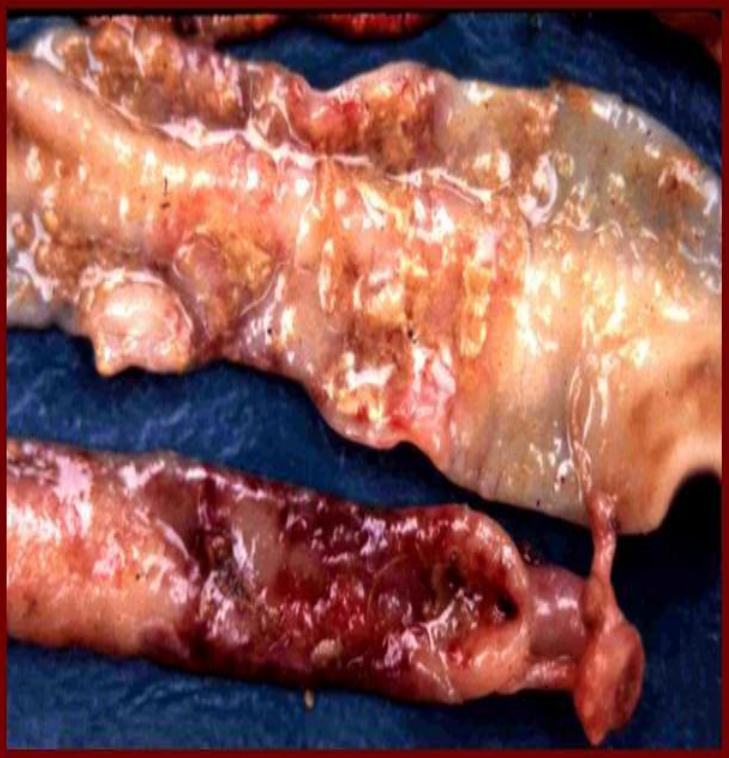
Evaluation of Coccidiosis Candidate Vaccine

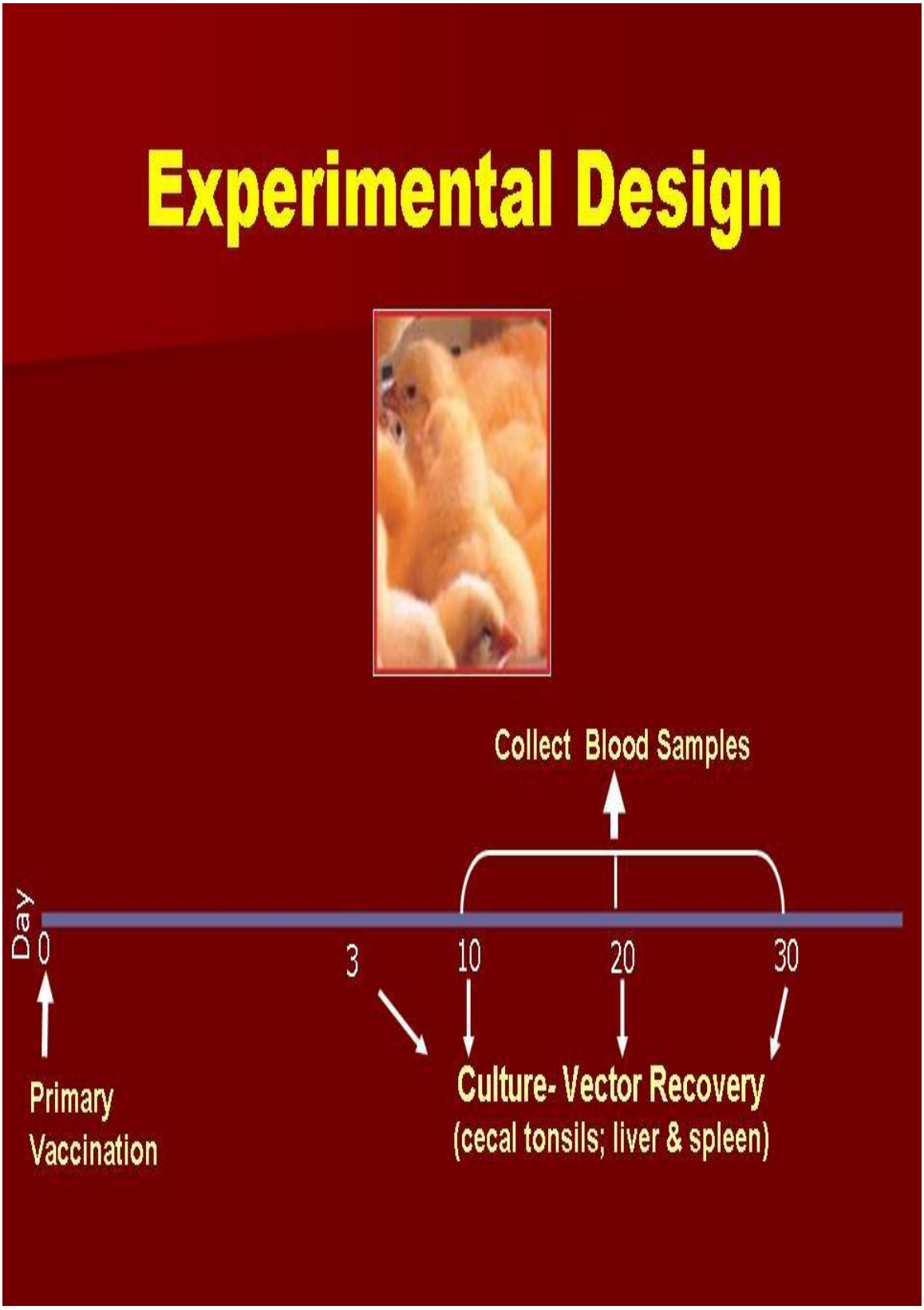
Challenge Group	Mortality at Day 26 (5 Days Post-Challenge)
Saline	10/46 (22%)
Vaccinated (TRAP)	1/46 (2%)


TRAP Serum Antibody Titers
S/P Ratio
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Control
TRAP Vaccinated

Necrotic Enteritis

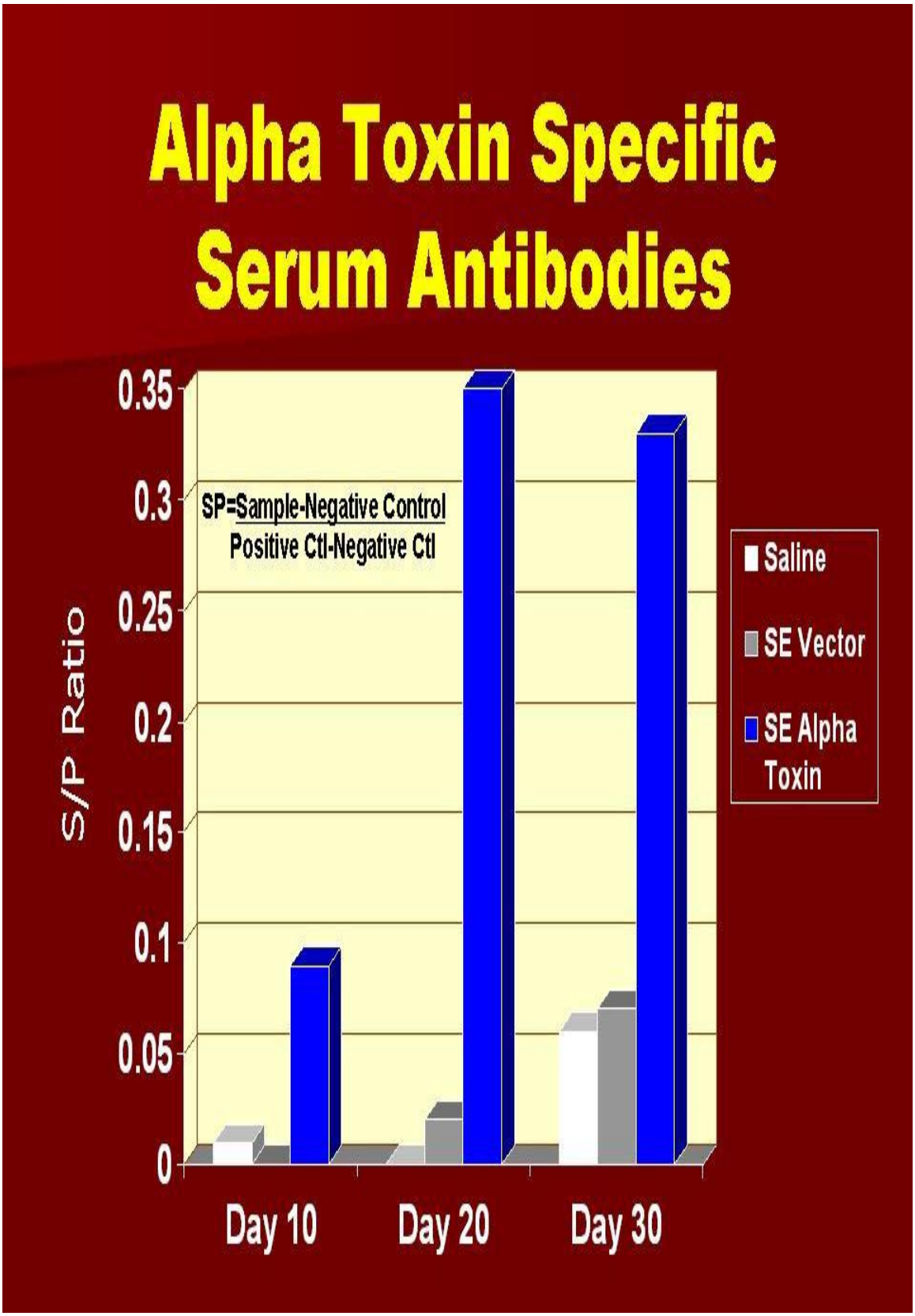
Clostridium perfringens




Experimental Design
Collect Blood Samples
Day
0
3
10
20
30
Primary
Vaccination
Culture- Vector Recovery
(cecal tonsils; liver & spleen)

Colonization, Invasion and Clearance of Salmonella Vaccine Vector

	Day 3 Liver/ Spleen	Day 3 Cecal Tonsils	Day 10 Liver/ Spleen	Day 10 Cecal Tonsils	Day 20 Liver/ Spleen	Day 20 Cecal Tonsils	Day 30 Liver/ Spleen	Day 30 Cecal Tonsils
Saline	0/15	0/15	0/15	0/15	0/15	0/15	0/14	0/14
SE Vector	1/15	12/15	10/15	10/15	7/15	14/15	1/15	3/15
Alpha Toxin Vaccine	9/15	14/15	8/15	12/15	13/15	12/15	1/14	9/14


Alpha Toxin Specific
Serum Antibodies
S/P Ratio
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
SP=Sample-Negative Control
Positive Ctl-Negative Ctl
Saline
SE Vector
SE Alpha Toxin
Day 10
Day 20
Day 30

Where to Now....

- Optimize the vaccine vector system to be used in Bacillus spp.
- Explore the use of a Type III secretion system to display large post-translationally modified proteins as antigens.
- Engineer current vaccine strains to display short epitope sequences from several different pathogens (multivalent ie...alpha toxin +TRAP)

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Srichaitanya Shivaramaiah

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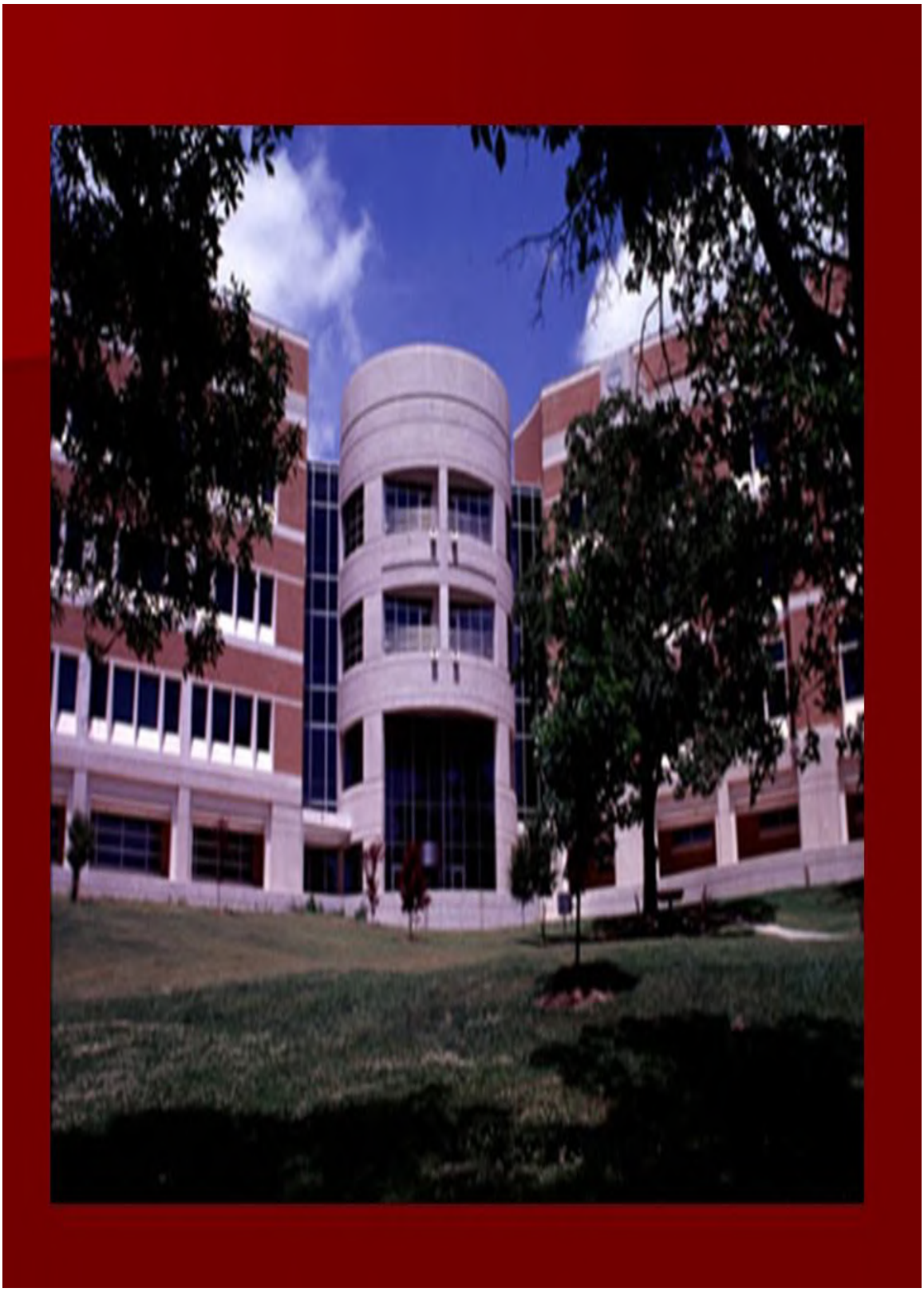
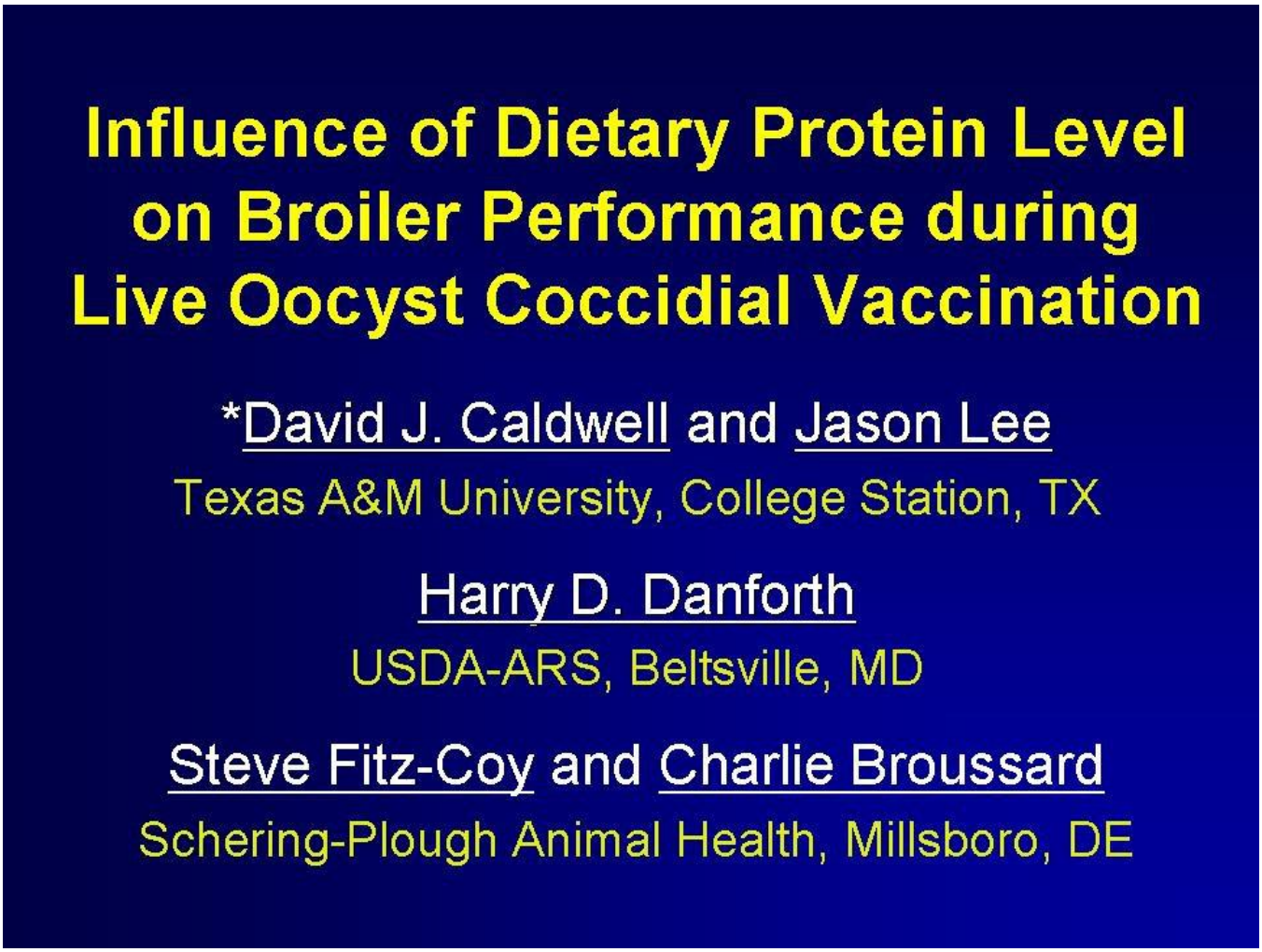
Marion Morgan
Guillermo Gaona
Ross Wolfenden



INFLUENCE OF DIETARY PROTEIN LEVEL ON BROILER PERFORMANCE DURING LIVE OOCYST COCCIDIAL VACCINATION

David J. Caldwell and Jason Lee[1]; Harry D. Danforth[2]; Steve Fitz-Coy and Charlie Broussard[3]
[1]Texas A&M University, College Station, TX, [2]USDA-ARS, Beltsville, MD; [3]Schering-Plough Animal Health, Millsboro, DE



Live Oocyst Vaccines

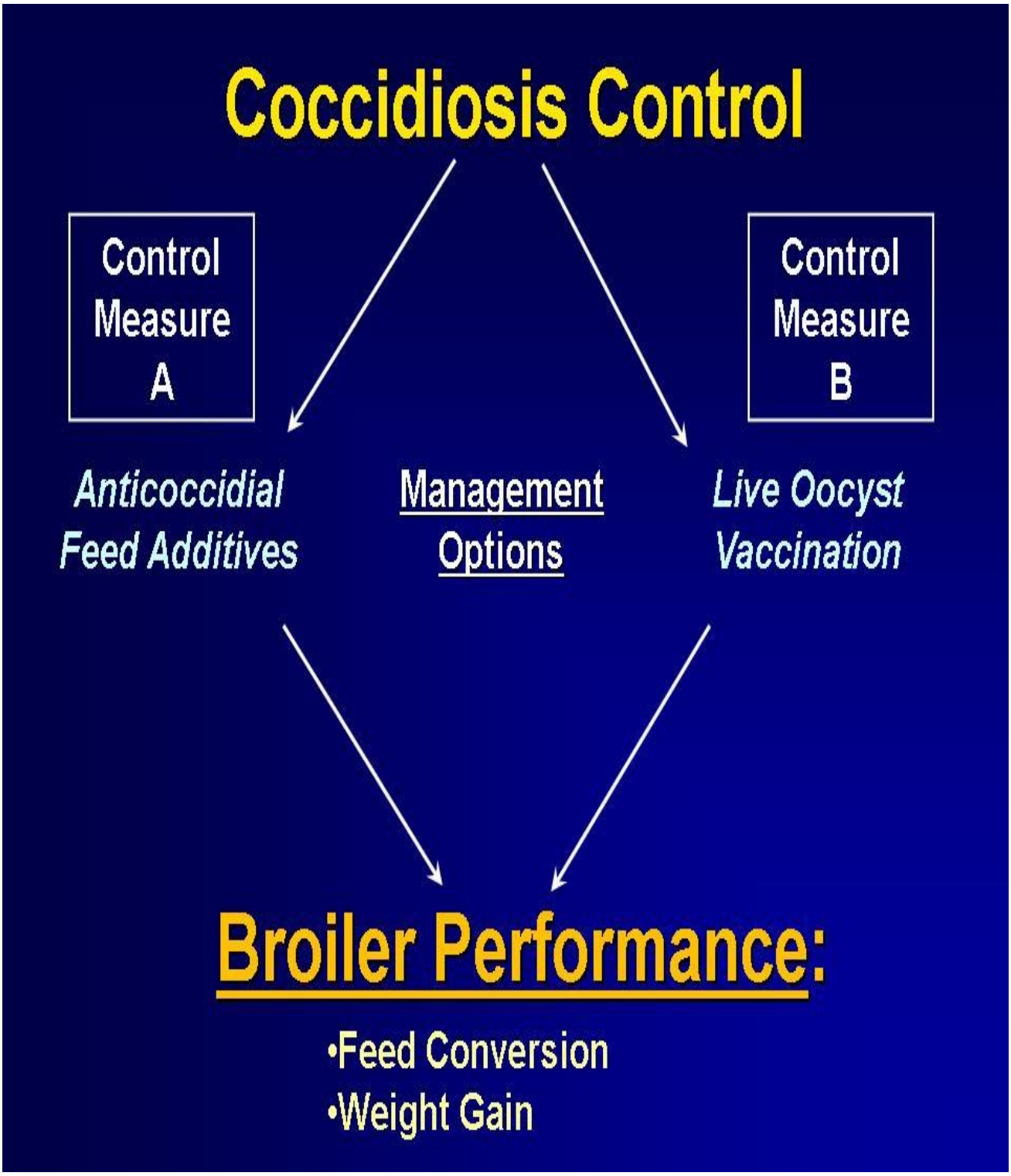
- Live oocyst vaccination has been used by the poultry industry for over 50 years. Initial use was primarily for broiler breeder and replacement layer stock.
  - Chapman *et al.*, (2002)
- The basis for vaccination is the host immunity that is generated against subsequent infections by the same species.
  - Yun *et al.*, (2000)

Efficacy of Vaccination

- Live oocyst vaccination affords protection against subsequent *Eimeria* challenge as evidenced by:
  - Increased body weight gain
    - Danforth (1998)
    - Crouch *et al.*, (2003)
    - Williams (2003)
    - Lee *et al.*, (2005)
  - Improved feed conversion ratios
    - Crouch *et al.*, (2003)
    - Lee *et al.*, (2005)
  - Reduced lesion development following challenge
    - Danforth (1998)
    - Crouch *et al.*, (2003)
    - Williams (2003)
    - Lee *et al.*, (2005)

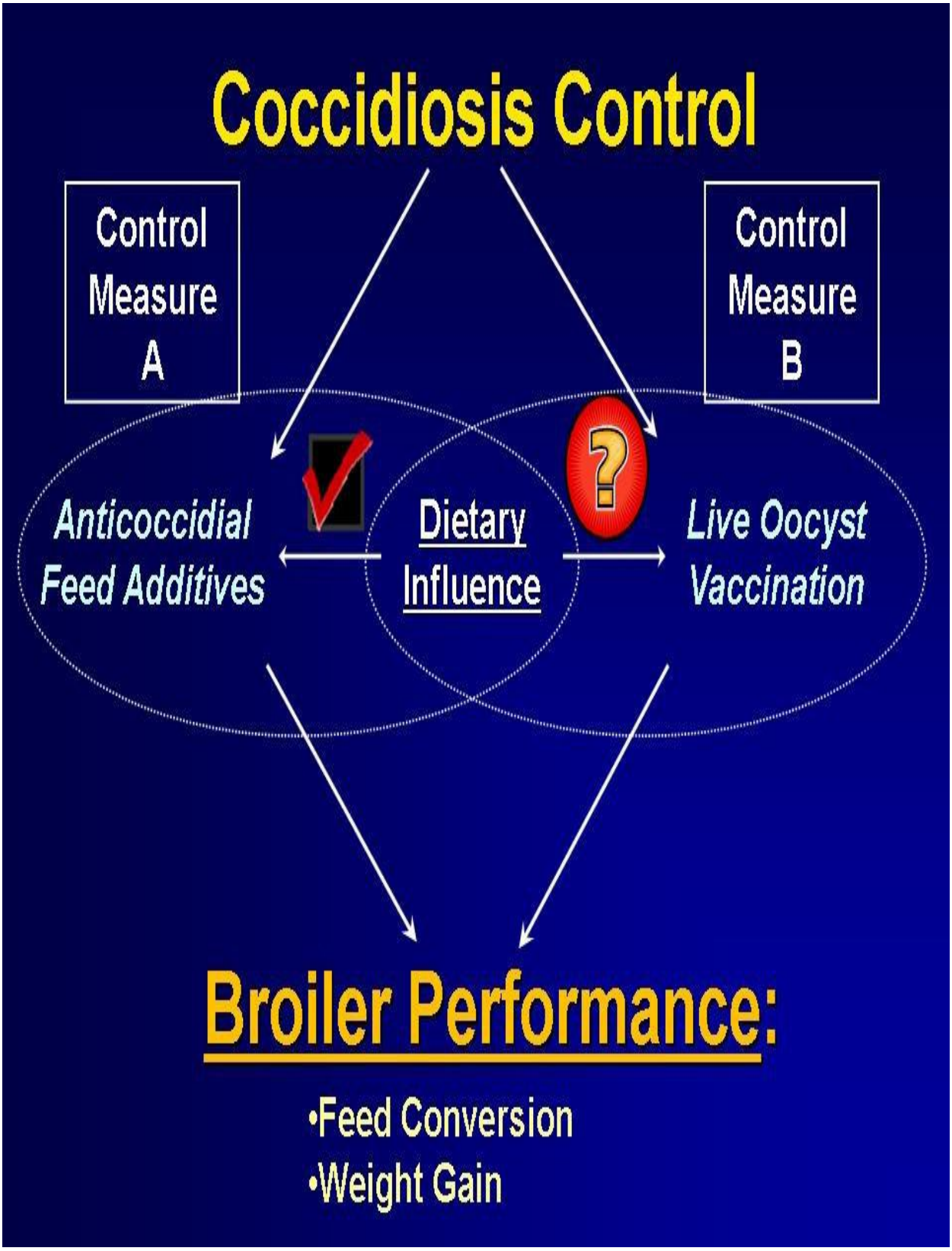
Limited Acceptance of Coccidiosis Vaccination within Main-Stream Broiler Production

- "Large-scale" broiler integrators within the U.S. have been reluctant to introduce vaccination programs due to reported reduced performance.
  - Allen and Fetterer (2002)
- Such reports involve decreased weight gain and increased feed conversion compared to feed-based anticoccidial usage during grow-out.
  - Williams (2002)
  - Waldenstedt *et al.*, (1999)
  - Danforth (1998)


Coccidiosis Control
Control Measure A
Control Measure B
Anticoccidial Feed Additives
Management Options
Live Oocyst Vaccination
Broiler Performance:
•Feed Conversion
•Weight Gain

Nutritional or Dietary Variability During Live Oocyst Vaccination

- Nutritional programs varied in the previously cited literature.
  - Dietary composition not described
  - NRC formulation
  - Diets formulated for anticoccidial usage used for vaccination
- A decrease in performance is due to impaired protein digestibility during periods of clinical coccidiosis.
  - Turk (1972)
- Previous research has indicated that varying protein levels can influence performance during clinical coccidial infection.
  - Sharma *et al.*, (1973)


Coccidiosis Control
Control Measure A
Control Measure B
Anticoccidial Feed Additives
Dietary Influence
Live Oocyst Vaccination
Broiler Performance:
•Feed Conversion
•Weight Gain

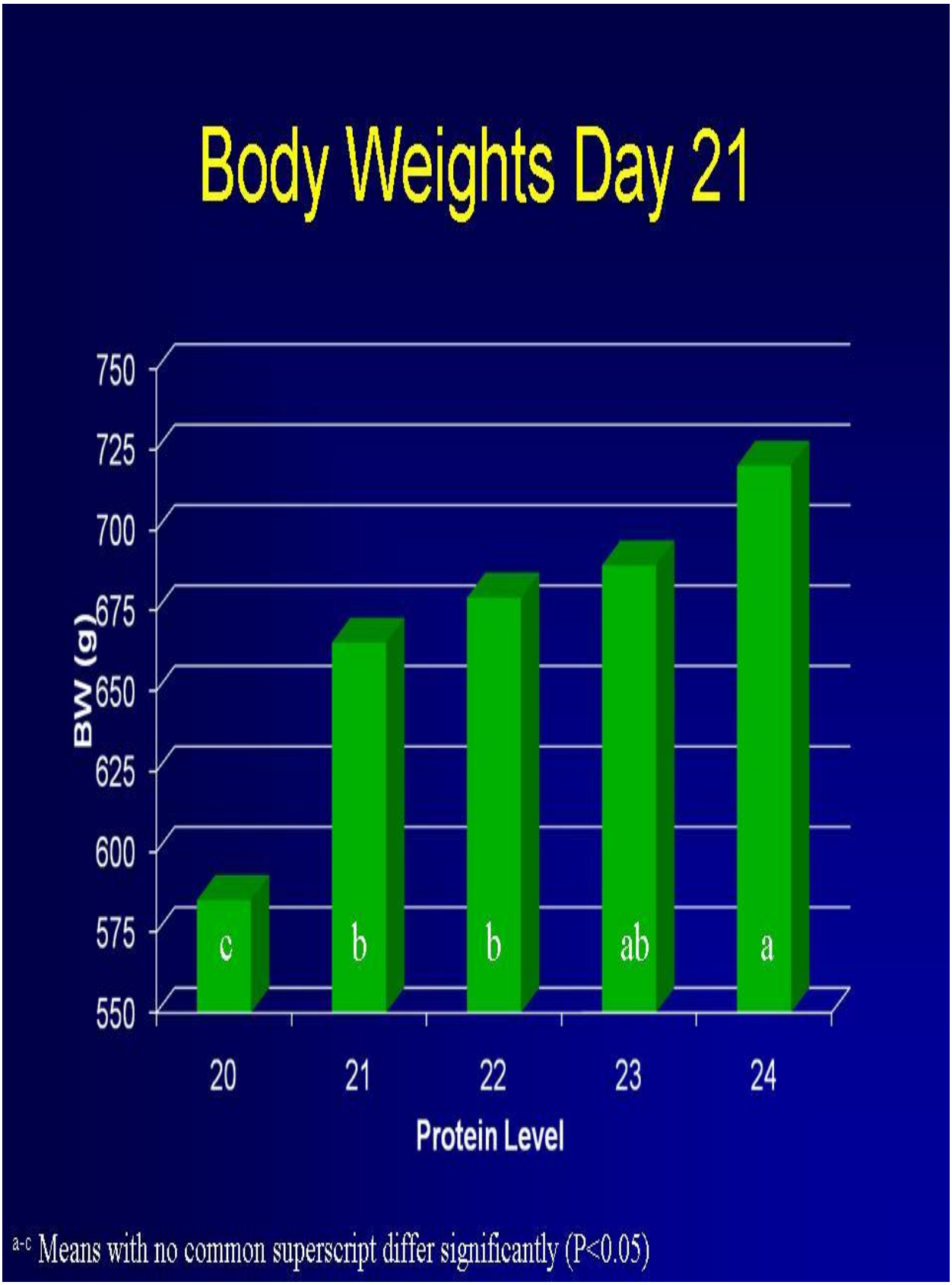
Working Hypothesis

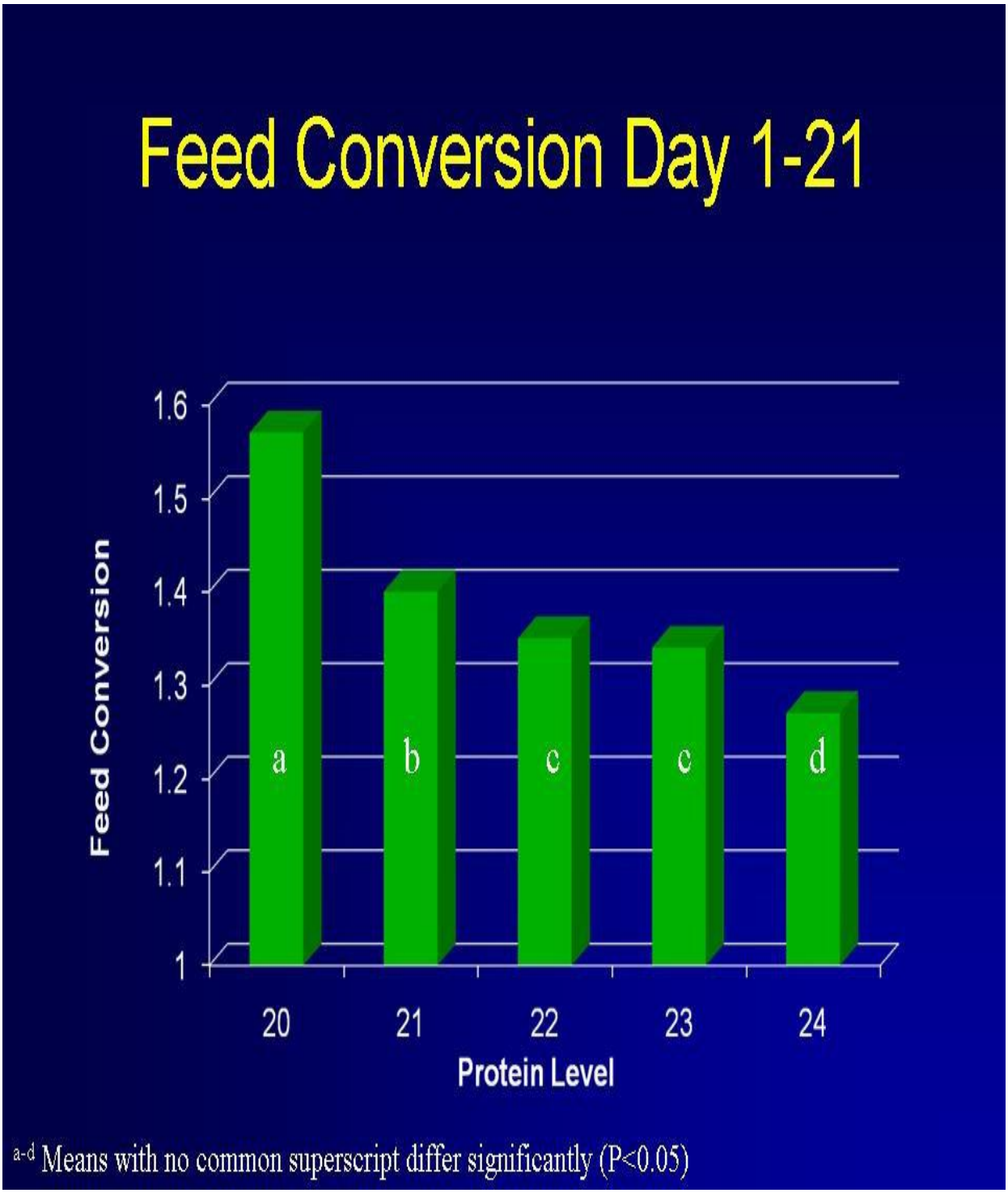
- Since extreme dietary variability exists in reports where live oocyst vaccination has been compared to anticoccidial drug usage, particularly in studies where vaccination has failed, we *hypothesize* that such failures are in part due to attempting to vaccinate against coccidiosis using a nutritional and management platform designed for anticoccidial drug usage.
- Experimental Objective: investigate the influence of dietary protein level and other micro ingredients on coccidiosis vaccine efficacy.

Materials and Methods
Trial 1

- Experiment 1 consisting of 5 dietary protein levels with eight replicates. Each replicate contained 25 chicks for a total of 1000 chicks.
  - 20, 21, 22, 23, and 24% protein
  - ME (3200 Kcal/Kg)
- On day of hatch, all chicks were individually weighed, wing banded and vaccinated with Coccivac®-B[1] by oral gavage.
- Once vaccinated, chicks were randomly assigned to treatment groups using chick weight.

[1] Schering Plough Animal Health Corp., Millsboro, DE.


Body Weights Day 21
750
725
700
675
650
625
600
575
550
BW (g)
c
b
b
ab
a
20
21
22
23
24
Protein Level
a-c Means with no common superscript differ significantly (P<0.05)

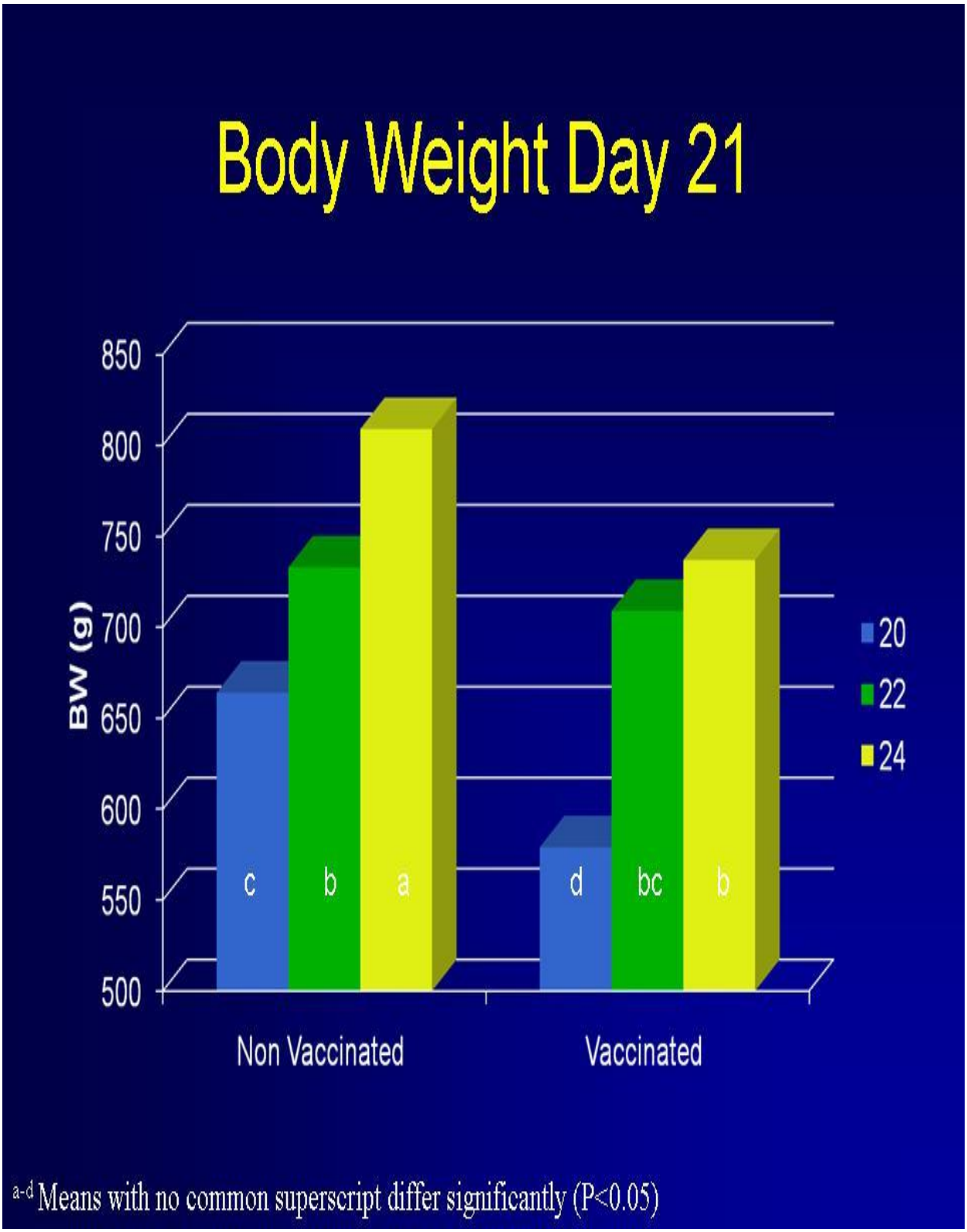

Feed Conversion Day 1-21
Feed Conversion
1.6
1.5
1.4
1.3
1.2
1.1
1
a
b
c
c
d
20
21
22
23
24
Protein Level
a-d Means with no common superscript differ significantly (P<0.05)

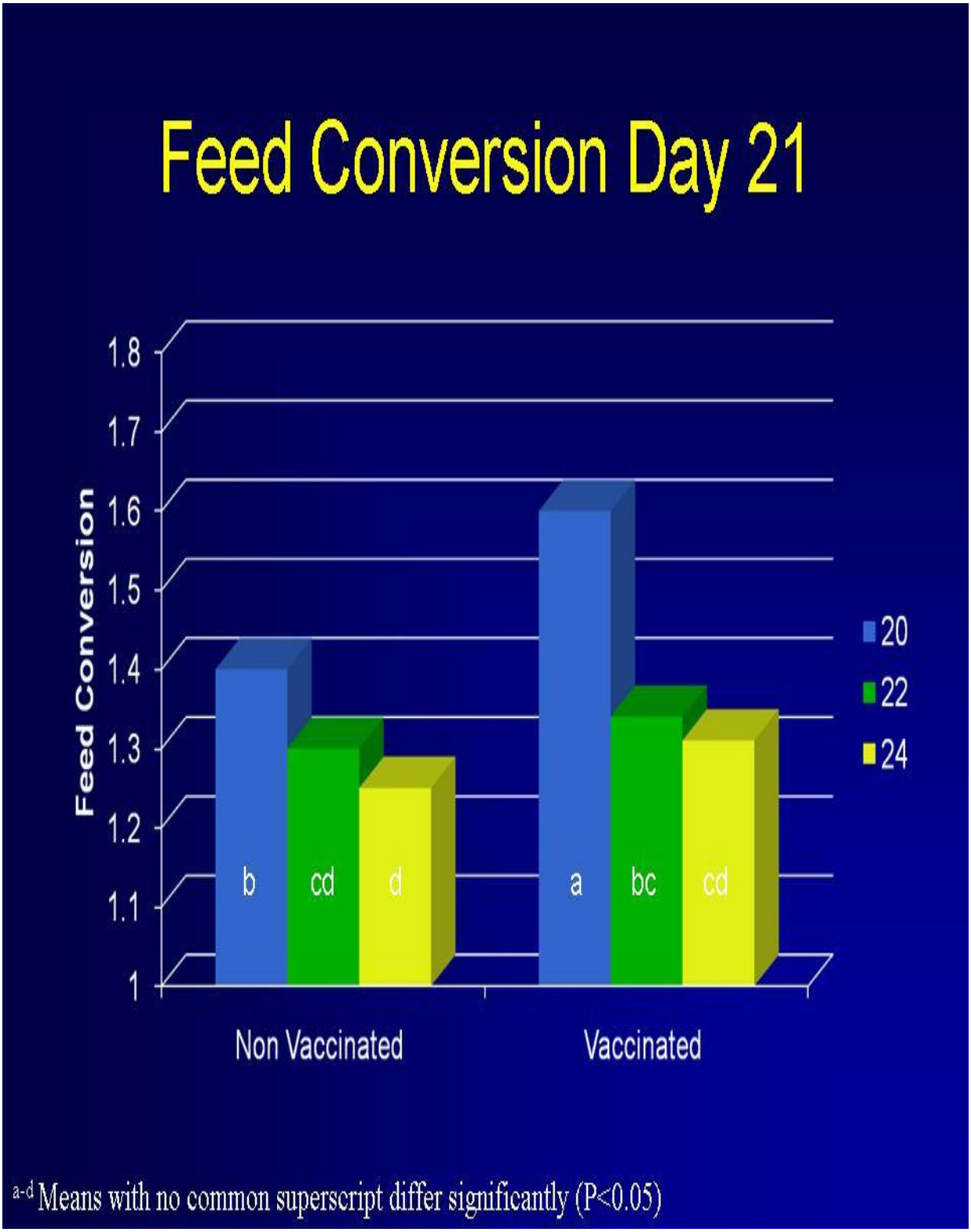
Summary

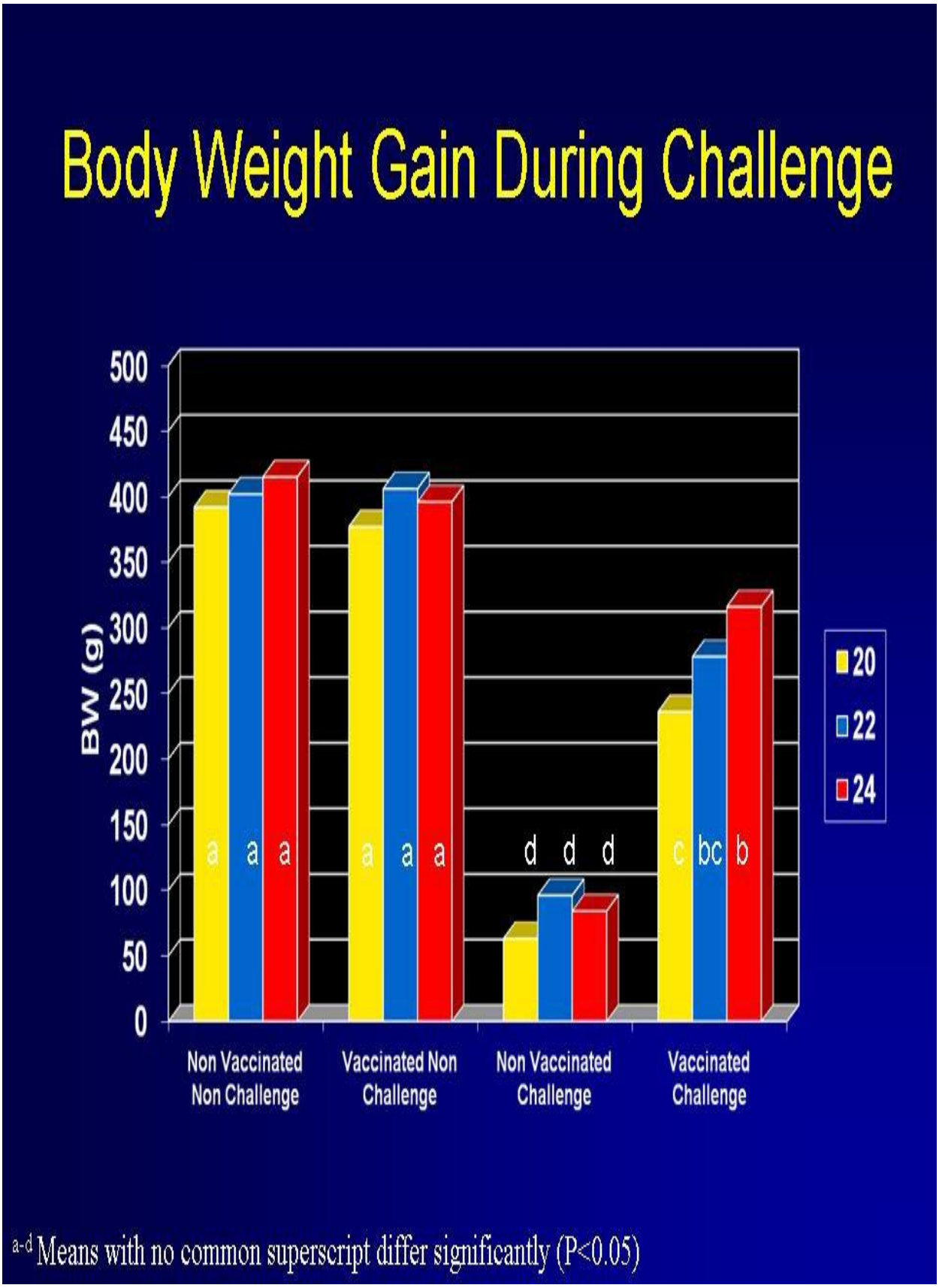
Trial 1

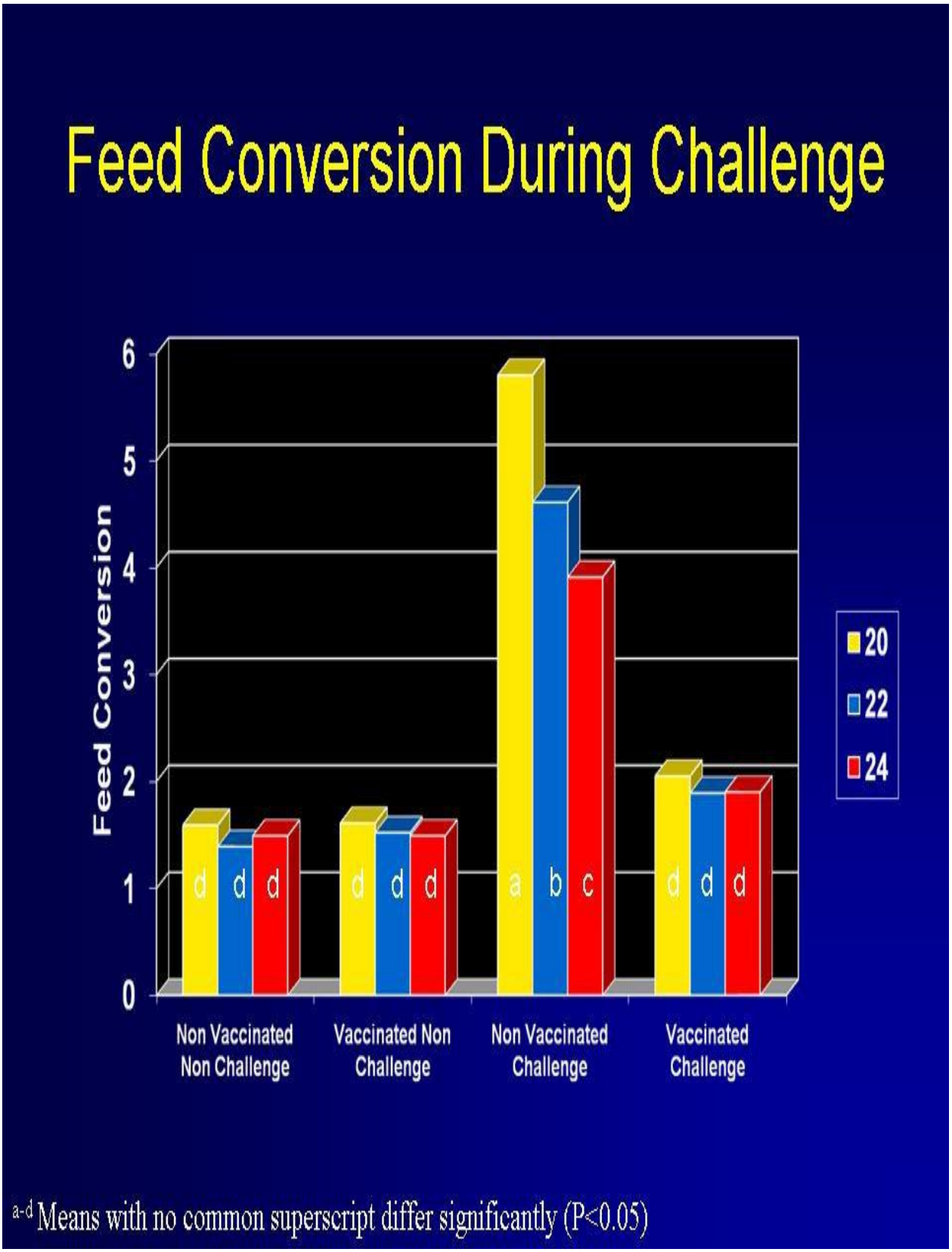
- In vaccinated chicks, body weight gain and feed conversion was directly related to increasing dietary protein levels.
- Broilers fed 20, 22, and 24% protein diets significantly differ in body weight and feed conversion values.
- These three proteins levels were chosen for use in subsequent experiments.


Materials and Methods
Trial 2
900 Randomly assigned broilers
Vaccinated
Non-Vaccinated
20%
22%
24%
20%
22%
24%
Non-Challenge
Non-Challenge
Non-Challenge
Non-Challenge
Non-Challenge
Non-Challenge
Challenge
Challenge
Challenge
Challenge
Challenge
Challenge


Body Weight Day 21
BW (g)
850
800
750
700
650
600
550
500
c
b
a
d
bc
b
Non Vaccinated
Vaccinated
20
22
24
a-d Means with no common superscript differ significantly (P<0.05)


Feed Conversion Day 21
Feed Conversion
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
b
cd
d
a
bc
cd
20
22
24
Non Vaccinated
Vaccinated
a-d Means with no common superscript differ significantly (P<0.05)


Body Weight Gain During Challenge
BW (g)
500
450
400
350
300
250
200
150
100
50
0
a a a
a a a
d d d
c bc b
Non Vaccinated Non Challenge
Vaccinated Non Challenge
Non Vaccinated Challenge
Vaccinated Challenge
20
22
24
a-d Means with no common superscript differ significantly (P<0.05)

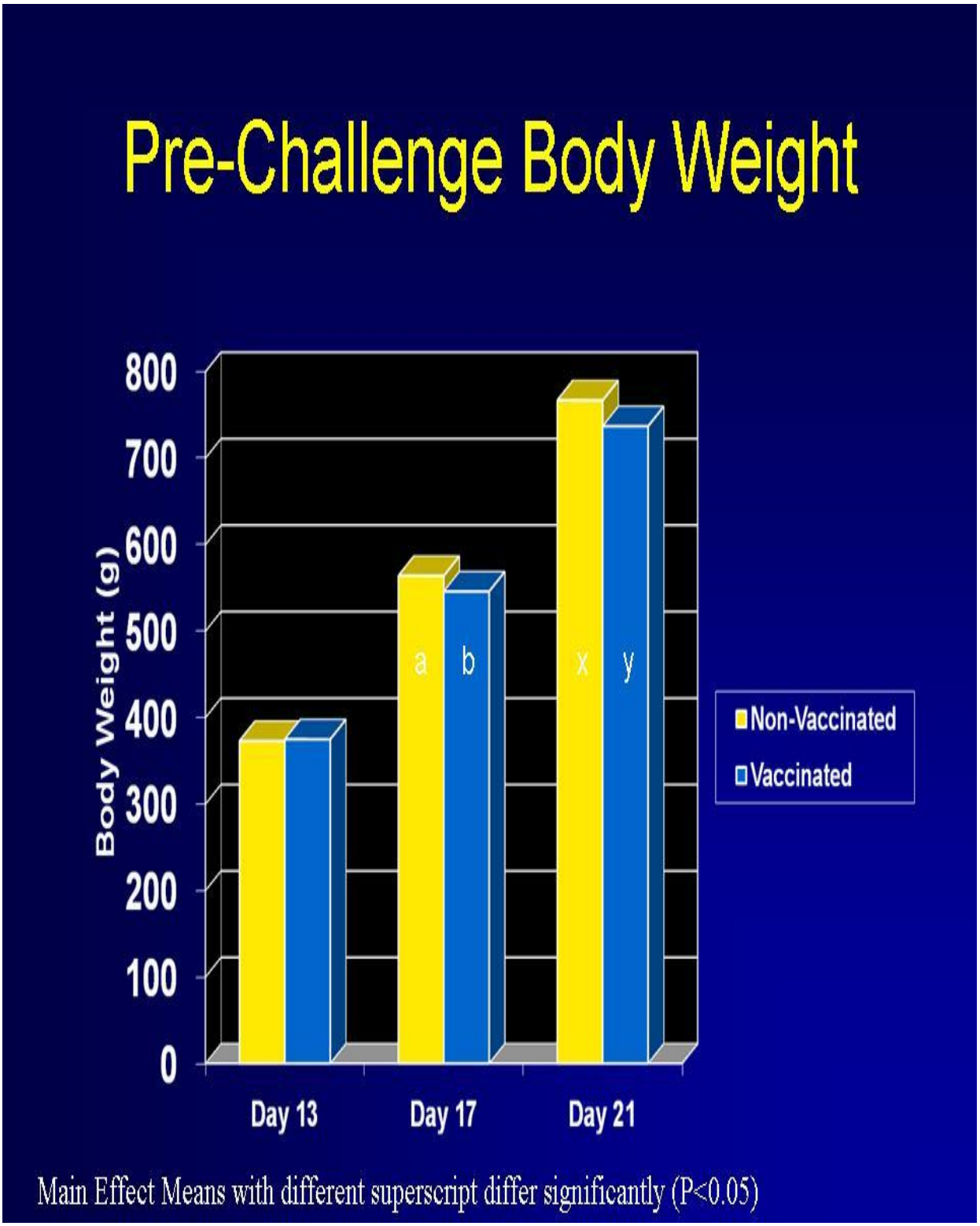

Feed Conversion During Challenge
Feed Conversion
0
1
2
3
4
5
6
d d d
d d d
a b c
d d d
Non Vaccinated Non Challenge
Vaccinated Non Challenge
Non Vaccinated Challenge
Vaccinated Challenge
20
22
24
a-d Means with no common superscript differ significantly (P<0.05)

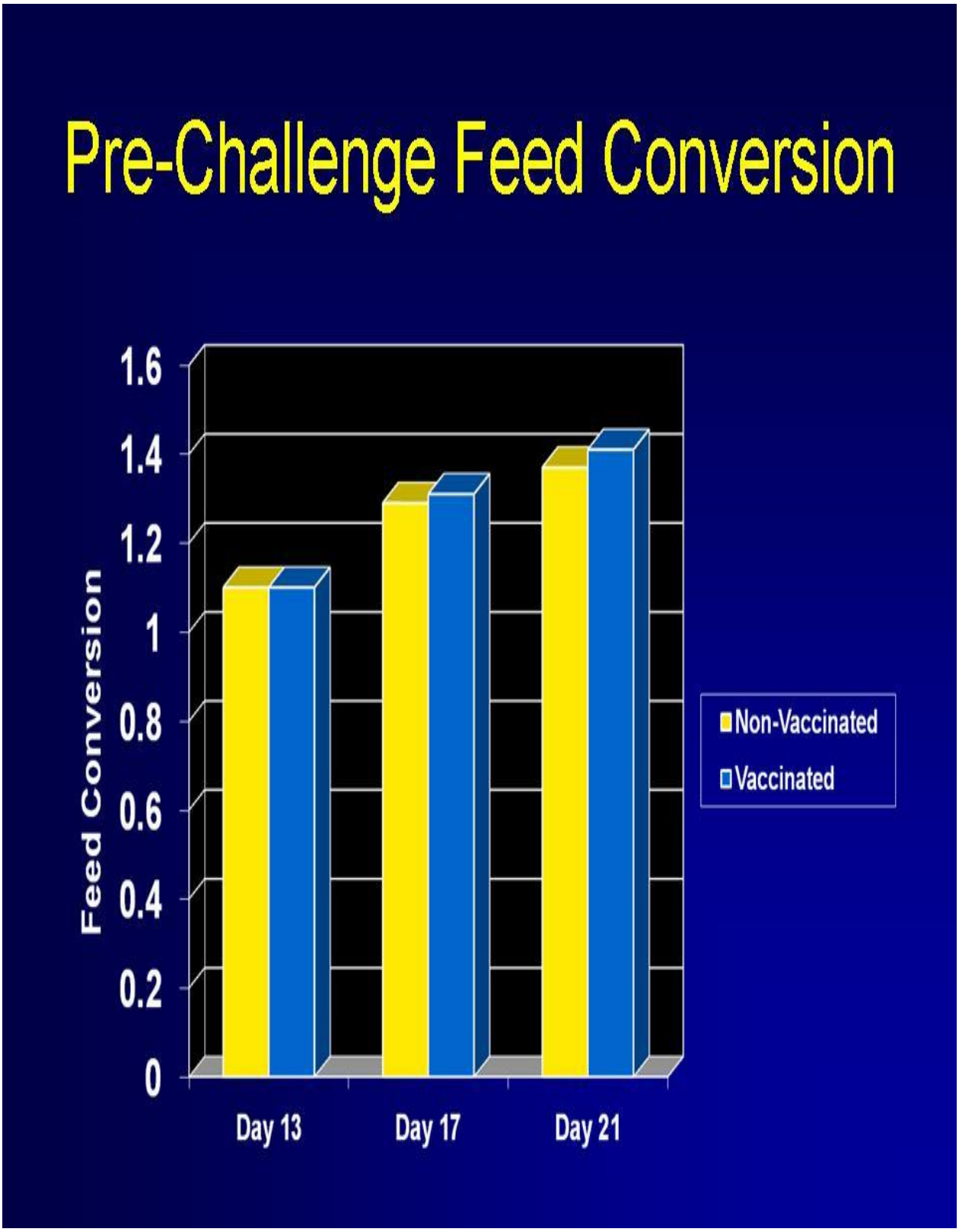
Summary Trial 2

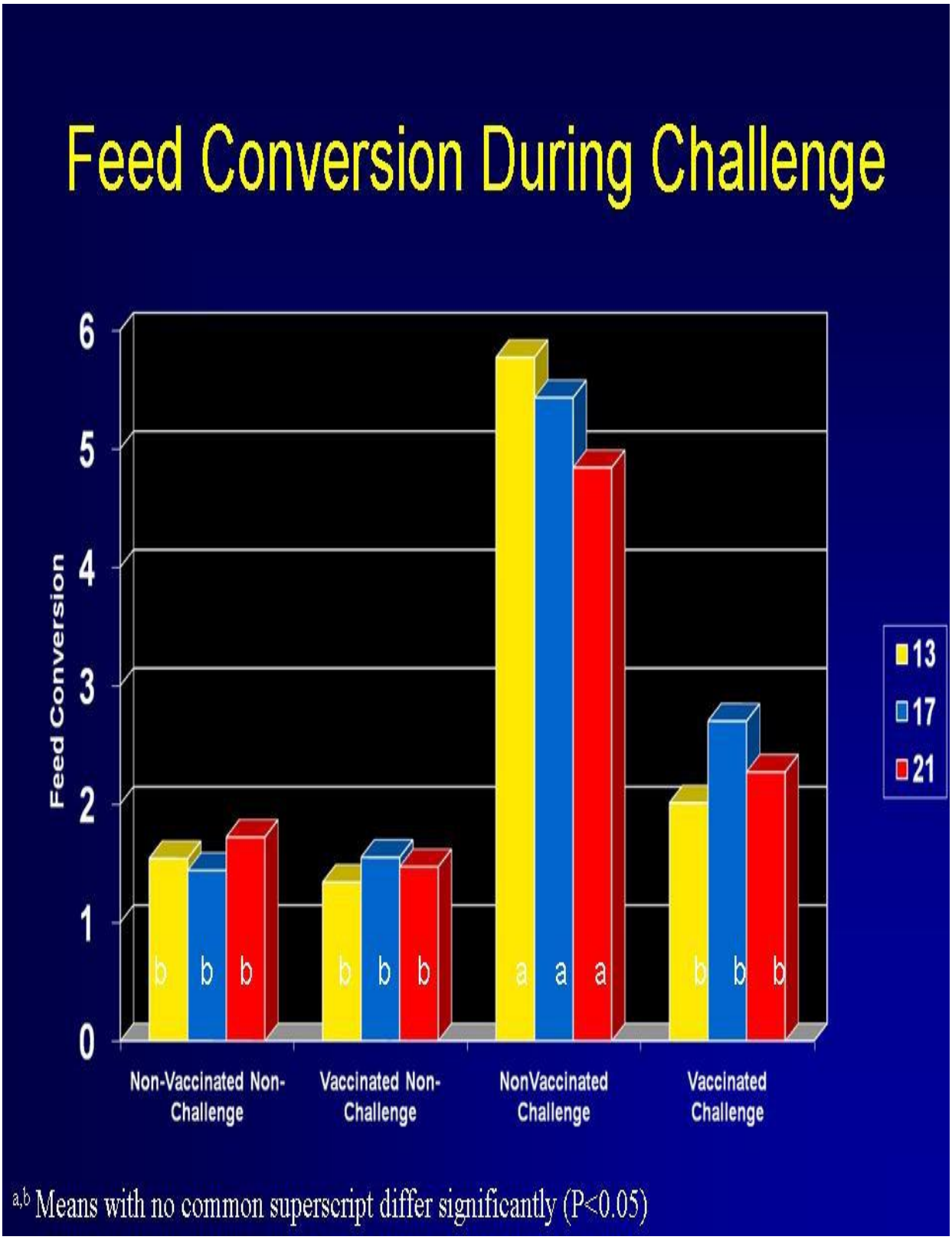
Weight Gain and Feed Conversion

- Vaccination increased body weight gain and decreased feed conversion of broilers during challenge when compared to non-vaccinated broilers.
- Vaccinated broilers fed 22% protein performed equal to the 24% dietary level post challenge.


Experimental Design
Trial 3
900 Randomly assigned broilers
Vaccinated
Non-Vaccinated
13 d
17 d
21 d
13 d
17 d
21 d
Non-Challenge
Non-Challenge
Non-Challenge
Non-Challenge
Non-Challenge
Non-Challenge
Challenge
Challenge
Challenge
Challenge
Challenge
Challenge


Pre-Challenge Body Weight
Body Weight (g)
800
700
600
500
400
300
200
100
0
a
b
x
y
Day 13
Day 17
Day 21
Non-Vaccinated
Vaccinated
Main Effect Means with different superscript differ significantly (P<0.05)


Pre-Challenge Feed Conversion
Feed Conversion
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Day 13
Day 17
Day 21
Non-Vaccinated
Vaccinated


Feed Conversion During Challenge
Feed Conversion
0
1
2
3
4
5
6
b b b
b b b
a a a
b b b
Non-Vaccinated Non-Challenge
Vaccinated Non-Challenge
NonVaccinated Challenge
Vaccinated Challenge
13
17
21
a,b Means with no common superscript differ significantly (P<0.05)

Summary Trial 3

Body Weights and Feed Conversion

- On day 13, vaccinated broilers had similar performance characteristics compared to non-vaccinated.
- Body weights were reduced due to vaccination on Day 17 and 21.
- Starter duration had no effect on broiler body weight.
- Feed conversion was unaffected by vaccination pre-challenge.

Summary Trial 3

Body Weights and Feed Conversion

- During challenge, vaccinated broilers gained significantly more body weight and had reduced feed conversions compared to non-vaccinated broilers.
- During the challenge period, non-challenged broilers had similar performance parameters regardless of vaccination status.
- Cumulative feed conversion for non-challenged broilers were similar for non-vaccinated and vaccinated.

Cumulative Summary of Results

- The previous experimental trials support the following conclusions:

  1. Vaccination efficacy is influenced by dietary protein level during the starter phase of broiler grow-out.
  2. Vaccination generates protective immunity against subsequent clinical *Eimeria* challenge and promotes growth performance during a challenge period.

- Data collected thus far does not include results from an entire growout period.

- The forthcoming experiment was designed to investigate vaccine efficacy under similar conditions during a complete growout period compared to a common ionophore used to control coccidiosis.

Materials and Methods
Trial 4

- Design: 2 x 2 factorial (treatment by diet)
- Animals: 1720 Cobb x Ross straight-run chicks with standard vaccination and processing
- House design: All treatments were blocked randomized into a total of 40 pens with 43 chicks placed per pen and 10 replicate pens per treatment.

Experimental Design
Trial 4

- Diets: all diets were corn/soy based rations and fed by the following schedule during grow out:
  - Starter: placement to 14 days
  - Grower: 14 to 29 days
  - Finisher: 29 to 40 days
  - Withdrawal: 40 to 50 days
- Starter, grower, and finisher diets contain growth promoters similar to industry standards.
  - Starter: Salin. (60g/ton) + BMD (50g/ton)
  - Grower: Salin. (60g/ton) + BMD (25g/ton) + roxarzone (45g/ton)
  - Finisher: Salin. (50g/ton) + BMD (25g/ton) + roxarzone (34g/ton)
- Chicks in Coccivac-B groups did not receive Salinomycin in diets.

Dietary Composition

- Diet A
  - Starter - 21.5% Protein and 3080 Kcal/Kg
  - Grower – 20.0% Protein and 3125 Kcal/Kg
  - Finisher – 16.5% Protein and 3190 Kcal/Kg
  - Withdrawal – 15.75% Protein and 3245 Kcal/Kg
- Diet B
  - Starter - 22.0% Protein and 3080 Kcal/Kg
  - Grower – 19.6% Protein and 3125 Kcal/Kg
  - Finisher – 17.8% Protein and 3190 Kcal/Kg
  - Withdrawal – 17.5% Protein and 3245 Kcal/Kg

Comparisons between Starter Diets: Diet A vs. Diet B

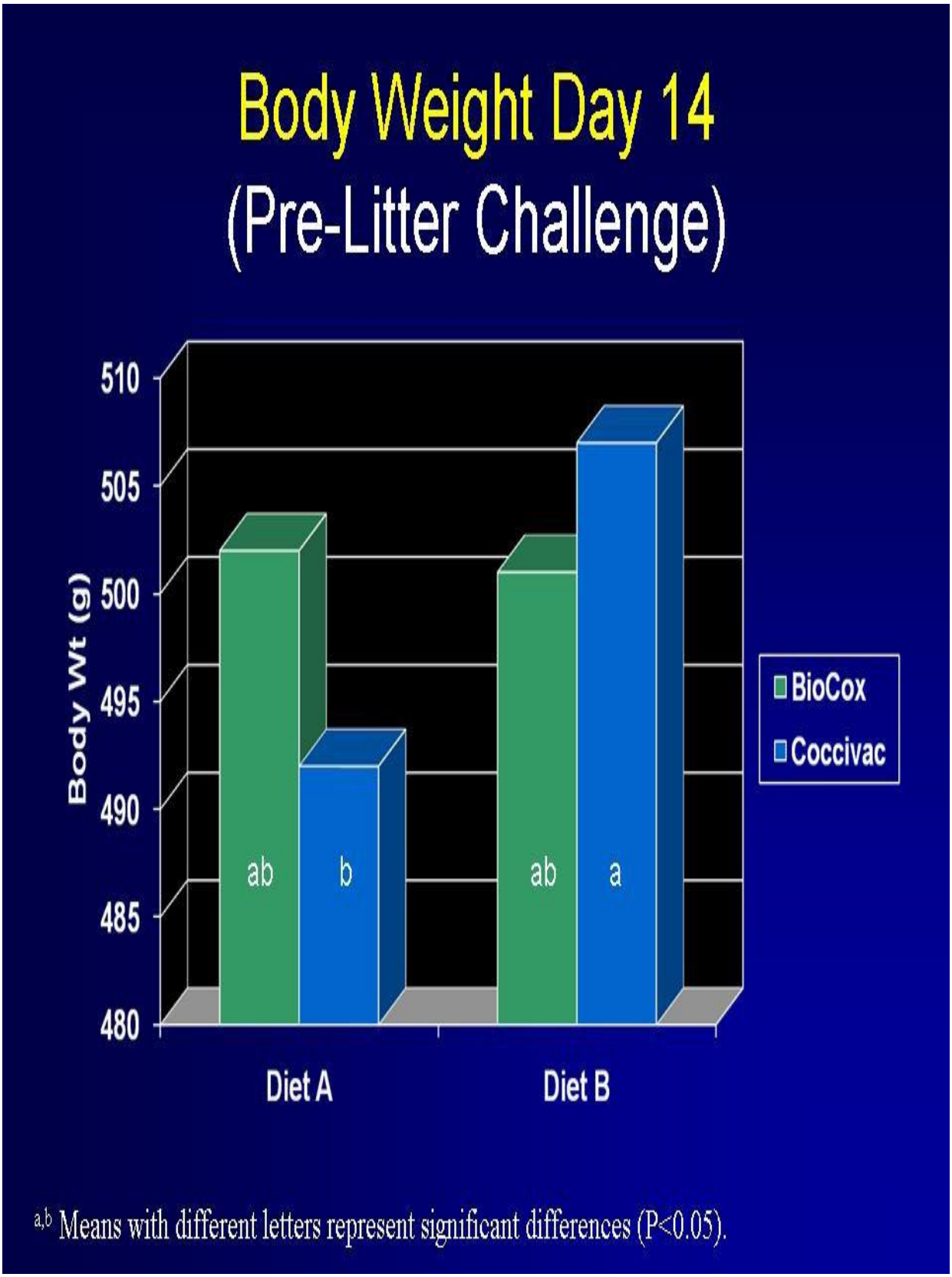
Dietary Component:	Diet A	Diet B
Total Protein:	21.5%	22.0%
Methionine	0.58	0.59
Lysine	1.22	1.27
TSAA	0.95	0.97
ME (kcal/kg)	3080	3080

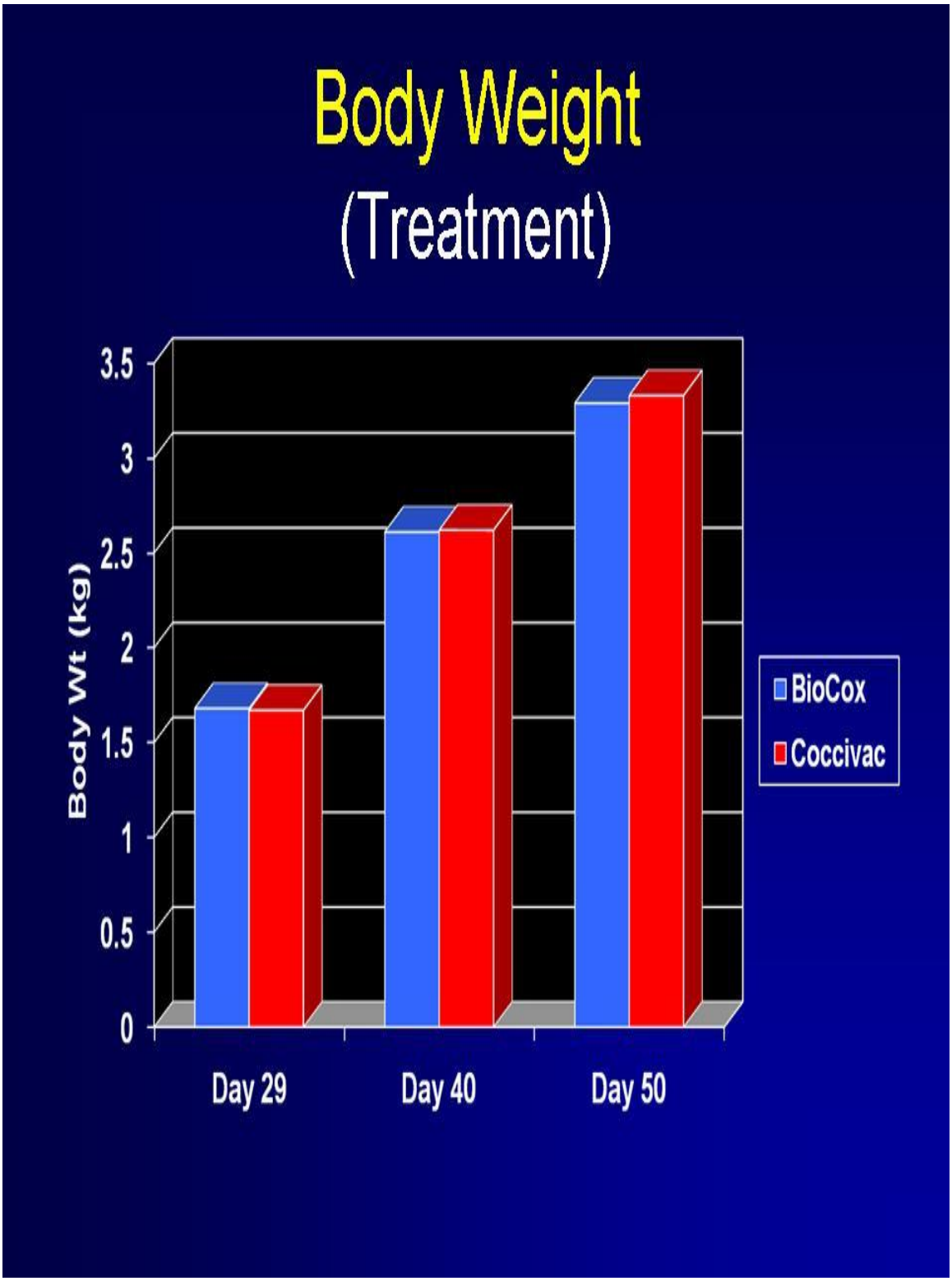
Comparisons between Grower Diets: Diet A vs. Diet B

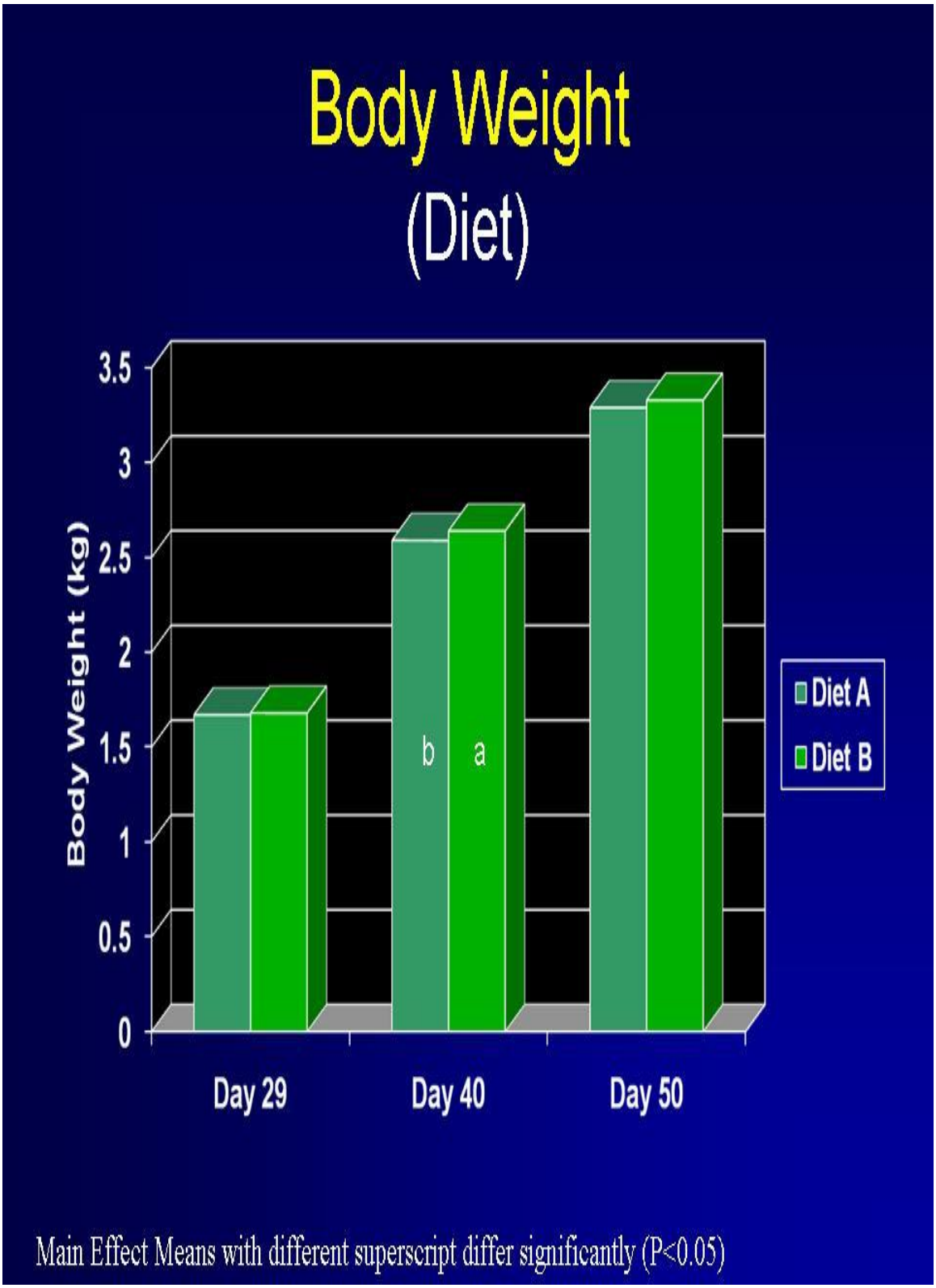
Dietary Component:	Diet A	Diet B
Total Protein:	20.0%	19.6%
Methionine	0.55	0.56
Lysine	1.14	1.12
TSAA	0.90	0.90
ME (kcal/kg)	3135	3135

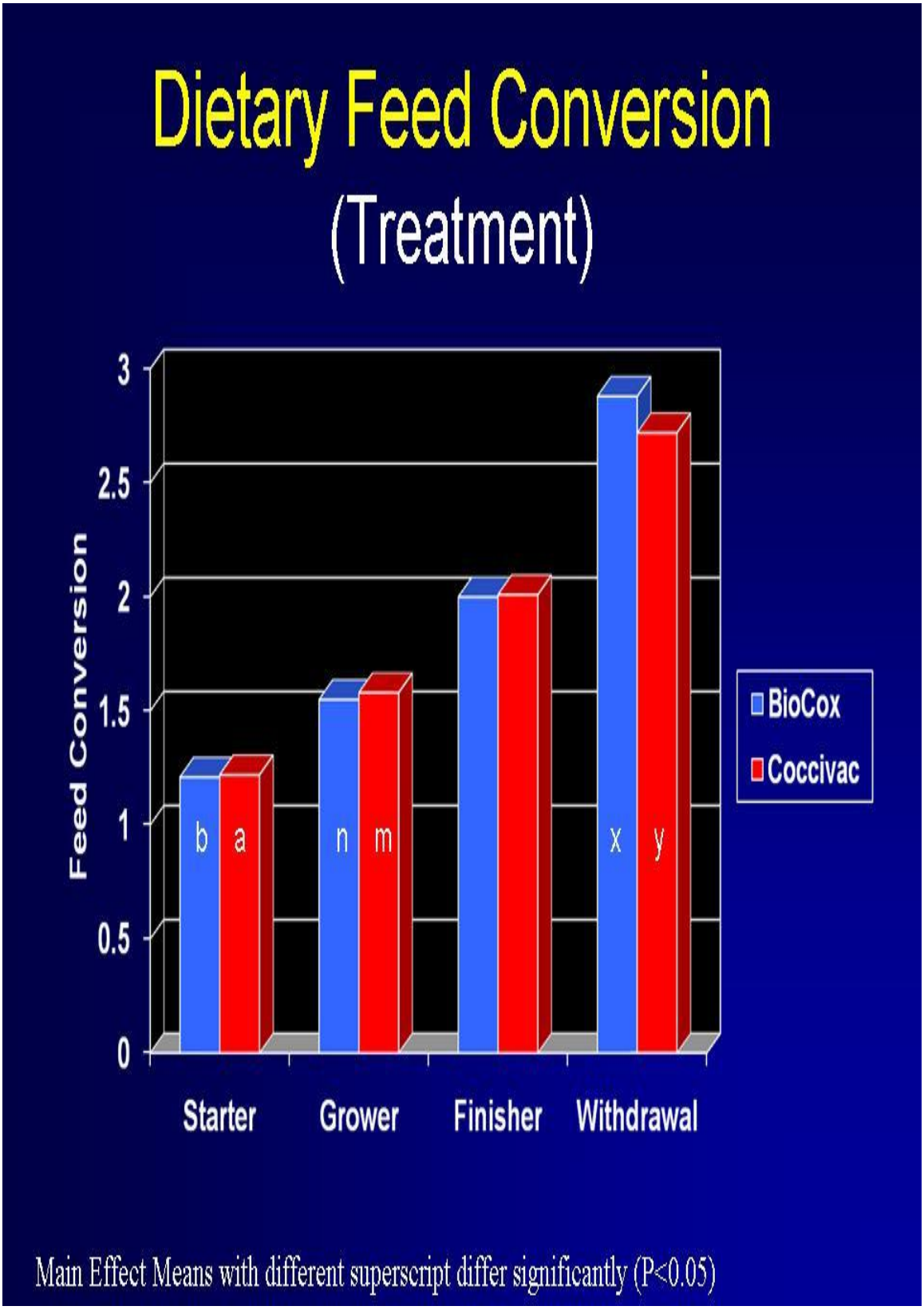
Challenge and Data Collection

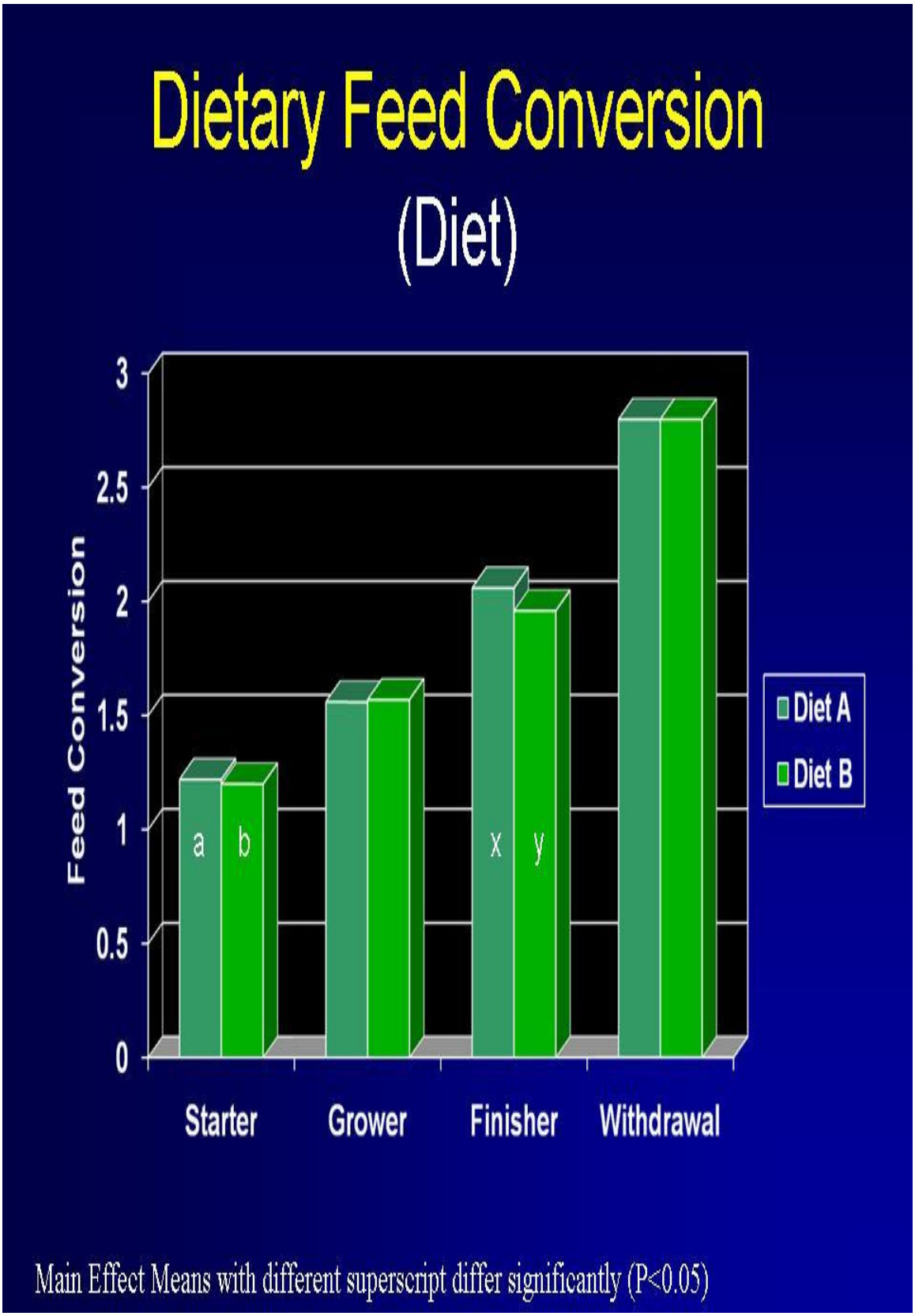
- On day 14, oocysts isolated from local commercial broiler farms were spray applied to the litter in each pen.
  - 40,000 oocysts/chick
  - *E. acervulina*, *E. maxima* and *E. tenella* present in inoculum
- Pen bulk weight and feed consumption was determined on days of dietary changes.

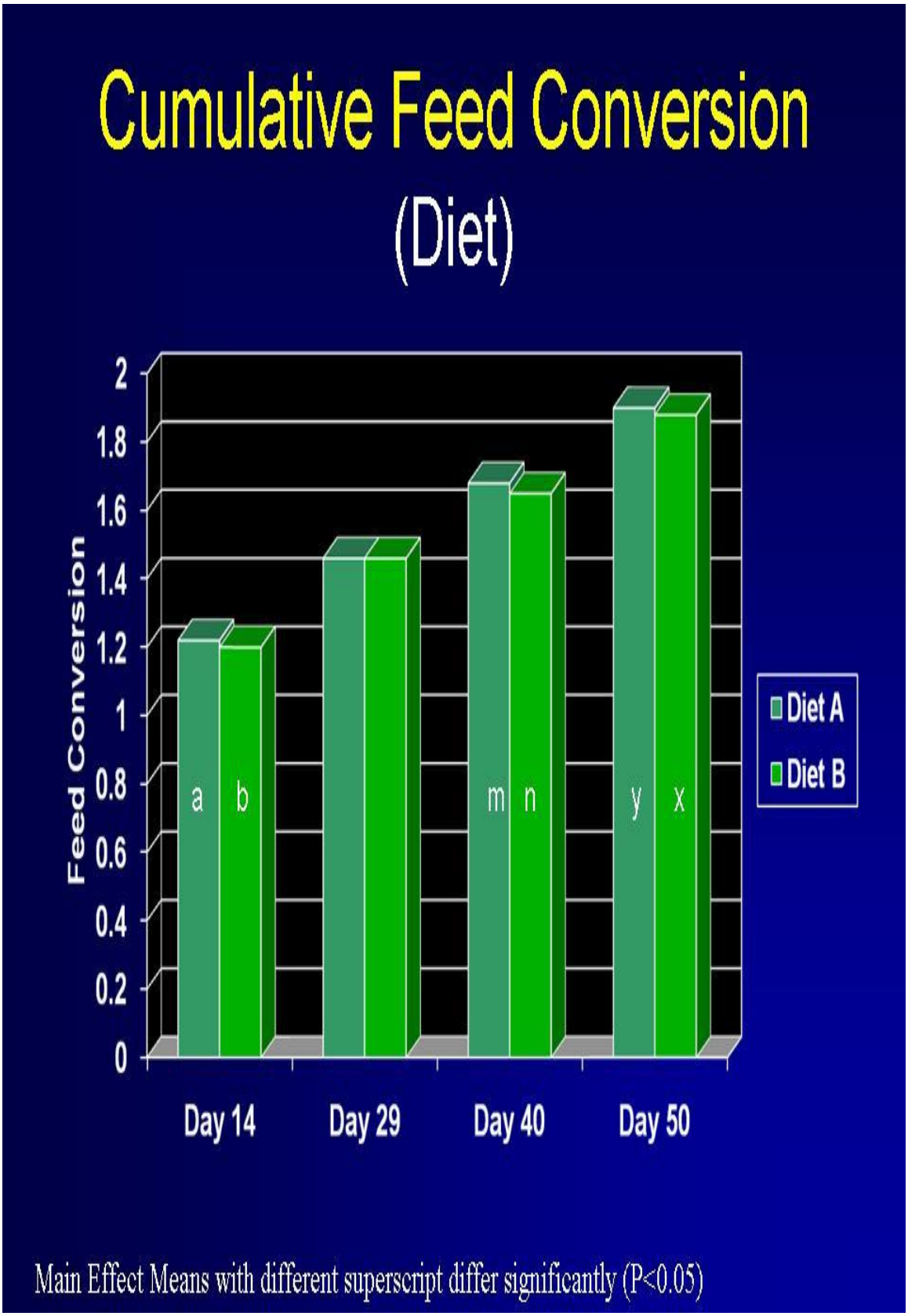

Body Weight Day 14
(Pre-Litter Challenge)
Body Wt (g)
510
505
500
495
490
485
480
ab
b
ab
a
Diet A
Diet B
BioCox
Coccivac
a,b Means with different letters represent significant differences (P<0.05).


Body Weight
(Treatment)
Body Wt (kg)
3.5
3
2.5
2
1.5
1
0.5
0
Day 29
Day 40
Day 50
BioCox
Coccivac


Body Weight
(Diet)
Body Weight (kg)
3.5
3
2.5
2
1.5
1
0.5
0
b
a
Day 29
Day 40
Day 50
Diet A
Diet B
Main Effect Means with different superscript differ significantly (P<0.05)


Dietary Feed Conversion
(Treatment)
Feed Conversion
3
2.5
2
1.5
1
0.5
0
b
a
n
m
x
y
Starter
Grower
Finisher
Withdrawal
BioCox
Coccivac
Main Effect Means with different superscript differ significantly (P<0.05)


Dietary Feed Conversion
(Diet)
Feed Conversion
3
2.5
2
1.5
1
0.5
0
a
b
x
y
Starter
Grower
Finisher
Withdrawal
Diet A
Diet B
Main Effect Means with different superscript differ significantly (P<0.05)


Cumulative Feed Conversion
(Diet)
Feed Conversion
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
b
m
n
y
x
Day 14
Day 29
Day 40
Day 50
Diet A
Diet B
Main Effect Means with different superscript differ significantly (P<0.05)

Summary

Body Weight

- Coccivac-B broilers fed Diet B had the heaviest ending body weights and were significantly heavier at day 14 compared to Coccivac-B broilers fed diet A. Both Bio-Cox groups were similar to both groups of Coccivac-B broilers at Day 14.
- There were no differences in body weight at day 50 with respect to coccidial control measure or dietary regimen.
- No significant differences were observed in mortality (data not shown).

Summary

Feed Conversion

- Diet A yielded a significantly higher cumulative mortality corrected feed conversion ratio compared to diet B.
- Cumulative mortality corrected feed conversion was similar for Bio-Cox and Coccivac-B broilers.
- Feed conversion was significantly increased in Coccivac-B broilers versus Bio-Cox during the starter and grower periods and significantly lower during the withdrawal period.
- Replacement of medication with the vaccination program had no significant effects on cost/kg of live weight.

Overall Conclusions

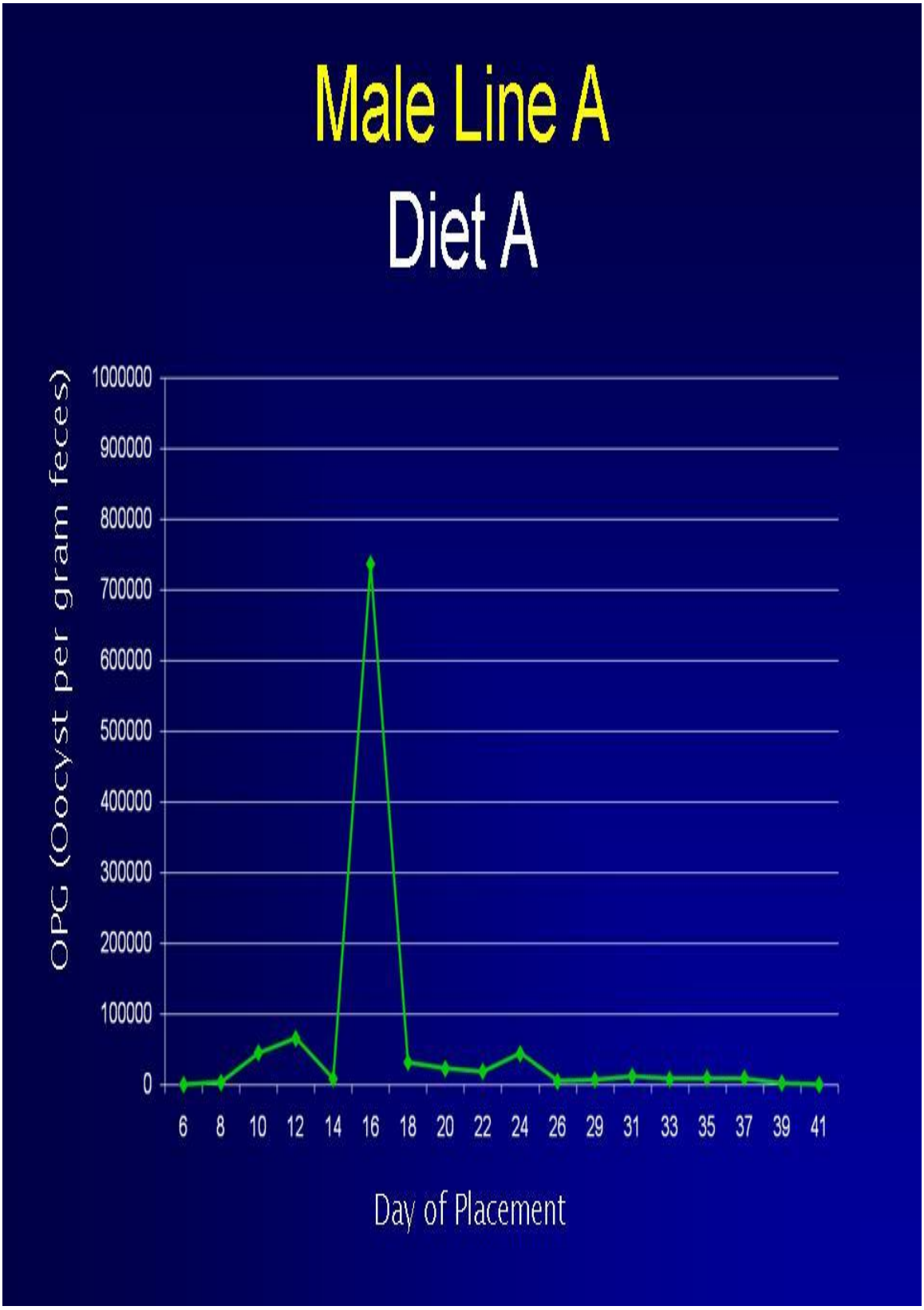
- Increasing dietary starter protein levels improves broiler performance.
- Vaccination is highly effective at generating immunity to *Eimeria* challenge.
- Vaccination improves broiler performance during an *Eimeria* challenge.
- Growth depression associated with vaccination appears between 13 and 17 days of age.

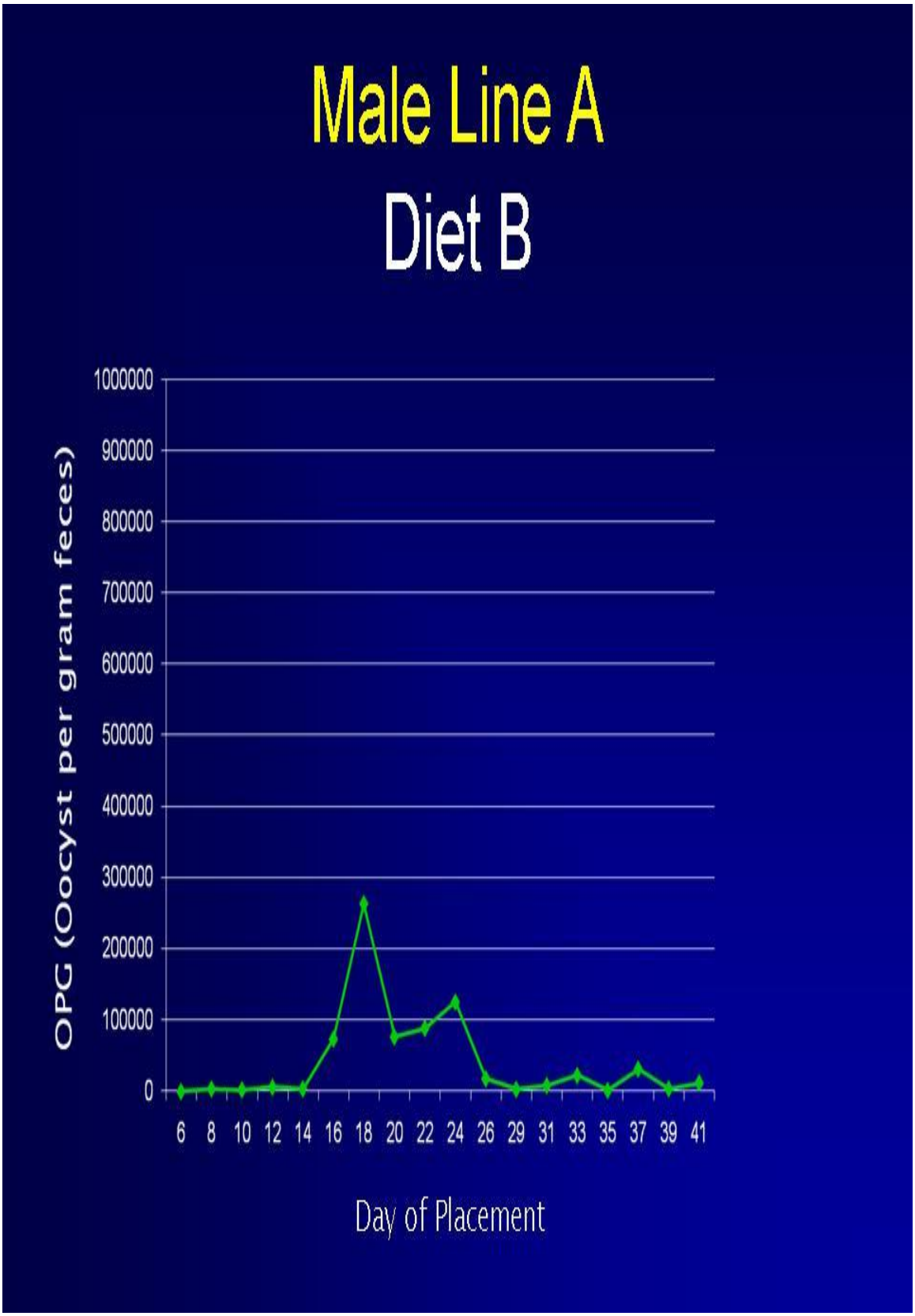
Recommendations

- Vaccination success depends upon early phase of production—specifically the starter period.
- Our research has shown a significant interaction between diet and vaccine efficacy / establishment when starter protein levels were specifically formulated and fed at a level of 22% protein.
- This enhanced establishment resulted in performance equivalent to ionophore usage in a less effective dietary platform.

Recommendations

- Other research offers insight into focusing early in production with vaccination—Teeter et al., 2007.
  - Majority of broiler growth occurs post-day 27
  - Insults to performance (*Eimeria* challenge) pre-day 27 can be overcome by compensatory gain.
  - Insults to performance post-day 27 result in permanent performance loss for the animal.
- Vaccination fits this model well:
  - Low-grade, controlled *Eimeria* infection early in production, peaks by Day 21, results in immunity development and improved performance during post-day 27 production.

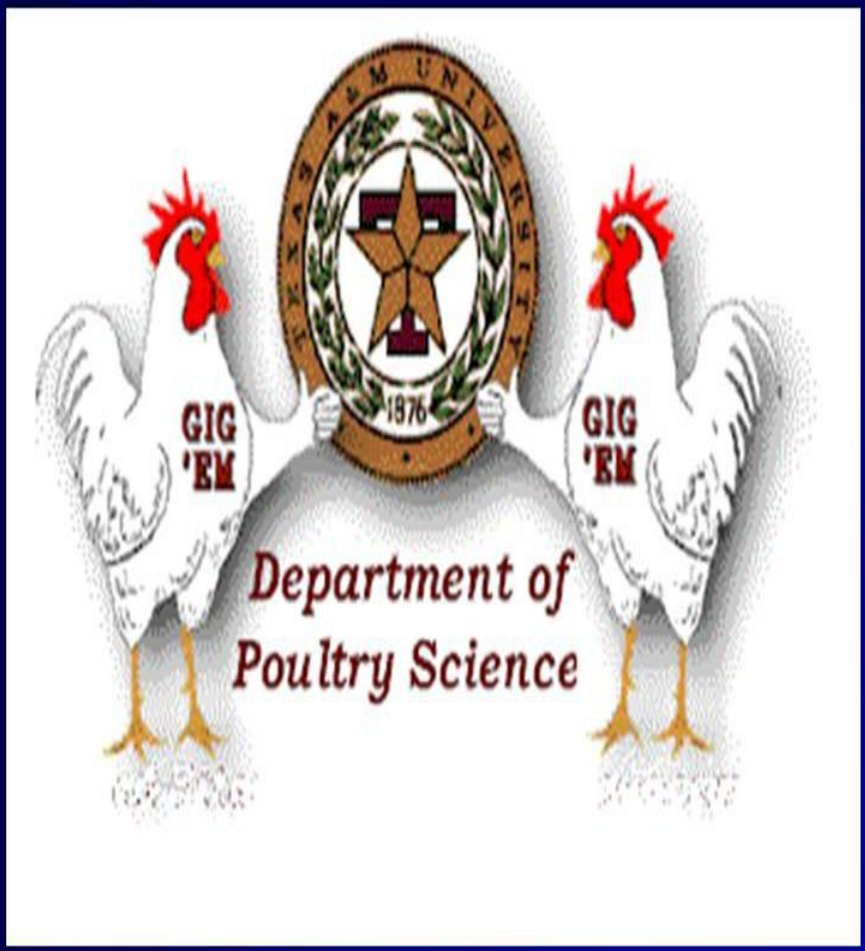

Male Line A
Diet A
OPG (Oocyst per gram feces)
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
6 8 10 12 14 16 18 20 22 24 26 29 31 33 35 37 39 41
Day of Placement


Male Line A
Diet B
OPG (Oocyst per gram feces)
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
6 8 10 12 14 16 18 20 22 24 26 29 31 33 35 37 39 41
Day of Placement

Acknowledgements

- Technical Assistance pertaining to vaccination:
  - Paul Burke, Schering-Plough Animal Health
- Technical assistance with formulation of experimental diets:
  - Dr. Phil Hargis, Hargis and Associates, Springdale Arkansas

Thank You



CARDIO-PULMONARY CHARACTERISTICS OF PULMONARY HYPERTENSION IN BROILERS INCLUDING THE IMPACT OF AEROSOLIZED LIPOPOLYSACCHARIDE

Gino Lorenzini.
University of Arkansas

Introduction

Pulmonary Hypertension (PH)

- PH is the sustained increase in pulmonary arterial pressure (PAP)

- PH develops when elevated PAP is necessary to propel required cardiac output (CO) through a non compliant pulmonary vasculature

Introduction
Pulmonary Hypertension (PH)

- PH has been extensively studied. However, there are areas where more research is needed to consolidate the understanding of PH

First chapter
Introduction

- Previous experiments confirmed a genetic component of PH in chickens (Anthony et al., 2001)

Objective

- To determine key differences in hemodynamics between experimental lines of broilers
  - PHS-resistant
  - PHS-susceptible
  - Relaxed (unselected)

Materials and methods

-Three lines selected by Dr. N. Anthony for >12 generations
Relaxed, PHS-Susceptible, PHS-Resistant

-A flow probe was positioned on the pulmonary artery → CO

-A catheter was introduced through the wing vein and advanced to the pulmonary artery → PAP

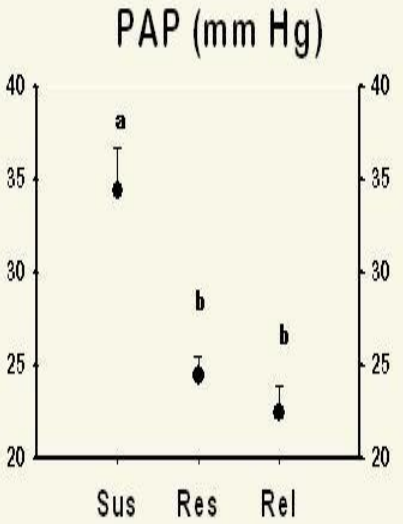
-Pulmonary vascular resistance (PVR) = PAP/CO

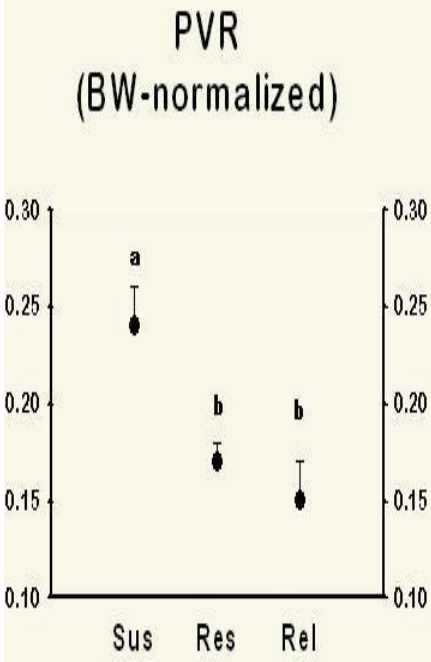
Results

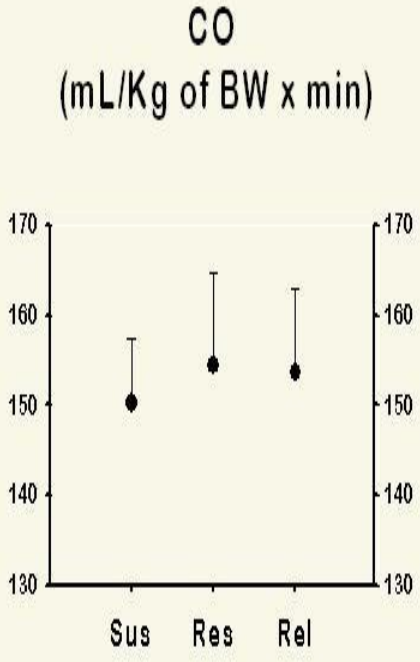
Sus = susceptible (n=12)

Res = resistant (n=12)

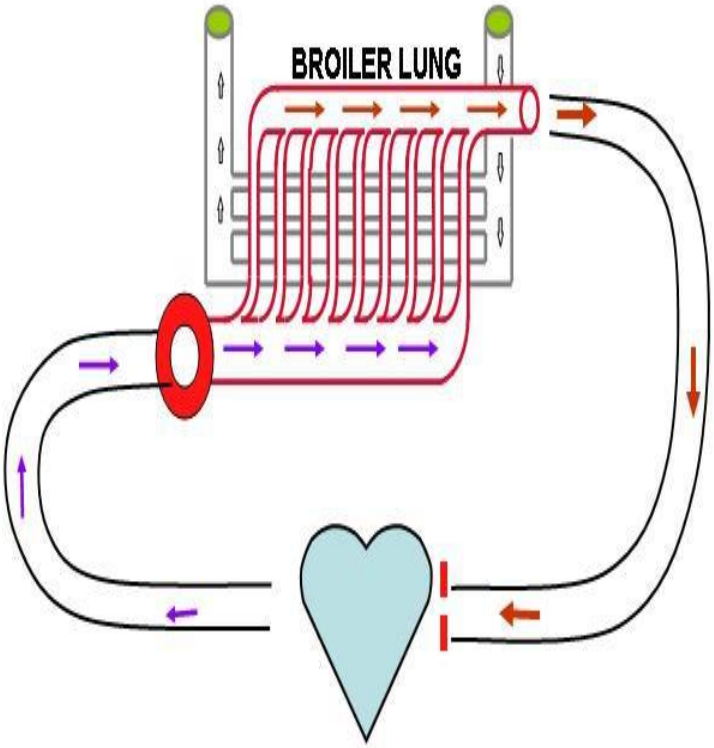
Rel = relaxed (n=12)







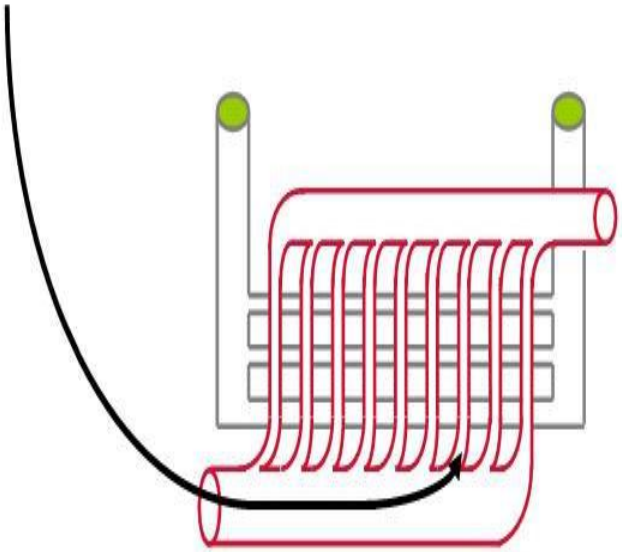
What Initiates Pulmonary Hypertension?



Hypothesis A: PAH
↑"Up-stream" Resistance

Hypothesis B: PVH
↑ "Down-stream" Resistance
(L. atrio-ventricular
valve failure; cardiomyopathy)

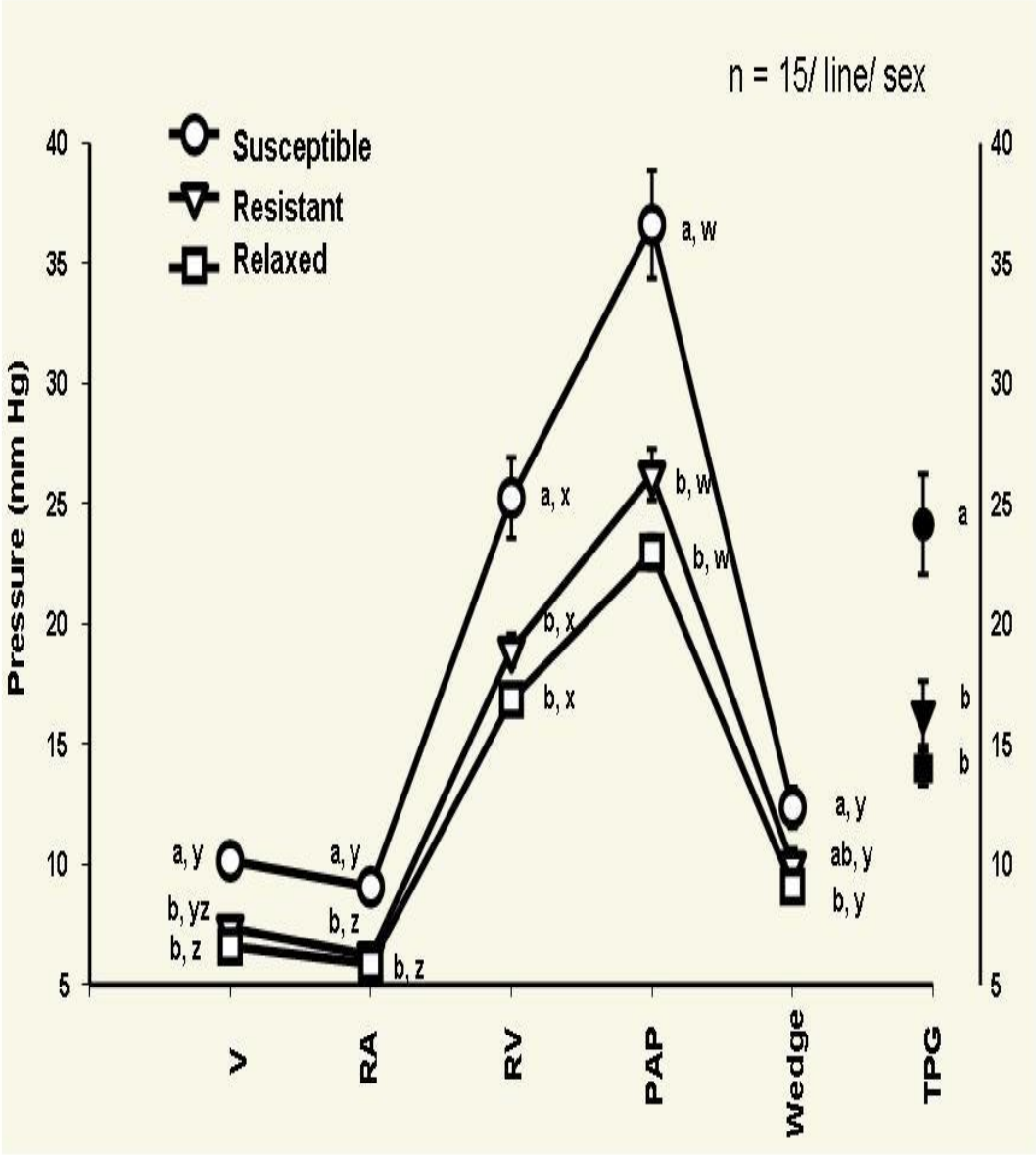
Materials and methods



Hypothesis A: PAH
↑PAP, ↓WP, ↑TPG

Hypothesis B: PVH
↑PAP, ↑WP, ↓TPG

Results


n = 15/ line/ sex
Susceptible
Resistant
Relaxed
Pressure (mm Hg)
40
35
30
25
20
15
10
5
a, w
a, x
b, w
b, w
b, x
b, x
a, y
a, y
ab, y
b, y
a, y
b, yz
b, z
b, z
b, z
a
b
b
V
RA
RV
PAP
Wedge
TPG

Conclusions

- Increase in PAP it is not due to increased CO
- Increase in PAP is due to increased PVR
- Due to the large TPG measured PVR is conclusively increased "up-stream"

Second chapter

Introduction

- Arterial blood of pre-ascitic and micro particle (MP) injected birds is not fully saturated with oxygen (<80 %)
- Diffusion limitation: blood flowing too rapidly through the gas exchange area does not achieve full saturation of hemoglobin with oxygen
- Arterial-venous shunts: a proportion of blood would leave the lungs without exposure to the gas exchange epithelia

Objective

- To define the cause of low arterial oxygenation in preascitic and in clinically healthy broilers injected with MP

- Hypothesis:

  If blood is not 100% exposed to the gas exchange area, arterial blood will not be 100% saturated with oxygen

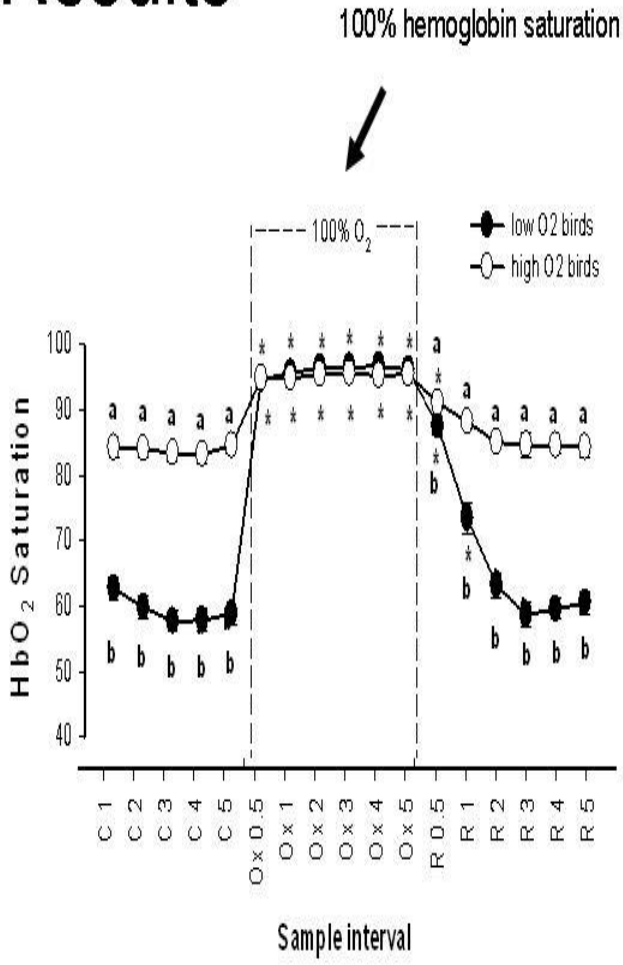
  If blood is fully exposed to the gas exchange area arterial blood will be 100% saturated with oxygen

Material and methods

- Blood gas analyzer
- Pulse oximeter
- 100% oxygen

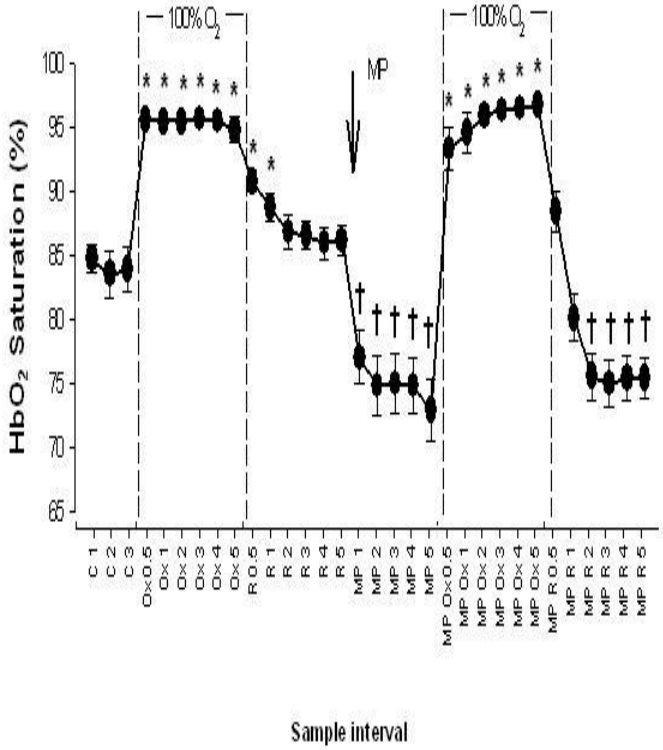
Results

Hypoxemia is eliminated by inhalation of 100% oxygen



Results

Inhaling 100% oxygen increases arterial blood oxygenation after MP injection



Conclusion

- Blood flowing through preascitic and clinically healthy broilers injected with MP is effectively exposed to ventilated gas-exchange surfaces

Third chapter

Introduction

- Intravenous LPS increases PAP (Chapman et al., 2005)
- In poultry houses LPS is found in large quantities on small size particles able to reach the respiratory parenchyma
- Respiratory surfaces are the most common route of entry

Objective

- Development of a controllable model for exposing the lungs of broilers to aerosolized LPS

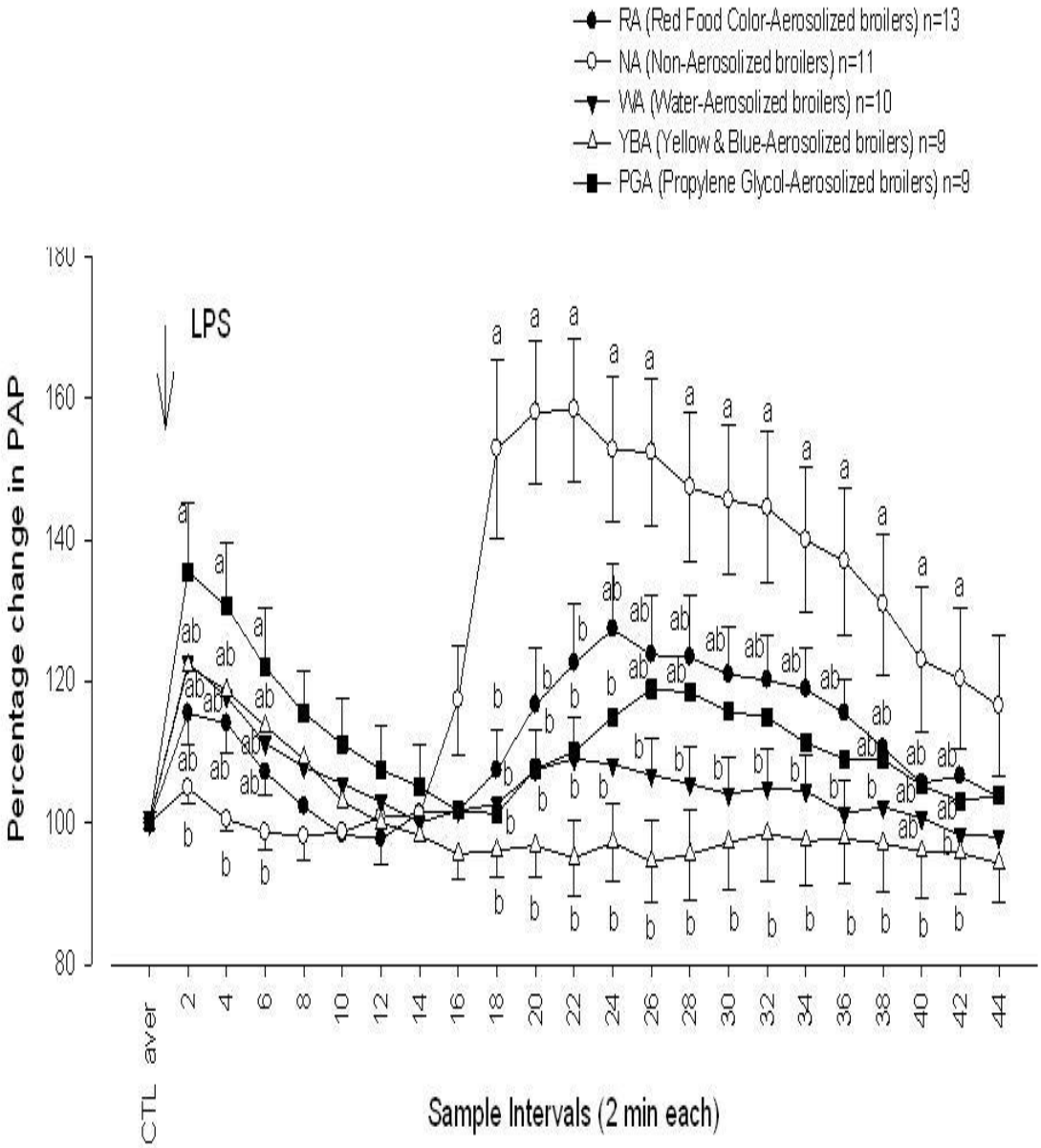
Materials and methods

- Birds aerosolized 40 min (box and nebulizer)
- 24 h period
- A catheter was introduced through the wing vein and advanced to the pulmonary artery → PAP
- 2 mg LPS challenge

Materials and Methods – Experiment 1

- Male broilers 43 to 55 d reared on clean litter in environmental chambers
- -Non-Aerosolized broilers (NA): LPS i.v.
- -Water-Aerosolized broilers (WA): i.t. LPS.
- -Red Food Color-Aerosolized broilers (RA): i.t. LPS.
- -Yellow & Blue Food Color-Aerosolized broilers (YBA): i.t. LPS.
- -Propylene Glycol-Aerosolized broilers (PGA): i.t. LPS.

Results Experiment 1



Conclusions

- The PAH responses to LPS were higher using i.v. than i.t. routes probably due to the direct interaction with immune cells

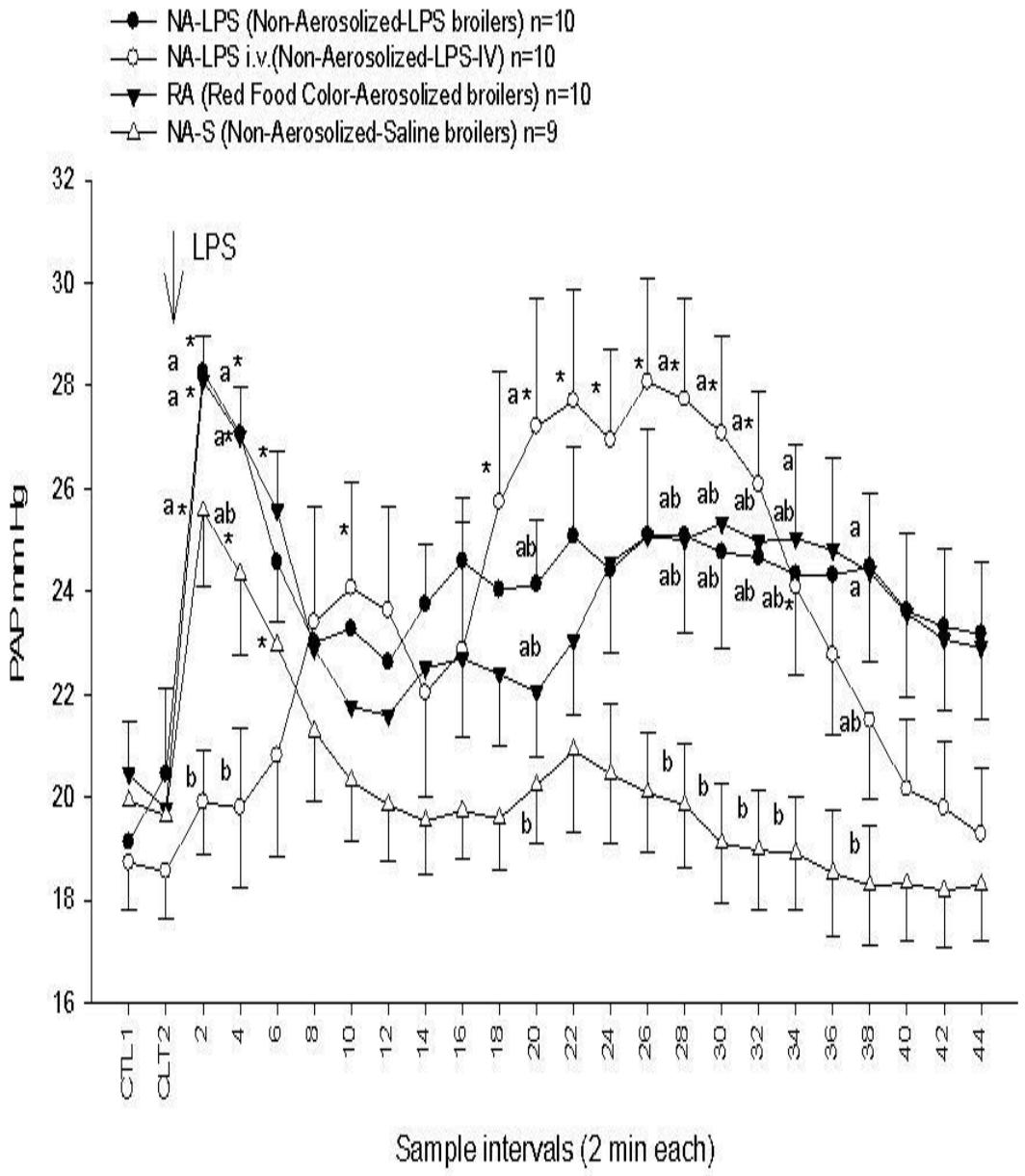
- Pulmonary mucosal defenses appear to apply a pattern of "tolerance" (by binding, inactivating or discriminating non-threatening agents) to minimize inflammation. Strong immune responses may produce more damage than protection (Ewaschul and Dieleman, 2006)

- Aerosol Red food colorant #3 and PG sensitized or primed the respiratory system allowing a more consistent pulmonary hypertensive response to i.t. LPS

Materials and Methods – Experiment 2

- Male broilers 35 to 53 d reared on previously used litter in a commercial type poultry house.
- -Non-Aerosolized-LPS-i.v. broilers (NA-LPS i.v.): LPS i.v.
- -Non-Aerosolized-Saline broilers (NA-S): 1 mL of i.t. 0.9% NaCl.
- -Non-Aerosolized-LPS broilers (NA-LPS): i.t. LPS.
- -Red Food Color-Aerosolized broilers (RA) red food color; i.t. LPS.

Results Experiment 2



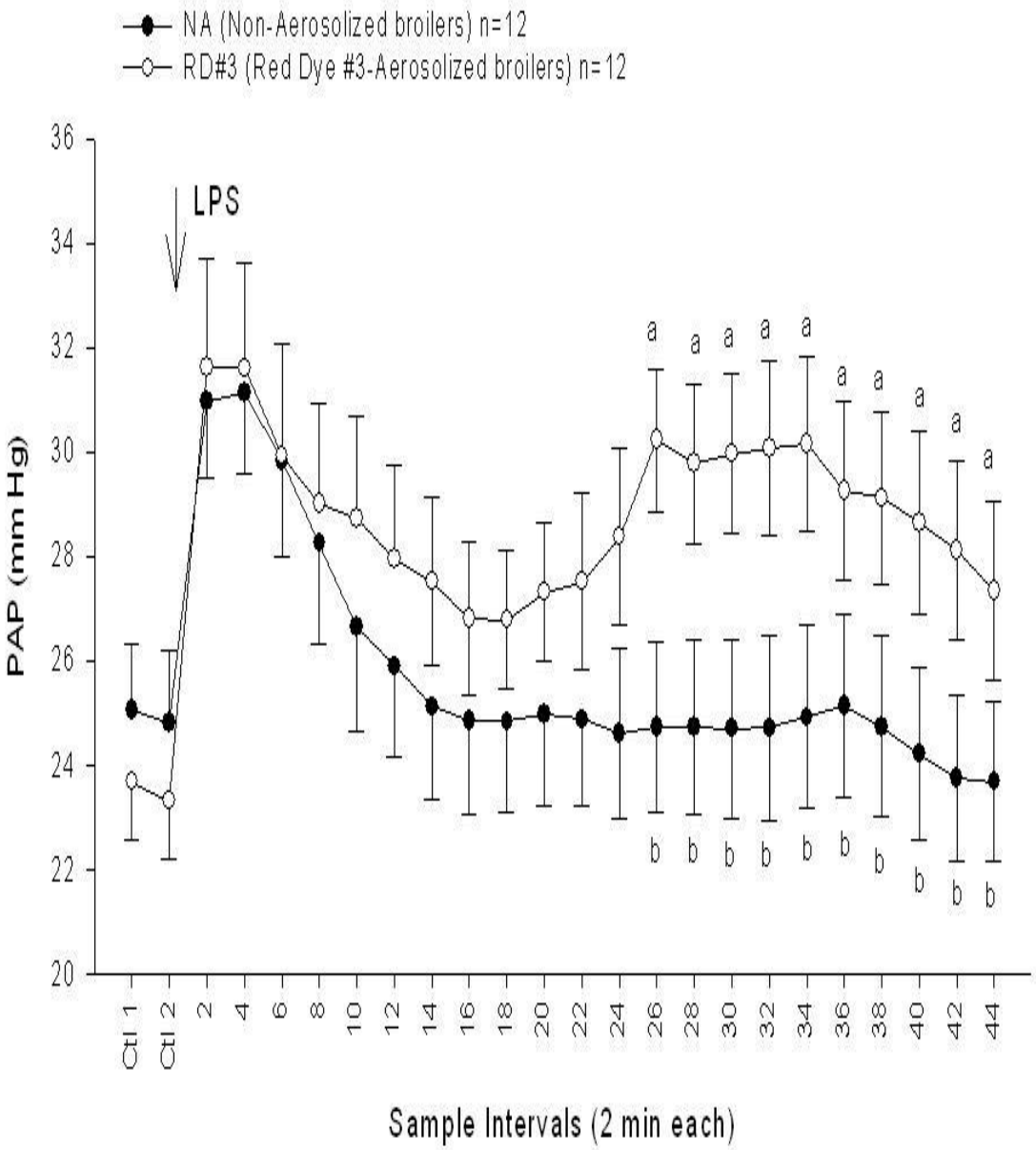
Conclusions

- Exposure to used litter appeared to naturally prime or sensitize the airways to react with hypertensive responses after i.t. LPS.

Materials and Methods – Experiment 3

- Male broilers 45 to 53 d reared on clean wood shavings litter in environmental chambers
- Non-Aerosolized broilers (NA): i.t. LPS.
- Red Dye #3 - PG Aerosolized broilers (RD#3) Red Dye #3, PG (0.04 g RD#3 / mL PG); i.t. LPS.

Results Experiment 3



Conclusion

- Treatment with aerosolized Red Dye #3 and PG appears to constitute a non-pathogenic, controllable experimental model for deducing the mechanisms by which air pollutants can enhance pulmonary hypertensive responses of broilers

Fourth chapter
Introduction

- Inhaling low doses of toxins and pathogens, or primers such as Red Dye #3 and PG, may induce sub-clinical levels of lung inflammation, unbalancing the mechanisms of "tolerance"

- Red Dye #3 and PG may unbalance the system by
  - Increasing the number of leukocytes in the airways
  - Shifting the proportion of different populations of leukocytes within the airways
  - Activating leukocytes from the airways
  - Changing the chemical structure of surfactant proteins

Objective

- Evaluate possible changes in the cellular components of the chicken airways after exposure to vaporized red #3 + PG
  - Number
  - Proportion

Materials and methods

- Bird vaporization with Red#3 and PG (40 min)
- 24 h period
- Birds were bled under deep anesthesia
- Lungs were lavaged with 60 mL of saline
- Red and white cells were counted (hemocytometer)
- White cells were classified in mononuclear cells, heterophils, basophils, and eosinophils (differential cell counting with Wright stain)
- Blood differential cell counting

Results

- The number (763.2 ± 158.7a and 402.9 ± 62.6b WC/µL) but not the proportion of leukocytes was higher in the lavage fluid of broilers from the Red#3+PG group compared to broilers from the control group, respectively

- Blood lymphocytes were lower in broilers from the Red#3+PG group compared to birds from the control group (53.1±2.8a and 60.2±3.2b %, respectively)

Discussion

- We propose that the number of leukocytes present within the airways is one of the components that enable pre-treated broilers to exhibit pulmonary hypertension in response to intratracheal LPS.

- However, the lavage fluid of control birds also contained a significant amount of leukocytes

Conclusions

- We support the existence of a mechanism of immune tolerance within the mucosa of the airways

- We propose that a multiple interaction between components of the mucosal immune system may be needed before an inflammatory response to aerosolized LPS can be elicited.

Future research to solve remaining questions

- Assess the activation of the immune cells present within the respiratory airways

  -LPS induced nitric oxide production

  -LPS induced IL 6 production

- Replace surfactant proteins after inhalation of Red #3 + PG

Future research practical approaches

- Vaccine design
- Evaluation of molecules that the mucosal pulmonary immune system can detect

The big picture

- Surfactant layer-immune cells from the airways - epithelial cells-macrophage interaction

Thank you

Dr. R. Wideman
Dr. G. Erf
Dr. N. Rath
Dr. N. Anthony