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Lista de contenidos

EVOLUTION OF COCCIDIA UNDER SELECTIVE PRESSURE OF VACCINES AND ANTICOCCIDALS

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Introduction

Evolution is not the first thing to come to mind when one thinks of coccidia in the commercial poultry house and the resulting clinical and sub-clinical coccidiosis that results. If one takes Darwin's (1859) classic view of evolution as "natural selection" and considers commercial poultry production, it becomes clear that the chickens and the organisms that infect them in the broiler house environment are under tremendous selective pressure. Through intense selective breeding, the genome of commercially successful lines of chickens has been modified significantly for robust growth in the commercial environment. Likewise, parasites and other infecting organisms are under constant selective pressure to adapt to environmental factors (e.g. temperature, humidity and litter characteristics), host immunity (including vaccination) and antimicrobial agents (e.g. coccidiostats, coccidiocides and other antimicrobials). Together, this "unnatural selection" makes for some interesting evolutionary changes in the parasites.

Origins of the *Eimeria* species infecting chickens

The domestic chicken has been shown to be derived from the red jungle fowl (Gallus gallus Linn. 1758) that lives on the floor of tropical and sub-tropical forests in Southeast Asia. Like domestic chickens (when given the opportunity), the jungle fowl will feed on a wide variety of vegetable matter and various invertebrates. In most of their range, jungle fowl will rear 2-4 broods of chicks annually with from 5 to 10 chicks in each brood. The chicks are fully fledged at about 12 days but will remain in a group with the hen for up to 12 weeks. Birds may live more than 10 years in captivity but are unlikely to do so in the wild.

Throughout each generation, coccidia probably infected virtually all of the new chicks as they began to feed from the ground during their association with the hen. During that time, oocysts shed by previous broods of chicks in the same area would be ingested and initiate infections. Usually, small numbers of oocysts from various *Eimeria* species would be ingested at random times as the chicks began to feed. For that reason, clinical coccidiosis in jungle fowl was probably non-existant. Instead, a mild enteritis or typhlitis would arise depending on the infecting species. No lasting damage would occur as a result of the infection and there would be only a modest impact on the growth and development of the young bird. To flourish in that environment, the coccidia needed to produce a large number of oocysts that would be scattered through the environment by the infected animals. Only a tiny proportion of these oocysts would ever successfully infect another chicken. The high productivity (more than 10^6 oocysts produced from a single infecting sporozoite in some cases) reflects the low chance of a single oocyst being ingested by a susceptible chicken.

Was evolution occurring in this natural situation? Absolutely. The parasites evolved to occupy all regions of the intestinal tract from the upper duodenum to the ceca. Parasites of similar genetic backgrounds tended to infect different regions of the intestinal tract (see Figure 1). The various species were partitioning the resource of the digestive tract and the most closely related parasite species evolved such that they did not directly compete in the same location in the chicken (Barta et al., 1997).

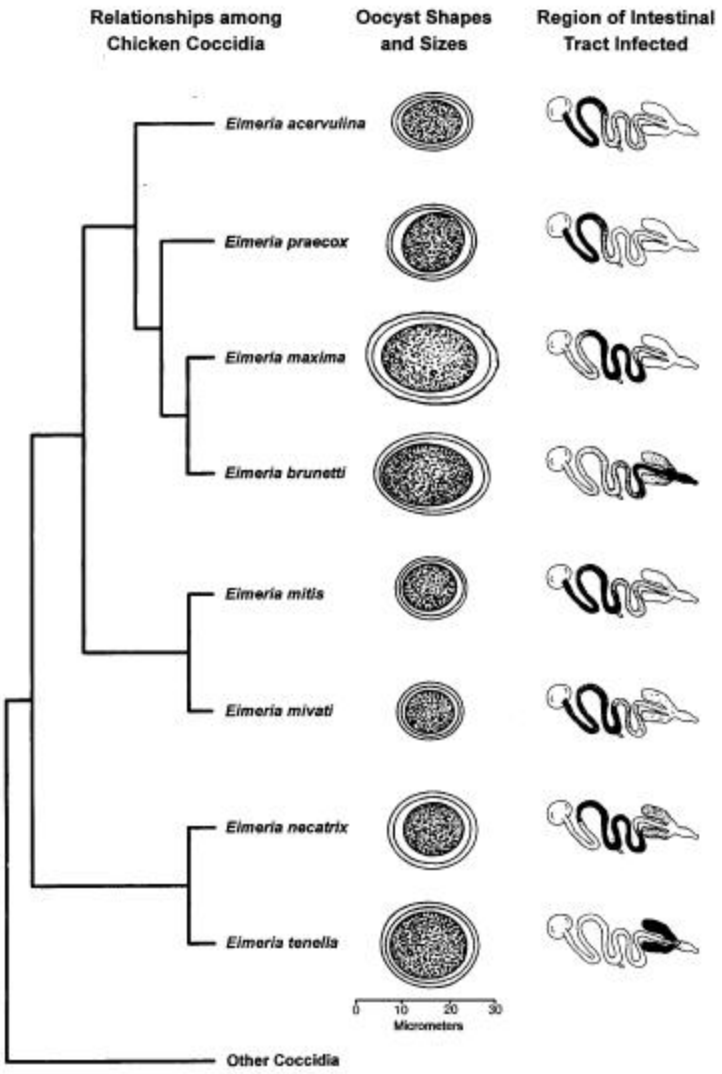

Figure 1. Molecular relationships among *Eimeria* species infecting chickens inferred from 18S ribosomal DNA sequences. Note that closely related *Eimeria* species have similarly shaped and sized oocysts but closely related species tend to occupy distinct regions of the digestive tract (from Barta et al., 1997)

Parasite responses to the selective pressure of anticoccidials

Anticoccidial resistance by *Eimeria* species has become a common problem in the poultry industry (Chapman, 1998). Reliance on continuous, in-feed prophylactic medication in intensively reared broiler production has generated profoundly drug-tolerant parasites to most, if not all, commercially available anticoccidials. Multi-drug resistance to more than one class of anticoccidial drug has become more common over time (Martin et al., 1997). The potential of the commercial broiler house to select for drug resistance is impressive. All animals in a house are treated similarly and so only parasites that have some innate resistance against a particular drug remain after drug treatment; essentially there are no refugia (see van Wyk, 2001) because the duration of drug pressure is frequently longer than the survival of any drug sensitive oocysts in the commercial broiler house (Williams, 1998). Live vaccination with drug sensitive lines of virulent parasites may act as artificial refugia in the commercial broiler house by seeding drug susceptibility back into the population of coccidia in the litter (Chapman et al., 2002).

Drug resistance can occur very quickly with such resistance already present at some low level in at least some coccidial

populations such as was observed for decoquinate (Williams, 2006) or much more slowly such as for ionophores. Resistance seems to be relatively specific to each class of anticoccidial drug. For example, a strain of *E. tenella* was shown to be resistant to monensin (and other monovalent ionophores such as narasin and salinomycin) but was still susceptible to maduramicin and lasalocid, which belong to different classes of polyether antibiotics (Bedrnik et al., 1989).

Parasite responses to the selective pressure of live vaccination

Selective pressure can certainly be immunologically based instead of physiologically or physically based. In the jungle fowl, coccidia had already evolved to be immunologically distinct at least at the species level and, most likely, at the strain level as well. Coccidia generally produce species-specific adaptive immune responses in birds that, after one or a few infections, are able to make the bird refractory to infection and prevent oocyst shedding by immune birds that ingest sporulated oocysts (Rose, 1976).

In the poultry house, there is actually little selective pressure for a particular strain of an *Eimeria* species already present in a broiler house to alter its antigens in response to the immune response of the chickens being reared there. Young birds that are infected will produce oocysts that will retain their infectivity for the few weeks necessary to infect the next crop of broilers reared in the same barn (Williams, 1998). Birds will often become immune to the local coccidial population before reaching market weight and thus no new oocysts will be shed by the birds, but sufficient oocysts will survive in the environment to continue the parasites' genetic lines.

Like any selective pressure, the immune system will actively select against any coccidia that have been encountered before. Thus, in the absence of such selection, there is little advantage for novel, immunologically distinct strains of particular parasites because the strain(s) present in the broiler house will successfully infect each new generation of birds as they are introduced into this environment. However, if vaccination is added to the mix, any locally abundant strains of the parasites that are immunologically distinct from the vaccine strain will be positively selected. In these cases, the immunologically distinct local strain may make itself known as a live vaccine "failure" (Martin et al., 1997). In reality, the vaccine was, in most cases, likely to have been properly delivered and efficacious; although the efficacy was not against the locally abundant parasites. Particularly with attenuated vaccines which produce relatively few oocysts, the reproductive potential of any immunologically distinct, local coccidial strains will be far greater and thus the local strain will have a distinct reproductive advantage. The immunologically distinct strain will likely persist and probably come to predominate under pressure of a continuous vaccination program. A potential future solution to this problem of live vaccine "failure" is to develop regional live vaccines that might incorporate locally relevant strains of parasites.

Subunit (non-viable) vaccines will exert the same selective pressure as live vaccination which suggests that such vaccines are likely to face the same challenges as live vaccines. That vaccination actually drives evolution is not unique to coccidia and has been recognized as a potential issue with other chicken pathogens, such as Marek's disease (Baigent et al., 2006)

Concluding Remarks

Coccidiosis continues to "succeed" in the commercial poultry house just as it succeeded in the original jungle environment of its jungle fowl host before domestication. Most importantly, evolution of the parasite was not stopped through domestication; indeed, if anything, the selective pressures of the broiler rearing environment, vaccination and anticoccidial usage have increased and rapid evolution of *Eimeria* species has occurred in the commercial poultry house as a result. Sustainable control of coccidia in the commercial broiler house will need to balance the number and types of oocysts in the litter through integrated use of anticoccidials and live vaccination.

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Differential Intestinal Responses to Eimeria Isolates in Broiler Chickens

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Coccidiosis in Commercial Poultry

Although the *Eimeria* species are among the most economically important pathogens in the poultry industry, there is a lack of knowledge on the importance of intestinal immune responses, specifically the host-pathogen interactions, to these enteric pathogens in chickens. While often the prophylactic chemotherapeutic control of poultry coccidiosis is effective, the costs of anticoccidial drugs and the increased emergence of drug-resistant parasites have prompted the need for more cost-effective and safer alternatives for control (Long, 1987; Danforth and Augustine, 1989). Although commercially available live oocyst vaccines will provide solid immunity when applied carefully under good rearing conditions, the problem of incomplete protection due to immunovariability (variability of immune protection to different strains of the same species of *Eimeria*) between coccidial species present in the vaccine and those found in poultry rearing facilities has emerged as a potential complicating factor associated with vaccination (Martin *et al.,* 1997; Danforth, 1998). Understanding the response of the intestinal immune system to the coccidial species and the interactions between the parasite and the host at the cellular level is crucial to the development of new approaches to coccidiosis control. Because the intestinal epithelium is the target tissue for *Eimeria* species, the interaction between the intestinal mucosa immune system and the pathogen is a key element in the defense of the chicken to these enteric pathogens.

Immunity to *Eimeria* in Chickens

While investigation of intestinal immunity to bacterial pathogens is not an easily approached task, coccidia present an added challenge to investigators due to their complex life cycle comprised of developmental stages both inside and outside the host (Lillehoj and Trout, 1996; Rose, 1996). The host-associated stage of the coccidian life cycle involves both asexual and sexual stages of reproduction, which contribute to a comparably complex immune response in the chicken intestine.

Eimeria species tend to be very site selective in the region of the intestine they infect, and the degree of immunologic response generated by the host varies with the different species (Ovington *et al.,* 1995; Lillehoj

and Trout, 1996). While the specifics of immunity to coccidia are poorly understood and there appear to be a variety of immune responses initiated in the host, it is commonly accepted that infection with the *Eimeria* parasite will result in induction of species-specific protection to limit, if not prevent, infection with a homologous subsequent challenge (Rose, 1987). Interestingly, distribution of sporozoites in the intestinal epithelium differs in naïve as compared to immune chickens. In naïve chickens the sporozoites are fully capable of reaching the crypt epithelium. However, in immune birds they appear to be arrested after penetration, with significantly fewer numbers found in the crypts (Jeurissen *et al*., 1996).

Coccidial infection in chickens stimulates components of both humoral and cell-mediated immunity (Ovington *et al.,* 1996; Lillehoj and Trout, 1996; Rose, 1996). Discussed in detail in these reviews, investigations have provided evidence that while humoral, or antibody-mediated, immunity appears to contribute to complete immunity to *Eimeria*, it is believed to play far less of a role compared to that of cell-mediated immunity. Additional studies have indicated a massive infiltration of macrophages and uncharacterized granulocytes in the lamina propria during the first days of a primary infection with *Eimeria,* which lead the investigators to propose a modulatory role for macrophages in the severity of primary infection (Jeurissen *et.al*., 1996). The initial contact with invading sporozoites appears to activate an immunological cascade of defense mechanisms which leads to inflammatory cell influxes with some populations contributing to primary modulation of the severity of infection and others generation of protective immunity.

As indicated by the referenced publications, the majority of studies have concentrated on the role of B and T lymphocytes in immunity to enteric pathogens in poultry. While these cell populations should by no means be slighted in their effector functions in mucosal immune responses, generally disregarded cells, such as mast cells, may also contribute to complete immunity to enteric pathogens, particularly with innate responses in naïve birds. Clearly, a multitude of roles for mast cells in mammalian intestinal immune responses to enteric pathogens have been proposed, however the involvement of mast cells in intestinal immunity in chickens has been largely overlooked. Perhaps, the phenotypic and functional heterogeneity of mast cells has contributed to the lack of effort to identify or characterize the effector functions they may play in chickens. Scientists have been plagued by the multitude of properties and appearances of mast cells, with variation not only with respect to tissue location in a single species, but also from one animal species to another. The preparation of tissues for gross microscopic evaluation of mast cells varies from one host species to another, and unless the appropriate fixatives and other histological reagents are used, mast cells will typically

degranulate, thereby making them indistinguishable from other intestinal immune cell types. In general, it appears that mast cells are highly adaptive, with morphology and function dependent on the specific microenvironment (Yong, 1997). The paucity of studies on mast cells in chickens, particularly with investigation into their functions in intestinal immunity, may be due to several of these factors. However, several studies have clearly documented the presence of mast cells in chicken intestinal tissue and correlated such presence with immunologic responses.

Of all avian species evaluated, mast cells have been identified in the greatest numbers in chickens and ducks in the lamina propria, epithelium, and lumen of the small intestine and ovary (Swayne and Wiesbrode, 1990; Daszak and Ball, 1993). Similar to mammalian mast cells, chicken mast cells have been shown to contain the same pre-formed biogenic amines and also apparently possess the capability for *de novo* synthesis of the arachidonic acid metabolites (Gray, 1976; Rose *et al.,* 1980). Studies are also suggestive of comparative responses of chicken mast cells to enteric pathogens. Elevated numbers of mast cells in the gut have been described in secondary cestode infections (Gray *et al*., 1976), primary infection with *Eimeria tenella* (ET; Daszak and Ball, 1993), and in response to secondary *Eimeria* challenge in chickens (Rose *et al*., 1980). Similar to reports of mammalian intestinal hypersensitivity in response to parasitic infection as described above, in chickens, gut hypersensitivity has been associated with secondary coccidial infection (Rose *et al*., 1975). More recent research has described the measurement of correlates of intestinal anaphylaxis in chickens immunized against *Eimeria* species (Caldwell *et al*., 2001; Reinap *et al*., 1999).

We have conducted several experiments to evaluate our hypothesis of the involvement of mast cells in the intestinal immune responses to *Eimeria* in chickens (McElroy *et al.,* 1999; Morris et al., 2004). In these preliminary investigations, challenge of broiler chickens with *Eimeria* resulted in significant ($P<0.05$) increases in lamina propria thickness and increased numbers of mast cells in the ceca or ileum of the intestine. These increases are suggestive of an inflammatory response in the intestine. Since our laboratories are investigating “non-classical” mechanisms of immunity to *Eimeria* in chickens, specifically mechanisms involving the mucosal epithelium of the intestine and the effector cells that mediate these responses, these data are particularly interesting. Data from these preliminary investigations identifies the responsiveness of mast cells to *Eimeria* infections in neonatal chickens. The influx of these cells into the lamina propria of the distal ileum and ceca apparently contributes to an increase in lamina propria thickness, suggestive of an inflammatory response. As mast cells are largely overlooked for involvement in mechanisms

of immunity in chickens, these observations are individually significant in suggesting these cells play a larger role in gut immunity to enteric pathogens in the chicken.

Furthermore, we find it particularly intriguing that the appearance of significantly increased numbers of mast cells temporally coincides with the appearance of lesions in the cecal tissue from the ET infection. Hypothetically this could correlate with a stimulated release of mast cell mediators. While purely speculative, if the mast cells are responsible for the reported intestinal gut hypersensitivity response to secondary coccidial infection (Rose *et al.,*1975; Reinap *et al.,* 1999), it would be expected to observe secretory diarrhea and increased fluid secretion as occurs with *Eimeria* infection in chickens. These disturbances in the mucosal environment could substantiate the malabsorption, decreased feed conversion, and decreased rate of growth as generally occurs with coccidial infections in chickens. Specutively, the multitude of mast cell mediators could contribute to the pathology of the disease, as their capacity to inflict damage on tissues is exemplified by the harmful inflammatory conditions they have been implicated in with mammalian studies.

As mentioned previously, immunogenicity and related immunovariability, varies among *Eimeria* species (Ovington *et al.,* 1996; Lillehoj and Trout, 1996; Rose, 1996). For example, with the same species, one strain will result in severe depression of weight gain and an absence of substantial lesions in the intestine, while another strain of the same species will result in severe lesion development and mild depression of weight gain (Danforth, 2000; Morris et al., 2004). This discrepancy with different strains of the same species may be related to differences in the host response to the pathogen, rather than variability in the pathogenicity of the *Eimeria* itself. Clearly, the host pathogen interaction and development of an immune response at the intestinal level must be investigated in much more detail to understand immunity and related pathophysiology to such enteric pathogens in chickens. Certainly the development of a competent immune system and general gut health and function are essential to allowing the bird to respond to *Eimeria* challenge and maintain a positive level of performance.

Genetics

Host genetics have been shown to play an important role in controlling host immunity to *Eimeria* infections (Ruff and Bacon, 1989; Martin et al., 1993; Lillehoj, 1994). Laan (2002) showed that through selection, future generations had an increase in a specific type of immune response (high antibody response, high cell-mediated immune response, and high phagocytic activity). Different lines of chickens have been shown to

have different immune and production responses when challenged with *Eimeria* (Lillehoj, 1994; Laan et al., 1998; Emara et al., 2002). Studies have also demonstrated that increased resistance to coccidiosis was possible through genetic selection (Johnson and Edgar, 1986). Jeffers and Shirley (1982) concluded that such resistance is mainly caused by the total of the genetic background resulting from the combination of genomes from diverse breeds and not so much by inheritance of one or more specific "resistance" genes. If resistance to disease could be enhanced through genetic selection this would be a possible alternative to using anticoccidials. The different species of *Eimeria* would need to be taken into account due to the fact that each species shows different immunovariability in chickens.

Data has also shown a difference in the immune response between genotypes of major histocompatibility (B) complex (Caron et al., 1997), which may possibly be attributed to the bird's ability to recognize the coccidia antigen. If lines that are naturally resistant to coccidiosis are found, then they could be incorporated into a breeding program in an effort to pass along the genes that demonstrate this resistance. Since resistance to *Eimeria* involves more then one type of immune response, different immune responses need to be selected for to provide this resistance. Selection criteria could include one or a combination of the following: high antibody response to foreign antigens, high cell-mediated immune responses, and MHC genes.

Layers that were selected for high antibody or low antibody response to sheep red blood cells did show a difference in antibody response when challenged with *E. acervulina*, but these responses did not show any difference in resistance to the *E. acervulina* in regards to termination of infection or expulsion of the parasite (Parmentier et al., 2001). This is in contrast to a study by Dunnington et al. (1992) that showed lines selected for high anitbody response were more resistant to an *E. tenella* challenge. Layers that showed a high cell-mediated immune response, determined by delayed hypersensitivity skin testing to *E. maxima* oocyst, had a more rapid development of immunity to *E. tenella* through vaccination compared to layers selected for a low cell-mediated immune response (Giambrone et al., 1984). However, the layers with a high cell-mediated immune response to *E. maxima* were susceptible to *E. necatrix* infection (Giambrone et al., 1984). Many studies have shown that different MHC genes have contributed to differences in the resistance or susceptibility to *Eimeria* infections (Lillehoj et al., 1989; Lillehoj et al., 1990; Dunnington et al., 1992; Caron et al., 1997).

A reason for the difference in the studies by Parmentier et al. (2001) and Dunnington et al. (1992) is that the layers used by Dunnington had different MHC haplotyes, while Parmentier did not mention a difference in other genetic parameters. As mentioned previously, antibody response appears to play a small role in the protection of chickens to an *Eimeria* challenge. Cell-mediated immune response plays a major role in prevention, but testing for a high cell-mediated immune response should involve the use of the same *Eimeria* species for which resistance is being generated. Selection for all three of the above mentioned criteria might be needed to provide resistance to coccidiosis, since they all play a role in the host immune response.

Research from our lab, which we will be presenting for the seminar, has indicated differential intestinal immune responsiveness and corresponding performance differences in commercial broilers from genetically divergent lines which emphasizes the importance of immune system competence in genetic selection programs in the industry (Morris et al., 2004; Wazlak et al., 2004). In current selection schemes by primary breeder operations in the poultry industry, some pure lines are being selected in the presence of an *Eimeria* challenge in the environment in an attempt to build resistance in the selection program. Further research needs to be conducted to evaluate the mucosal immune response associated with differences in resistance between commercial lines of broilers.

Experimental Results

Experiments were conducted to evaluate variability in the broiler host response to a single challenge with one of two EA isolates, as well as determine if responses were influenced by the genetics of the bird. These experiments demonstrated that immunovariability does exist between different isolates within the same species of *Eimeria*. Differences in body weight gains PC, duodenal lesion scores, villus height and crypt depth, and localized mast cell influx were dependent upon which EA strain was administered; however, quantified and observed effects were not always consistent and were possibly influenced by the challenge dosages of EA.

In all experiments conducted, lesions of the duodenal mucosa of birds challenged with *Eimeria acervulina* isolate 1 (EA1) consisted of typical white, ladder-like plaques, which were consistent with the findings of Johnson and Reid (1970) upon which the current method of lesion scoring was based. However, Eimeria acervulina isolate 2 (EA2) produced significantly fewer classical lesions but exhibited signs of an anaphylactic-like secretory-type response. Differences in lesion scores found between EA1 and EA2 challenged birds within the same breed could possibly be related to the fact that the two isolates are

generating a differential host response due to the presence of different surface antigens, which may or may not cause edema and the presence of intestinal lesions (Lillehoj and Trout, 1993). Similar findings suggest that immunological mechanisms responsible for protecting birds against weight loss may differ from mechanisms that protect against lesions of the intestinal mucosa (Augustine, et al., 1991).

Coccidiosis is known to produce different histopathological features depending on the *Eimeria* species causing the infection (Johnson and Reid, 1970; Lillehoj and Trout 1993; McDougald and Reid, 1997). However, examination of the duodenal tissues taken from EA inoculated birds 6 days PC confirmed a differential host response to different EA isolates as well. The intestinal epithelium acts as a selective barrier allowing nutritional ions and macromolecules to be absorbed while resisting harmful pathogens. Once this protective barrier is removed, the exposed lamina propria undergoes morphological changes due to the affects of inflammation. The villus is then unable to adequately absorb nutrients and is subject to secondary infections (Schat, 1991). At the mucosal level, the villus structure may be altered directly by the infectious agent, or indirectly, by the host's immunoinflammatory response (Barker, 1993). Crypt hyperplasia is a well-known histopathologic consequence of coccidiosis resulting in increased metabolic activity to compensate for epithelial destruction (Rose et al., 1992). In the present experiments, increases observed in intestinal crypt depth were in agreement with results from experiments conducted by Fernando and McCraw (1973). Crypt hyperplasia was found to be present in EA1 challenged birds when compared to controls. Crypt length increased to a maximum at 6 days PC for both challenged groups, whereas no detectable increases in controls were found over the 5 day period after challenge. It appeared that the crypt measurements were inversely related to villus height in primary EA infection and that severe alterations coincided with the heightened period of infection. Results from the present experiments indicated that the host responded to EA2 in a different manner than EA1 at the level of the duodenal mucosa.

From these results, it is suggested that there was a differential immune response elicited to the two strains of EA. However, the mechanisms responsible for these acute differences are unknown. Arguably, the differential responses could have been due to the parasite itself, the host's reaction to the parasite, or a combination of both. Observed changes in mast cell populations could contribute to differences in the host response. These results, along with data from other investigators, support the role of mast cell involvement in *Eimeria* infections. It appeared that increases in mast cells were related to secretory responses in the

intestine; especially those seen in EA2 challenged birds. However, mast cell counts were inconsistent, and counts were highly variable in these experiments.

In other experiments, the objective was to measure performance and intestinal response among different genetic stocks of commercial broilers that results in differential host responses to *Eimeria* infection. The effects of a mixed *Eimeria* challenge on productivity of broilers from three different commercial broiler stocks through measurement of body weight gain, feed conversion, and lesion scores were evaluated. The effects of a mixed *Eimeria* challenge on intestinal response were evaluated by analyzing villus height, crypt depth, and quantitating mast cell numbers in three commercial broiler genetic stocks. The study indicated variability in the immune response and performance parameters among different lines of commercial broilers. This was in agreement with previous studies that demonstrated genetics to have a role in the bird's immune function and ability to deal with coccidial parasites (Lillehoj, 1998; Uni et al., 1995). The experiment reported differences in body weight gain, feed conversion, spleen weights, lesion scores, villus height, crypt depth, villus/crypt ratio, and mast cells that were dependent upon the genetic stock of broiler challenged.

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PROTEIN LEVEL, LIVE OOCYST VACCINATION, AND *EIMERIA* CHALLENGE

The Effect of Starter Dietary Protein Level on Broiler Performance during Live Oocyst Coccidial Vaccination

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ABSTRACT

A series of experiments were conducted to investigate the effect of starter diet protein level on the performance of broilers vaccinated with Coccivac-B® and subsequently challenged with a mixed species *Eimeria* challenge compared to non-vaccinated broilers. The data indicates that increasing protein level in the starter diet improves broiler performance during the starter phase. The observed reduction in performance of vaccinated broilers versus non-vaccinated broilers was eliminated by the conclusion of the experiments (27 d) in the higher protein diets in this study. Vaccination was effective at generating protective immunity against the *Eimeria* challenge evidenced by significantly increased body weight gains, improved feed conversions, reduced post-challenge mortality, and reduced lesion development in vaccinated broilers compared to non-vaccinated. These data indicate that vaccination can be utilized as an anticoccidial preventive and are suggestive that reduced protein concentration of starter diets can lead to significant losses in broiler performance when utilizing a vaccination program to prevent coccidiosis.

INTRODUCTION

Coccidiosis is an intestinal disease of intensively reared livestock cause by coccidial parasites of the genus *Eimeria*. The disease causes intestinal epithelium lesions, reduction of body weight, reduced feed efficiency and several degrees of mortality (Guzman et al., 2003). Economically, the most important members of this genus in chickens worldwide are *E. acervulina, E. brunette, E. maxima, E. mitis, E. praecox, E. necatrix,* and *E. tenella* (Shirley et al., 2005). Infections with *E. acervulina, E. maxima,* and *E. tenella* are diagnosed frequently in intensively reared poultry (McDougald et al., 1997). It has been estimated that coccidiosis costs the world's commercial chicken producers at least $800 million each year (Williams, 1998) with approximately 80% of this cost due to poor performance (Williams, 1999). As the world's poultry production continues to grow so do the concerns about the control of coccidiosis since commercial

chicken flocks free from coccidia are extremely rare (Williams, 2002). Historically, the poultry industry has relied on anticoccidial drugs for the control of coccidiosis but resistance of coccidia has developed to all of the anticoccidial drugs introduced so far (Chapman, 1997) along with consumers desires to eliminate drugs from animal feeds has led to increased interest in the use of alternative methods of control.

Vaccination against coccidiosis is not a new concept and has been used in the poultry industry for around 50 years (Shirley et al., 2005). Live oocysts vaccination, attenuated and non-attenuated strains, is currently the only commercially available anticoccidial vaccination option. Attenuation can be obtained by passing parasites through embryonated eggs or by selection for precocity (Williams, 2002). These vaccines provide solid immunity to coccidial infection when applied carefully under good rearing conditions (Shirley and Long, 1990). Despite their proven success in eliciting effective protection against coccidiosis, especially in replacement and breeding flocks, these vaccines have not been universally accepted by the U.S. poultry industry for meat producing broiler and heavy roaster bird flocks (Danforth, 1998). The reluctance of broiler producers to adapt anticoccidial vaccination strategies has been that bird performance, as measured by weight gains and feed efficiency, has not always equaled that of medicated broilers (Danforth, 1998; Williams, 2002). The reduced performance is related to a mild coccidia infection associated with live oocysts vaccination. Increasing protein level during periods of clinical coccidiosis has been shown to improve broiler performance (Sharma et al., 1973). The objective of the current research is the evaluation of the effect of dietary protein level on broiler performance and lesion development during live oocysts vaccination with Coccivac-B®[1] and subsequent mixed species *Eimeria* challenge.

MATERIALS AND METHODS

A series of 2 experiments were designed to identify the effect of dietary protein level on broiler chick performance, identified by body weight and feed conversion ratios, while utilizing a vaccination program for the prevention of coccidiosis and subsequent mixed species challenge of *Eimeria* as compared to non-medicated non-vaccinated control broiler chicks.

For each of the following experiments, broiler chicks were provided age appropriate supplemental heat and given access to feed and water *ad libitum*. All animal care procedures were conducted in accordance with an Animal Use Protocol approved by the Texas A&M University

[1] Schering-Plough Animal Health, 556 Morris Avenue Summit, NJ 07901-1330 USA

laboratory animal care committee, and grow out facilities were thoroughly cleaned and disinfected and fresh pine shavings were used for bedding material. Pens were equipped with one 30 lb tube feeder and nipple drinkers. In experiments that included a mixed species *Eimeria* challenge, dose titration was performed prior to experiment to identify a challenge dose that would significantly reduce weight gain in vaccinated broilers.

Experiment 1

Experiment 1 was a randomized block design consisting of 5 dietary protein levels (20, 21, 22, 23, and 24%) with eight replicates of each protein level for a total of 40 pens. Each replicate contained 25 chicks for a total of 1000 chicks. During diet formulation, careful consideration was given to maintain constant amino acid to protein ratios throughout all 5 dietary treatments and diets were formulated on an isocaloric basis (Table 1). The 23% dietary treatment met or exceeded NRC (1994) specifications for a broiler starter diet. On d of hatch, all chicks were individually weighed (top and bottom 5% discarded), wing banded and vaccinated with Coccivac®-B by oral gavage. Once vaccinated, chicks were randomly assigned to treatment groups using chick weight. Broiler chicks were fed dietary treatment for 21 d at which pen weights were taken and feed consumption determined for the calculation of feed conversion ratios.

Experiment 2

The experimental design utilized for Experiment 2 was a 3 x 2 x 2 factorial designed to determine the effect of one of three dietary protein levels (20, 22, and 24%), vaccination (vaccination compared to non-medicated non-vaccination), and mixed species *Eimeria* challenge (21 d of age) on broiler chick performance. This experiment yielded a total of 12 treatment groups and each group was replicated in triplicate. Each replicate contained 25 chicks for a total of 900 chicks. Dietary treatment groups were identical to those utilized in Experiment 1 (Table 1). At d of age, chicks were individually weighed (top and bottom 5% discarded), wing banded, and assigned to treatment groups. Chicks assigned to vaccinated treatment groups were vaccinated using Coccivac-B® in a commercial spray type cabinet that vaccinates 100 bird chick trays. A red food coloring agent was included in the vaccination solution and chicks were allowed to preen for 1 hr before placement. On d 21, pen weights were taken, feed consumption determined for the calculation of feed conversion ratios, and chicks were challenged with a mixed species challenge containing *Eimeria acervulina* (6×10^5), *Eimeria maxima* (4×10^5), and *Eimeria tenella* (2×10^5) sporulated oocysts. On d 27 (6 days post-challenge), pen weights were taken, feed consumption

determined, and 10 broilers from each replicate necropsied for the determination of intestinal lesion (Johnson and Reid, 1970).

STATISTICAL ANALYSIS

Experiment 1

Data were analyzed using SPSS Version 11.0 for Windows (SYSTAT, 2001) for all experiments. Statistical significance was determined by one-way analysis of variance and means were separated by Duncan's multiple range test. The threshold for statistical significance was $P \leq 0.05$.

Experiment 2

Body weights and feed conversions for d 21 data were analyzed using a one-way analysis of variance due to the presence of a significant interaction present between protein level and vaccination. Significant differences were determined at $P \leq 0.05$ and means were separated using a Duncan's Multiple Range Test. Similarly, due to an interaction with challenge and vaccine, data collected on d 27 including body weight gain, feed conversion ratios, post-challenge mortality, and lesion scores were analyzed using a one-way analysis of variance with differences deemed significant at $P \leq 0.05$, and means were separated using a Duncan's Multiple Range Test. Post-challenge mortality was subjected to a square root arcsin transformation before analysis.

RESULTS

Experiment 1

Average body weights at d 21 of broilers increased as protein level in the diet increased (Table 2). The 20% protein starter diet yielded significantly lower average body weights compared to all other treatments and the 24% protein diet yielded significantly higher average body weights of broilers compared to the 21 and 22% protein starter diets while the 21, 22, and 23% protein starter diets were similar in average body weights. Feed conversion results yielded an inverse relationship as body weight gain with increasing protein level reducing mortality corrected feed conversion of vaccinated broilers at 21 d of age. The 20% protein diet resulted in a significantly higher mortality feed conversion ratio compared to all other treatments. Increasing dietary protein concentration to 21% significantly lowered mortality corrected feed conversion ratio compared to the 20% protein level while a further increase in protein level to 22 and 23% further significantly reduced mortality corrected feed conversion ratios. The 24% protein starter diet resulted in a

significantly lower mortality corrected feed conversion ratio compared to all other dietary protein levels. Average body weights and mortality corrected feed conversion ratios of vaccinated broilers fed 20, 22, and 24% dietary protein levels were significantly different from each at 21 d of age. Therefore, these three protein levels were selected for use in Experiments 2 and 3.

Experiment 2

Average body weights of broilers at 21 d followed a similar trend as reported in Experiment 1, increasing dietary protein level increased average body weights of broilers (Table 3). In the non-vaccinated broilers, significant increases in average body weights were observed with each increase. The 20% protein level resulted in the lightest body weight and the 24% protein level resulted in the highest average body weights. Body weights for vaccinated broilers followed a similar trend with the 20% protein level resulting in significantly lower body weights than the 22 and 24% starter diets. An increase in body weight due to increased protein concentration from 22% to 24% was not observed in vaccinated broilers. Vaccinated broilers fed the lowest protein concentration in the starter diet resulted in significantly lighter broilers compared to all other treatments. Vaccination of broilers significantly reduced body weights at the 20 and 24% protein levels compared to the non-vaccinated broilers fed the same protein level while the vaccinated broilers fed 22% protein were similar to non-vaccinated broilers fed the same protein concentration. Mortality corrected feed conversion ratios at 21 d also followed similar trends as in Experiment 1, increasing protein level reduced feed conversion ratios of both vaccinated and non-vaccinated broilers (Table 2). Feed conversion for 20% vaccinated broilers was significantly increased compared to all other treatment groups. Non-vaccinated broilers fed 22 and 24% protein diets yielded similar feed conversions ratios and vaccinated broilers fed 22 and 24% protein starter diets did not differ significantly from non-vaccinated broilers fed the same dietary protein levels.

Body weight gains and mortality corrected feed conversion ratios during the challenge period (21 d to 27 d) were similar for non-vaccinated and vaccinated non-challenge broilers at all protein levels (Table 4). In challenged broilers, vaccination significantly increased body weight gains compared to non-vaccinated broilers at all protein levels investigated. Non-vaccinated challenge broilers had similar weight gains at all protein levels during the challenge period which were significantly lower than all other treatments. Within the vaccinated challenge broilers, significantly increased weight gains were observed in broilers fed the 24% protein diet compared to the 20% protein level while the 22% level was intermediate. Mortality corrected feed

conversion ratios during the challenge period for vaccinated challenge broilers were similar at all protein levels and were significantly lower compared to all non-vaccinated challenge broilers and were similar to non-challenged treatments. In non-vaccinated challenged broilers, post challenge feed conversion ratios were significantly decreased with each increase in dietary protein level.

Cumulative mortality corrected feed conversion ratios for 1 to 27 d were similar in non-vaccinated and vaccinated non-challenged broilers at both 22 and 24% protein levels. Non-vaccinated non-challenge 20% protein fed broilers had a significantly higher feed conversion ratio compared to all broilers fed 22 and 24% level with a further increase observed in the 20% protein level for vaccinated non-challenged broilers. In challenged broilers, vaccination led to a significant decrease in cumulative feed conversion ratio in the 22 and 24% protein fed broilers compared to non-vaccinated challenge broilers while the 20% protein level was similar for both vaccinated and non-vaccinated challenged broilers. Increased dietary protein led to significant decreased cumulative feed conversion ratios in non-vaccinated challenged broilers. Vaccination resulted in decreased mortality in the 22% protein fed challenged broilers compared to the 20 and 22% non-vaccinated challenge broilers while all other challenge groups resulted in similar mortality rates.

Overall, lesion development was decreased in vaccinated challenged broilers compared non-vaccinated challenged broilers (Table 5). Lesions in the upper small intestine indicative of *E. acervulina* were significantly lower in all vaccinated challenged broilers compared to the 22 and 24% protein fed non-vaccinated challenged broilers although lesion scores observed in the upper small intestine were minimal. Mid intestinal lesion development associated with *E. maxima* was significantly decreased in the 24% protein fed vaccinated challenged broilers compared to the 24% non-vaccinated challenged broilers, and numeric decreases in mid intestinal lesion scores were observed in vaccinated challenge broilers at the 20 and 22% levels compared to non-vaccinated challenged broilers. Lower intestinal lesion development associated with *E. tenella* challenge were significantly decreased in vaccinated challenge broilers compare to non-vaccinated challenge broilers at all protein levels. Lower intestinal lesion scores were similar for all non-vaccinated challenge broilers while vaccinated challenged broilers fed 24% protein had significantly increased lower lesion scores compare to the 20 and 22% vaccinated challenge broilers. An inverse relationship existed between mid and lower intestinal lesion development in vaccinated challenged broilers fed different dietary protein levels. Mid intestinal lesions decreased

with increasing protein level while lower intestinal lesion development increased with increasing protein level. Lesions were observed in a small percentage of non-challenged broilers. The lesions present in the non-vaccinated non-challenge broilers may be attributed to the close proximity in which broilers were reared while the lesions present in the vaccinated non-challenged broilers may be due to continued cycling of the vaccine or the close proximity. Housing both challenged and non-challenged broilers in close proximity to each other was essential in order to assure no environmental effects on performance throughout the duration of the experiment.

DISCUSSION

Increasing dietary protein level improved broiler performance at 21 d of age, determined by body weights and feed conversions, regardless of vaccination. This observation was expected as many reports have correlated crude protein level of diets to broiler performance (Sterling et al. 2003; Vieira et al., 2004). Crude protein level of diets is of extreme importance due to the cost associated with increasing the protein level in diets and thereby strongly affects costs as well as revenues in broiler meat production (Eits et al., 2005). Increasing dietary protein levels can be one route of eliminating the reduction in broiler performance due to anticoccidial vaccination during the starter period. In Experiment 1, a linear relationship was observed with respect to body weight and feed conversion associated with increased crude protein level to 21 d of age in vaccinated broilers. Broilers fed 20, 22, or 24% protein in the starter diet to 21 d of age differed significantly in performance characteristics and therefore, these protein levels were selected to use in the subsequent experiments to compare growth characteristics to non-vaccinated broilers during a field strain challenge of *Eimeria*.

Non-vaccinated broilers indicated similar patterns in performance characteristics as vaccinated broilers with improvement in performance characteristics related to increased protein level of the diet. Vaccination tended to reduce body weight and increase feed conversion compared to non-vaccinated broilers prior to challenge. However, in Experiment 2 where an interaction was observed and data were analyzed by one-way analysis, vaccinated broilers fed 22% protein had similar growth characteristics compared to the non-vaccinated broilers fed the 22% starter diet. Reduced body weights and increased feed conversion ratios during the early stages of growth due to vaccination have been reported by other investigators (Danforth, 1998; Williams, 2002), although other published reports have indicated that compensatory gain in vaccinated broilers during subsequent dietary periods results in similar if not improved performance characteristics at

the completion of grow-out (Danforth, 1998; Williams et al., 1999; Williams and Gobbi, 2002; Williams, 2002).

The generation of immunity through vaccination through improved performance of broilers during *Eimeria* challenge has been widely reported (Brake et al., 1997; Weber and Evans, 2003; Williams, 2003; Shirley et al., 2005) and was evident by significantly higher body weight gains, reduced feed conversion ratios, reduced lesion develop, and in some cases reduced mortality post challenge compared to non-vaccinated broilers. Improved growth characteristics during the challenge period in vaccinated broilers led to significantly improved cumulative feed conversion ratios (d 1-27) in broilers fed the two higher protein levels in Experiment 2.

In Experiment 2, increasing dietary protein tended to reduce lesion development associated with *E. maxima* while tending to increase lesion development associated with *E. tenella* in vaccinated chickens. The results obtained from these experiments indicate that a dietary protein inclusion rate of 22% in the starter diet allows for the many times similar growth characteristics of vaccinated and non-vaccinated broilers. Within these experiments, broilers immunized with Coccivac®-B had significant protection against lesion development associated with *E. acervulina* and *E. tenella*. Reductions in lesion scores, both significant and numeric, in the mid intestinal segments were observed but not to as great an extent as observed in the other two sites of infection. This is most likely attributed to the immunogenic variability observed within *E. maxima*. In order to gain significant immunization with the use of a commercially available live oocyst vaccine, Danforth et al. (1997) altered the vaccine with the addition of *E. maxima* strains locally isolated.

Data from this series of experiments indicate that vaccination with a live oocysts vaccine is an effective tool for the generation of immunity to subsequent field strain *Eimeria* challenge resulting in improved growth parameters in immunized compared to non-immunized broilers following challenge. Further observations indicate that dietary protein level is an important factor to consider when utilizing a vaccination program for the prevention of coccidiosis in order to maximize growth characteristics.

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Table 1. Calculated nutrient composition of experimental diets used in Experiments 1 through 4.

Nutrient					
Protein (%)	20	21	22	23	24
Methionine	0.44	0.47	0.50	0.52	0.55
TSAA	0.78	0.82	0.86	0.90	0.94
Lysine	1.06	1.12	1.19	1.26	1.33
Threonine	0.75	0.79	0.83	0.87	0.91
Arginine	1.32	1.40	1.48	1.55	1.63
Tryptophan	0.24	0.25	0.27	0.28	0.30
Calcium	0.90	0.90	0.90	0.90	0.90
Available Phos.	0.45	0.45	0.45	0.45	0.45
Sodium	0.20	0.20	0.20	0.20	0.20
ME (kcal/kg)	3200	3200	3200	3200	3200

Table 2. Average body weights (g) and mortality corrected feed conversion ratios ± SEM of live oocysts vaccinated (Coccivac-B®) broilers at day 21 fed varying concentrations of protein (Experiment 1).

Protein (%)	Body Weight (g)	Feed:Gain
20	585 ± 12[c]	1.57 ± 0.03[a]
21	665 ± 5[b]	1.40 ± 0.02[b]
22	679 ± 11[b]	1.35 ± 0.01[c]
23	689 ± 14[ab]	1.34 ± 0.02[c]
24	720 ± 9[a]	1.27 ± 0.01[d]

[a-d] Means with different superscripts within columns differ significantly at $P<0.05$.

Table 3. Average body weights (g) and mortality corrected feed conversion ratios ± SEM of non-vaccinated and vaccinated (Coccivac-B®) broilers at day 21 fed diets containing three different protein concentrations (Experiment 2).

Protein (%)	Treatment	Body Weight (g)	Feed:Gain
20	Non-vaccinated	664 ± 18^{c}	1.40 ± 0.03^{b}
20	Vaccinated	579 ± 8^{d}	1.60 ± 0.02^{a}
22	Non-vaccinated	733 ± 33^{b}	1.30 ± 0.01^{cd}
22	Vaccinated	709 ± 14^{bc}	1.34 ± 0.02^{bc}
24	Non-vaccinated	808 ± 10^{a}	1.26 ± 0.01^{d}
24	Vaccinated	737 ± 17^{b}	1.31 ± 0.02^{cd}

$^{a-d}$ Means with different superscripts within columns differ significantly at $P<0.05$.

Table 4. Average body weight gains (g), mortality corrected feed conversion ratios (FCR), and mortality ± SEM of non-vaccinated (NV) and vaccinated (V) (Coccivac-B®) broilers six days post mixed species *Eimeria* challenge at day 21 fed diets containing three different protein concentrations (Experiment 2).

Protein (%)	Vaccine	Chall[1]	BW Gain (g)	FCR Day 21-27	FCR Day 1 -27	Post-challenge Mortality[2]
20	NV	No	392 ± 7[a]	1.59 ± 0.02[d]	1.49 ± 0.03[d]	0.07 ± 0.07[bc]
20	V	No	377 ± 32[a]	1.61 ± 0.05[d]	1.60 ± 0.02[c]	0.00 ± 0.00[c]
22	NV	No	402 ± 8[a]	1.39 ± 0.16[d]	1.38 ± 0.01[e]	0.00 ± 0.00[c]
22	V	No	406 ± 16[a]	1.52 ± 0.04[d]	1.41 ± 0.02[e]	0.00 ± 0.00[c]
24	NV	No	415 ± 15[a]	1.49 ± 0.02[d]	1.35 ± 0.01[e]	0.07 ± 0.07[c]
24	V	No	396 ± 12[a]	1.49 ± 0.02[d]	1.35 ± 0.01[e]	0.00 ± 0.00[c]
20	NV	Yes	63 ± 17[d]	5.80 ± 0.46[a]	1.78 ± 0.03[a]	0.35 ± 0.09[a]
20	V	Yes	236 ± 21[c]	2.05 ± 0.09[d]	1.74 ± 0.05[a]	0.16 ± 0.08[abc]
22	NV	Yes	96 ± 6[d]	4.61 ± 0.22[b]	1.67 ± 0.01[b]	0.33 ± 0.03[a]
22	V	Yes	278 ± 19[bc]	1.89 ± 0.03[d]	1.50 ± 0.01[d]	0.07 ± 0.07[bc]
24	NV	Yes	84 ± 28[d]	3.91 ± 0.60[c]	1.59 ± 0.05[c]	0.23 ± 0.12[ab]
24	V	Yes	316 ± 24[b]	1.90 ± 0.03[d]	1.50 ± 0.01[d]	0.19 ± 0.10[abc]

[a-d] Means with different superscripts within columns differ significantly at $P<0.05$.

[1] Mixed species challenge contained *Eimeria acervulina* (6×10^5), *Eimeria maxima* (4×10^5), and *Eimeria tenella* (2×10^5) sporulated oocysts.

[2] Reported values are a result of a square root arcsin transformation of the observed mortality rates.

Table 5. Lesion scores of non-vaccinated and vaccinated (Coccivac-B®) broilers six days post mixed species *Eimeria* challenge at day 21of half of the treatment groups fed diets containing three different protein concentrations (Experiment 2).

Protein (%)	Vaccination	Challenge[1]	Upper	Mid	Lower
20	Non-vaccinated	No	0.26 ± 0.08^{bc}	0.40 ± 0.02^{e}	0.00 ± 0.00^{d}
20	Vaccinated	No	0.00 ± 0.00^{d}	0.87 ± 0.05^{de}	0.23 ± 0.12^{d}
22	Non-vaccinated	No	0.00 ± 0.00^{d}	0.64 ± 0.16^{e}	0.04 ± 0.04^{d}
22	Vaccinated	No	0.00 ± 0.00^{d}	0.87 ± 0.04^{de}	0.03 ± 0.03^{d}
24	Non-vaccinated	No	0.10 ± 0.05^{cd}	0.47 ± 0.02^{e}	0.00 ± 0.00^{d}
24	Vaccinated	No	0.00 ± 0.00^{d}	0.60 ± 0.02^{e}	0.07 ± 0.04^{d}
20	Non-vaccinated	Yes	0.13 ± 0.08^{cd}	2.13 ± 0.46^{a}	2.17 ± 0.11^{a}
20	Vaccinated	Yes	0.00 ± 0.00^{d}	1.90 ± 0.09^{ab}	0.60 ± 0.15^{c}
22	Non-vaccinated	Yes	0.33 ± 0.09^{b}	1.83 ± 0.22^{ab}	2.03 ± 0.11^{a}
22	Vaccinated	Yes	0.03 ± 0.03^{d}	1.57 ± 0.03^{bc}	0.77 ± 0.17^{c}
24	Non-vaccinated	Yes	0.53 ± 0.15^{a}	1.93 ± 0.60^{ab}	2.03 ± 0.11^{a}
24	Vaccinated	Yes	0.00 ± 0.00^{d}	1.20 ± 0.03^{cd}	1.40 ± 0.20^{b}

[a-e] Means with different superscripts within columns differ significantly at $P<0.05$.

[1] Mixed species challenge contained *Eimeria acervulina* (6×10^5), *Eimeria maxima* (4×10^5), and *Eimeria tenella* (2×10^5) sporulated oocysts.

EMERGING TECHNOLOGIES IN MICROBIAL ECOLOGY: CHALLENGE OF COCCIDIOSIS AND NECROTIC ENTERITIS TO THE HEALTH OF THE DIGESTIVE SYSTEM

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Necrotic Enteritis is caused by the gram positive, anaerobic bacterium *Clostridium perfringens. Clostridium perfringens* (CP) can be readily found in soil, dust, feces, feed and, especially, in poultry litter.

Clinical signs of the disease include droopiness, lack of appetite, diarrhea, ruffled feathers and, in severe cases, mortality in infected birds. A characteristic of necrotic enteritis is that the onset of the disease can happen quickly. If the disease persists in a flock for five to 10 days, mortality is expected to range from 2% to 50%. The subclinical form of necrotic enteritis does not cause mortality and often goes untreated, yet it is estimated by the U.S. Poultry and Egg Association to cost poultry producers worldwide over $2 billion annually. The disease is one of the more multifaceted and difficult problems that face poultry production worldwide.

CLINICAL DIAGNOSIS

Diagnosis of advanced cases of necrotic enteritis is relatively simple by examining postmortem lesions. Lesions from milder cases or from early postmortem diagnosis may be confused with coccidiosis, dysbacteriosis or viral enteritis. Using Giemsa or gram stains will show many bacilli in necrotic enteritis. In addition, histological sections of the intestines will show necrotic mucosa. And, bacterial cultures incubated under anaerobic conditions also will show typical clinical examples of CP bacterial colonies.

In clinical cases, postmortem examination frequently reveals thin and transparent intestines filled with gas. Spots are frequently seen on the inner surface of the intestines. In severe cases, necrotic spots coalesce and the entire inner surface of the intestines becomes bloody and necrotic.

CAUSAL AGENT

Clostridium perfringens (CP) causes enteritis in many animal species, including man. Necrotic enteritis in chickens is caused by a *Clostridium perfringens* which produces either alpha and/or beta toxins. Under optimum disease conditions, CP forms spores that are very resistant to environmental changes and can persist over long periods of time under harsh conditions.

The disease-causing bacteria are ubiquitous in the environments of chickens and turkeys grown in confinement. Subclinical necrotic enteritis significantly impairs feed efficiency, often causing birds to have diarrhea and an overall listless appearance. The disease may manifest itself as hepatitis or cholangeohepatitis found in broilers at processing.

Worldwide, there are over 800 known serotypes of CP. Multiple serotypes combined with the ubiquitous nature of disease-causing spores and the universal presence of spores in areas of the world where chickens are grown in close proximity explain why necrotic enteritis occurs so commonly in poultry production.

However it does not explain one of the many quirks of this disease, which is that healthy, highly productive birds often have low levels of *Clostridium perfringens* bacteria in their intestines. These bacteria contain the same toxins that cause both the clinical and subclinical forms of the disease. For some reason, not fully understood by the scientific community, the bacteria in healthy birds do not release enough of the toxin and to affect the productivity of the bird.

NUTRITION AND ENVIRONMENT ARE KEY ISSUES

As with any disease in which the cause is multifactorial, no single component can be determined to be THE most important. However, the continually high level of necrotic enteritis in areas where 20% to 25% corn or soybean is NOT maintained in the diet of broilers is compelling evidence that nutrition is one of the key factors in the development and spread of the disease.

Unlike corn and soybean meal, small grains (wheat, barley, oats) become highly viscous and move slowly through the digestive tract of chickens, allowing CP to increase in numbers and ferment. Fish meal and other feeds high in protein also are believed to be contributing factors to development of necrotic enteritis in chickens.

Ionophore coccidiostats help prevent or reduce the impact of necrotic enteritis. In countries in which grains are routinely fed to chickens, the use of digestive enzymes included as feed additives in feed have been used successfully to combat necrotic enteritis. Probiotics fed or sprayed over chickens, sometimes throughout their production cycle, have likewise been effective in reducing negative effects of necrotic enteritis. Perhaps the most successful dietary combatant to necrotic enteritis is the use of high corn concentrations in the diet of chickens, which allows more complete and easy digestion and absorption of feed nutrients.

SOLUTIONS

While causes of necrotic enteritis are many, solutions are few and getting fewer. The elimination of Avoparcin and other antibiotic growth promoters in Europe and elsewhere, including the United States, has created a predictable rise in necrotic enteritis and coccidiosis.

Coccidiosis is the most common agent to predispose birds to necrotic enteritis. The use of vaccination of broiler chicks with live vaccines causes a mild and temporary appearance of coccidians in the intestines, but does not normally enhance necrotic enteritis.

Drastic feed changes should be avoided and feed and water should be monitored for contaminants that alter intestinal motility or disrupt intestinal mucosa.

Bacitracin methylene disalicylate has proved effective in preventing and/or treating necrotic enteritis. The preventative level is 50 grams per ton in feed. Treatment rate for treating existing cases of necrotic enteritis is 100 to 200 grams per ton of feed, or about 110 to 220 parts per million. Penicillin, erythromycin and the tetracyclines have also proven effective in reducing the severity of necrotic enteritis.

INTESTINAL MICROFLORA

The intestinal microflora is the first line of defense for the intestine and the better we understand the complex interaction of the flora and the bird, the better we will be able to prevent intestinal disease. All of the above conditions, either alone but especially in combination, result in significant alterations of the intestinal microflora. The intestinal microflora is part of a complex ecosystem that is involved in augmenting intestinal development, immune surveillance, and competitive exclusion against pathogenic organisms. However, when the microflora fails to protect the mucosa from pathogens, antimicrobials are used to reduce the numbers of pathogenic organisms or to prevent their colonization of the intestine. We studied the effects of feed additives on the bacterial community within the ileum of chickens. We used two 16S ribosomal DNA community analysis protocols, terminal restriction fragment length polymorphism analysis combined with 16S rDNA clone libraries. These methods showed that feed additives caused significant alterations in the microbial community structure of the ileum.

FUTURE

There have been many non-antibiotic products that have been studied for prevention and control of necrotic enteritis. These include defined probiotics, undefined competitive exclusion products, complex carbohydrates, such as MOS, enzymes (digestive enzymes and lysozymes), essential oils and organic/inorganic acids. All appear to have some efficacy but, as expected, none work as consistently well in prevention/treatment as the antibiotics. However, it is this author's opinion that use of some of these products along with better understanding of the role of the microflora will help the poultry industry manage necrotic enteritis with no or minimal use of antibiotics.

Unraveling Feed Efficiency: A Mitochondrial Perspective

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Abstract

Studies have been conducted to assess associations of mitochondrial function and biochemistry with the phenotypic expression of feed efficiency (FE) in male broilers within the same genetic line and fed the same diet. Frequently, mitochondria obtained from broilers with low FE exhibited greater uncoupling of the electron transport chain (ETC). This respiratory chain uncoupling was apparently due to site-specific defects in electron transport that resulted in greater amounts of reactive oxygen species (ROS) being generated in mitochondria obtained low compared to high FE broilers. Higher amounts of ROS production in Low FE mitochondria were likely responsible for higher protein carbonyl levels that are indicative of higher protein oxidation. In turn, higher protein damage in Low FE mitochondria may have contributed to lower respiratory chain complex activity relative to values observed in high FE mitochondria. However, low FE mitochondria did not exhibit a compromised ability to carryout oxidative phosphorylation. While there were differences in expression of certain electron transport chain proteins, there was no indication that differences in coupling and respiratory chain activity could be attributed to a general decrease in protein expression between low and high FE mitochondria. We are currently investigating the expression of messenger RNA encoding for mitochondrial transcription factors or protein synthesis in low and high FE broilers. Such information will be useful in unraveling the role that mitochondria play in the phenotypic expression of feed efficiency.

Abbreviation Key: ANT1, adenine nucleotide translocase-1; ETC, electron transport chain; FE, feed efficiency; H_2O_2, hydrogen peroxide; RCR, respiratory control ratio; ROS, reactive oxygen species.

Introduction

An overview of the history of mitochondria was recently provided by Nisoli et al. (2004). It is interesting that the first description of mitochondria as discrete organelles occurred over 150 years ago by a cytologist, Kölliker, in 1850 (see Lehninger, 1965) and that the importance of mitochondria in the production of energy by oxidative phosphorylation for the cell was first reported over 50 years ago (Kennedy and Lehninger, 1949). Nisoli et al. (2004) indicated that the construction of the intricate components of the complete mitochondria as well as regulation of biogenesis and growth of existing mitochondria is complex and controlled by the activation of specific transcription factors and cell signaling pathways. Thus, understanding of mitochondrial physiology has obviously advanced in 50 years, but there are still much that remains to be explored. This paper will provide a short overview of mitochondrial function and physiology, and then will focus on studies being conducted that provide a linkage between mitochondrial function and biochemistry with the phenotypic expression of feed efficiency in broilers.

Mitochondrial Function and Biochemistry

The oxidative phosphorylation system consists of five multi-protein complexes (I to IV) and ATP synthase (Complex V) (Lehninger et al., 1993). As shown in Figure 1, electrons enter the electron transport chain (ETC) from nicotinamide adenine dinucleotide, reduced (NADH-) or flavine adenine dinucleotide, reduced ($FADH_2$-) linked substrates at Complex I and II, respectively. Electron movement in the ETC to the terminal electron acceptor, O_2, is coupled to proton pumping into the intermembrane space. The resulting proton motive force drives ATP synthesis (from ADP and P_i) as protons move back to the matrix through ATP synthase. Mitochondrial inefficiency may occur as a result of electron leak from the respiratory chain. Rather than being completely reduced to water, 2 to 4% of oxygen consumed by mitochondria may be incompletely reduced to reactive oxygen species (ROS) such as superoxide and hydrogen peroxide (H_2O_2) due to univalent reduction of oxygen by electrons that leak from the respiratory chain before they reach the terminal electron acceptor (Boveris and Chance, 1973; Chance et al., 1979). The mitochondrial formation of ROS makes this organelle a major source of oxidative stress in the cell. If not metabolized by antioxidants, ROS can cause oxidation of critical biomolecules (e.g. lipids, proteins, and DNA) in the mitochondrion and/or cell, that can lead to

further inefficiencies and accentuate additional ROS production.

Increased mitochondrial ROS production has been linked to various metabolic diseases (Fiegel and Shapiro, 1979; Hagen et al., 1997; Kristal et al., 1997; Herrero and Barja, 1998; Lass et al., 1998; Cawthon et al., 2001; Iqbal et al., 2001a; Tang et al., 2002). The use of respiratory chain inhibitors can be employed to identify site-specific defects in electron transport within mitochondria. Whereas electron leak occurs mainly within Complex I or III of the respiratory chain (Turrens and Boveris, 1980; Nohl et al., 1996; Herrero and Barja, 1998), Kwong and Sohal (1998) demonstrated that sites of H_2O_2 production are tissue dependent. For example, in broilers with pulmonary hypertension syndrome, increased ROS production was associated with Complex I and III in heart, muscle, and lung (Iqbal et al., 2001a; Tang et al., 2002) and Complex II in liver mitochondria (Cawthon et al., 2001).

Mitochondrial function and biochemistry is dependent upon the careful orchestration of protein synthesis occurring by nuclear (n) DNA encoding as well as synthesis of proteins by a discrete mitochondrial (mt) DNA that encodes 22 tRNA, 2 rRNA, and 13 ETC proteins (Anderson et al., 1981; Desjardin and Morais, 1990). Thus, expression of respiratory chain proteins is under control of both n and mt DNA (Sue and Schon, 2000). Mitochondrial function also requires import of hundreds of proteins including ETC proteins synthesized by nDNA (Rabilloud et al., 2002). Some proteins are part of the mitochondrial import machinery, whereas others are needed for expression of its genome and metabolism. While other proteins are important for apoptosis (Liu and Kitsis, 1996), redox cell signaling and homeostasis (Bogoyevitch et al., 2000; Levonen et al., 2001; Droge, 2002). Consequently, *"... mitochondrial function in general, and mitochondrial protein synthesis in particular, depend on the coordinated expression of both mitochondrial and nuclear genomes*" (Rabilloud et al., 2002).

Mitochondria and Feed Efficiency

A. Overview: Feed efficiency (FE) remains an important trait for commercial breeding companies as feed represents 50 to 70% of the cost of raising an animal to market weight. Genetic selection for FE has been responsible for more than 80% of the improvement in feed efficiency in modern broilers (Havenstein et al., 1994, 2003). As mitochondria are responsible for producing 90% of the energy needed for cells, we have conducted a series of studies to understand relationships of mitochondrial function and biochemistry with the phenotypic expression of feed efficiency in broilers

(Bottje et al., 2002, 2004; Iqbal et al., 2004, 2005; Ojano-Dirain et al., 2004, 2005ab; Tinsley et al., 2004; Lassiter, 2005).

It is well known that genetics and diet have profound influence over mitochondrial function. For example, differences in oxygen utilization rates between breeds of chicken (Mukherjee et al., 1970; Dziewiecki and Kolataj, 1976) have been observed and mitochondria have been hypothesized to be part of the basis for heterosis observed in plants (McDaniel and Sarkissian, 1966; Srivistava, 1981, sheep (Wolanis et al., 1980), swine (Dzabo and Wassmuth, 1983), and chicken (Brown et al., 1986). Dietary manipulations of fat and protein levels have also been shown to have effects on mitochondrial function (Renner et al., 1979; De Schrijver and Privett, 1984; Toyomizu et al., 1992abc). Yet in each of these studies, mitochondrial function was investigated in response to a dietary difference or with respect to different breeds. The studies outlined below will summarize results obtained in our lab in which mitochondrial function and biochemistry were determined in a single line of broilers fed the same diet, thus eliminating dietary effects or differences in breed (e.g. slow vs. fast growing or fat vs. lean lines). These studies have helped provide a better understanding of the cellular basis of feed efficiency.

In each of the studies in our lab, birds with the lowest or highest FE (6 to 8 per group) were identified within a group of 100 breeder male replacement stock (Bottje et al., 2002). In all studies summarized in this review, the high FE birds exhibited similar weights as low FE birds at the start of the week of the feed efficiency determination, but gained more during the week on the same amount of feed as the Low FE birds. Typical differences in FE between broilers with Low and High FE in these studies is provided in Figure 1 taken from Bottje et al. (2002). The dependency of body weight gain on feed intake is clearly indicated. Feed efficiency (FE, g gain /g feed) in this study was 0.64 ± 0.01 and 0.83 ± 0.01 for low and high FE groups, respectively. Tissues that have been investigated include breast muscle (*pectoralis superficialis*), leg muscle (*quadriceps femoris*), liver, the upper duodenum, heart and lymphocytes. Mitochondria were isolated by differential centrifugation.

B. Coupling and Oxidative Phosphorylation in Low FE Mitochondria: Relationships in muscle mitochondrial function and FE in broilers were presented (Bottje et al., 2002). There were no differences in mitochondrial function provided succinate ($FADH_2$- linked substrate). However, when provided NADH-linked substrates, the respiratory control ratio (RCR, an index of respiratory chain coupling [Estabrook, 1967]) was higher in high FE breast and leg muscle

mitochondria compared to Low FE mitochondria. These results indicate more efficient coupling of electron transport in High FE than in Low FE muscle mitochondria and provide indirect evidence that functional differences (i.e., differences in respiratory chain coupling) in muscle mitochondria between the two groups might be due to differences in electron transport associated with Complex I. Regression analysis revealed that breast mitochondria RCR values were highly correlated with FE similar to that reported in rats (Lutz and Stahly, 2003). There were no differences in the ADP:O with either energy substrate. Thus, Low FE mitochondria did not exhibit a compromised ability to carry out oxidative phosphorylation.

Studies were also conducted to determine relationships between intestinal mitochondrial function and FE in broilers (Ojano-Dirain et al., 2004). In this study, duodenal mitochondrial function was assessed following repeated additions of ADP; a paradigm of repeated energy demand that revealed mitochondrial dysfunction in pulmonary hypertension syndrome in broilers (Cawthon et al., 1999; Iqbal et al., 2001b). Unlike muscle, there were no differences in the initial RCR in duodenal mitochondria obtained from broilers with Low and High FE provided either NADH or $FADH_2$-linked energy substrates, but after a second addition of ADP, tighter coupling (i.e., higher RCR values) was observed in High FE duodenal mitochondria provided succinate but not when NADH-linked energy substrates were provided. These findings suggest that there was a defect in electron coupling associated with Complex II in Low FE duodenal mitochondria. As Low FE duodenal mitochondria provided NADH-linked energy substrates exhibited a significantly higher ADP:O ratio with the second addition of ADP, the ability to synthesize ATP may actually be superior in low FE duodenal mitochondria to that observed in high FE mitochondria under some conditions. Possibly, there is a greater demand for ATP in Low FE mitochondria, e.g., increased ATP needed to repair oxidatively damaged proteins (see Oxidative Stress below).

C. Increased Mitochondrial ROS Production and Site-Specific Defects in Electron Transport in Low FE Mitochondria. To determine if ROS production plays a role in inefficiencies associated with low FE mitochondria, hydrogen peroxide (H_2O_2) was monitored in freshly isolated mitochondria according to Iqbal et al. (2001a) to assess electron leak and to identify site-specific defects in electron transport in muscle, liver and intestinal mitochondria (Bottje et al., 2002; Iqbal et al., 2004; 2005; Ojano-Dirain et al., 2004). In these studies, basal electron leak represents H_2O_2 production in untreated mitochondria. A summary of relative

differences in basal ROS production in High and Low FE mitochondria provided either NADH or $FADH_2$-linked energy substrates is shown in Figure 2. With the exception of leg muscle, these findings indicate a generalized increase in ROS production, suggesting an inherently greater oxidative stress in Low FE mitochondria.

According to Barja (1999), increased radical production following electron transport chain inhibition indicates a defect in electron transport between the site of inhibition and entry of substrate into the electron transport chain. Thus, an increase in radical production in mitochondria with a specific inhibitor would indicate a site-specific defect at that site of the respiratory chain.

Bottje et al. (2002) reported increased electron leak (H_2O_2 production) in Low FE breast muscle mitochondria following electron transport inhibition of Complex I and III with rotenone and antimycin A, respectively, indicating that these are likely areas of site-specific defects in electron transport contributing to the higher basal H_2O_2 production in Low FE breast muscle mitochondria. No increase in H_2O_2 production in High FE mitochondria was observed following electron transport inhibition at Complex I and III suggesting High FE mitochondria have lower electron leak *in vivo.* No differences were observed when electron transport was inhibited at Complex II or the Q cycle of Complex III. Complex I may also be a potential site of electron leak in Low FE leg muscle mitochondria (Bottje et al., 2002).

Electron transport defects were also investigated in duodenal mitochondria (Ojano-Dirain et al., 2004). Similar that observed in muscle, basal radical production was higher in Low FE duodenal mitochondria provided NADH- or $FADH_2$-linked substrates. However, unlike muscle mitochondria, Low FE duodenal mitochondria provided with succinate or pyruvate-malate as energy sources exhibited site-specific defects in electron transport at Complex I, II, and III.

C. Lower Complex Activities in Low FE mitochondria. With the exception of one study (Ojano-Dirain et al., 2005a), we have observed a general reduction in the activities of Complexes I, II, III, IV, and/or V of the respiratory chain in Low FE mitochondria (Bottje et al., 2002; 2004; Iqbal et al., 2004; 2005; Ojano-Dirain et al., 2005b). Bottje et al. (2002) reported that activities of Complex I and II in breast and leg muscle mitochondria from broilers with Low FE were 63 to 79% of the levels of activity observed in High FE broilers. Additional studies by Iqbal et al. (2004; 2005) indicated that complex activities in Low FE muscle and liver mitochondria were all significantly lower than in High FE mitochondria. The biggest difference in was in Complex IV activity with values in the Low FE mitochondria that were 44 ± 8 and 59 ± 4 % compared to high

FE values in muscle and liver, respectively. These data suggest that a generalized decrease in respiratory chain complex activity in muscle and liver mitochondria is associated with low FE. In contrast to an earlier study (Ojano-Dirain et al ., 2005a), Ojano-Dirain et al. (2005b) reported that all respiratory chain complex activities, with the exception of Complex IV, were lower in Low FE duodenal mitochondria. The reason for differences between these studies is not apparent at this time.

D. Oxidative Stress and Complex Activities: With the evidence of increased ROS production occurring in Low FE mitochondria described above, one mechanism that might contribute to the generally lower activity of respiratory chain complexes that we have observed in Low FE mitochondria could be oxidative stress and subsequent damage to critical proteins in the respiratory chain.

Mitochondrial antioxidant protection from ROS includes reduced glutathione (GSH), vitamin E, Mn superoxide dismutase (MnSOD), GSH peroxidase and GSH reductase (Yu, 1994). GSH is vital to mitochondria by metabolizing ROS through the action of GSH peroxidase or by donating reducing equivalents directly. MnSOD converts superoxide to H_2O_2. In turn, GSH peroxidase uses reducing equivalents of GSH to convert hydro- and lipid peroxides to water or lipid alcohols. Metabolism of H_2O_2 is particularly important due to the propensity to be converted to the highly reactive hydroxyl radical in the presence of transition metals (e.g. Fe^{2+}, Cu^{2+}) via the Haber-Weiss and Fenton reactions, respectively. GSH reductase is vital in reducing oxidized GSH (GSSG), formed by GSH peroxidase, back to GSH to prevent thiol toxicity in mitochondria (Olafsdottir and Reed, 1988).

Although there were no differences in GSH peroxidase or reductase activities, Ojano-Dirain et al. (2005a) observed that GSH levels were lower ($P < 0.08$) and the GSSG/GSH ratio (an index of oxidative stress) was higher ($P < 0.08$) in low FE compared to high FE duodenal mitochondria. Regression analysis revealed positive correlations between GSH levels and the activities of Complex II, IV, and V. This would suggest that mitochondrial GSH in broilers may protect critical thiol groups in the respiratory chain complexes from oxidative damage as previously reported (Cardoso et al., 1999; Jha et al., 2000).

A major indicator of oxidative damage of proteins is the formation of protein carbonyls. Besides a consistent finding of increased ROS production in Low FE mitochondria, another

consistent observation we have observed is that of increased protein carbonyl formation in Low FE mitochondria or tissue (Fig. 3). Preliminary results indicate that there may be increased protein carbonyl levels associated with Complex III (Higgins et al., 2004).

E. Respiratory Chain Complex Activities and Protein Expression. Because the activities of respiratory chain complexes may depend on the amounts of protein of the protein subunits within each respiratory complex, breast muscle mitochondria from Low and High FE broilers were probed with antibodies for specific ETC proteins and their expression determined by Western blot analysis. Lower complex activities in Low FE muscle and liver were not due to a general decrease in ETC protein expression as several ETC proteins were expressed at levels equal to or higher than in High FE mitochondria (Iqbal et al., 2004; 2005). Similar findings have also been observed in lymphocytes (Lassiter, 2005) and heart muscle (Tinsley et al., 2004). In the duodenum, 6 of 8 nuclear-encoded proteins were higher in Low FE while 3 of 6 mitochondrial-encoded proteins were higher in High FE mitochondria (Ojano-Dirain et al., 2005b). The expression of ATP synthase α-subunit was higher in High FE liver and lymphocytes (Iqbal et al., 2005; Lassiter, 2005), was lower in High FE compared to Low FE duodenal mitochondria (Ojano-Dirain et al., 2005b), and was not different between Low and High FE groups in muscle (Iqbal et al., 2004).

In summary, while differences in expression of proteins were observed between Low and High FE mitochondria within a tissue, the differences did not hold for any particular ETC protein from tissue to tissue. Thus, differences in expression of mitochondrial proteins between Low and High FE mitochondria may depend whether they are encoded by mt- or n- DNA and apparently vary from tissue to tissue. From these findings, it does not appear that there is any decrease in expression of individual proteins in the respiratory complexes that can account for the generalized reduction in respiratory chain complex activities observed in Low FE mitochondria.

Another protein that should be mentioned is the adenine nucleotide transporter (ANT1). ANT1, located on the mitochondrial inner membrane, is responsible for exchange of ADP and ATP between the mitochondrial matrix and the cytosol (Li et al., 1989). As such, ANT1 is important for energy production, as membranes are impermeable to adenine nucleotides. Indeed, decreased mitochondrial function was observed in ANT1 knockout mice that lacked the ability to exchange ADP and ATP between the mitochondria and cytosol (Graham et al., 1997). Thus, differences in expression of ANT1 could be hypothesized to play a role in the phenotypic expression of feed

efficiency as well. The expression of ANT1 was higher in low FE muscle (Iqbal et al., 2004) and heart (Tinsley et al., 2004) mitochondria, but not in liver (Iqbal et al., 2005) or duodenum (Ojano-Dirain et al., 2004).

SYNOPSIS

Investigation of respiratory chain protein expression in muscle and liver did not reveal a consistent pattern in protein expression between broilers with low and high feed efficiency. As there are many other proteins that are important for function of mitochondria and the respiratory chain (e.g. transport proteins, scaffolding proteins), it is possible that the differences we seek may lie in this direction; i.e., proteins other than those associated with the respiratory chain. For this reason, we have initiated a proteomic approach directed towards identifying candidate proteins that are differentially expressed according to FE type. We have also begun to investigate transcription factors that influence mitochondrial protein synthesis that may be related to feed efficiency such as that reported in rats by Nisoli et al. (2004). In comparison to High FE, mitochondria obtained from Low FE broilers appear to exhibit decreased electron transport chain coupling, increased electron leak with subsequent increased ROS production, increased protein oxidation, and lower respiratory chain complex activities. We believe that the lower complex activities may be the result of increased protein oxidation. The positive correlation observed between mitochondrial GSH and Complex II, IV and V activity in the duodenum appear to support this hypothesis. Thus, the consistent findings of higher mitochondrial ROS production and protein oxidation suggest that these are important mechanisms contributing to the phenotypic expression of Low FE in broilers in our studies. While we have identified higher ROS production and protein oxidation in broilers with low feed efficiency, the important question of what causes these processes to occur in the first place remains unanswered.

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FIGURE 1. Diagrammatic representation of the electron transport chain adapted from Lehninger et al. (1993) (p. 559). The electron transport chain consists of 4 protein complexes (Complex I, II, III and IV). Electrons (e-) that enter the electron transport chain from energy substrates such as malate (Complex I) and succinate (Complex II) are passed down the electron transport chain (solid arrows) to the terminal electron acceptor, oxygen that is reduced to water. Coenzyme Q (CoQ, ubiquinone) accepts electrons from both Complex I and II and passes them to Complex III. Associated with the movement of electrons along the electron transport chain is the movement of protons (H+, dashed arrows) from the mitochondrial matrix into the intramembranous space, creating a proton motive force. The movement of protons through the ATP synthase (ATPase) provides the energy to support ATP synthesis.

FIGURE 1

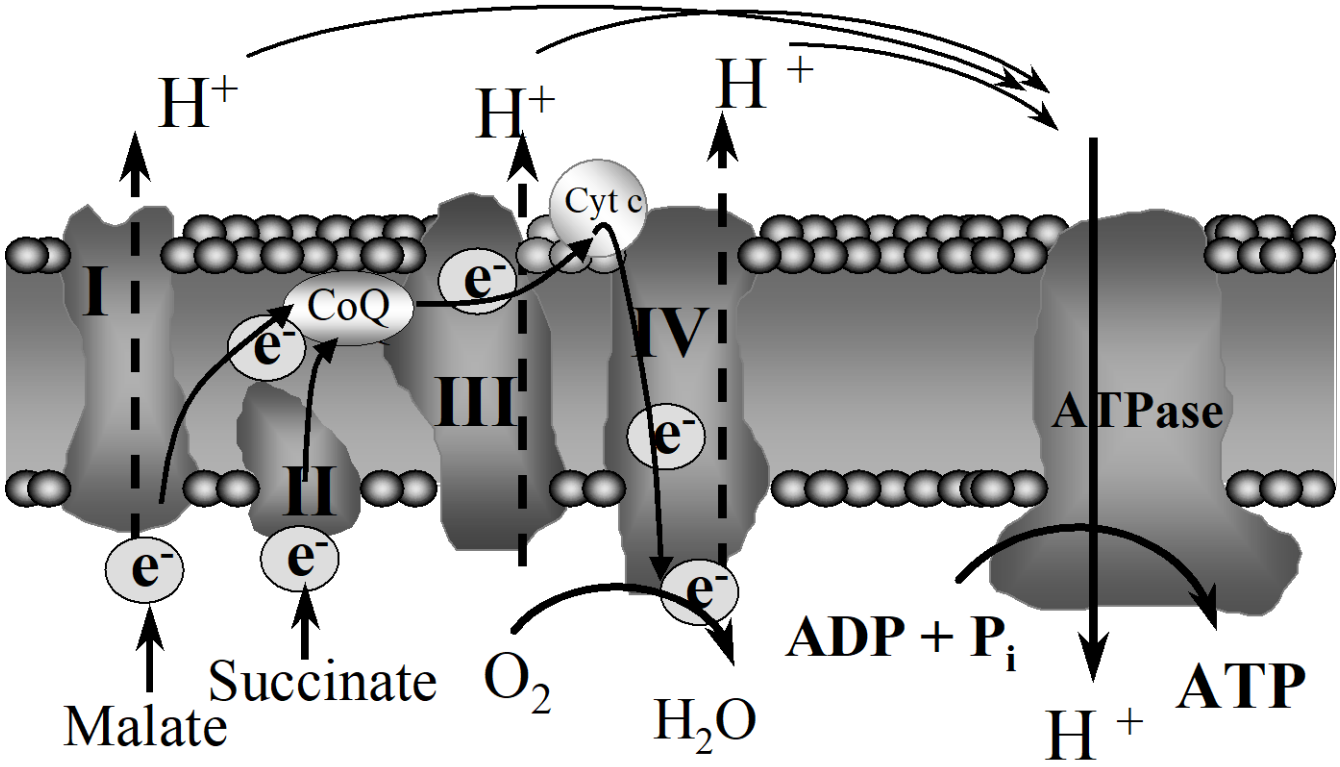
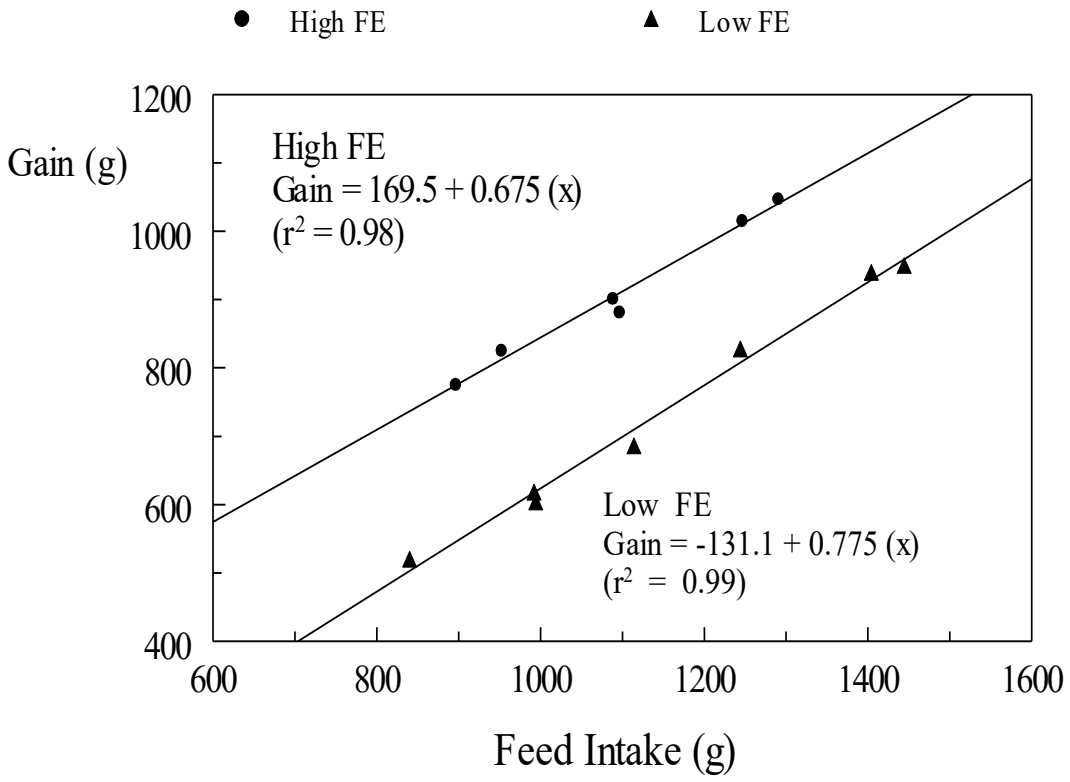


FIGURE 1. Relationships between total feed intake (Feed Intake, g) and body weight gain (Gain, g) between 6 and 7 weeks of age in male broiler breeders with high and low feed efficiency (FE) (from Bottje et al. 2002). Regression equations shown were significant ($P < 0.05$).

FIGURE 2



(from Bottje et al., 2002)

FIGURE 2. Relative levels of basal hydrogen peroxide (H_2O_2) production in mitochondria obtained from broilers with high feed efficiency (FE, solid bars) and low FE (open bars). The data was obtained from breast and leg muscle (Bottje et al., 2002), from duodenum (Ojano-Dirain et al., 2004) and liver (Iqbal et al., 2005) (See Figure 6). Comparisons were made between low FE mitochondrial H_2O_2 production expressed as a percent of values obtained in high FE mitochondria. Energy sources used in these studies shown in parentheses below each tissue were glutamate (Glut), succinate (Succ) and malate (Mal). Each of the values represent the mean $\pm$ SE of 5 to 8 observations.

FIGURE 2

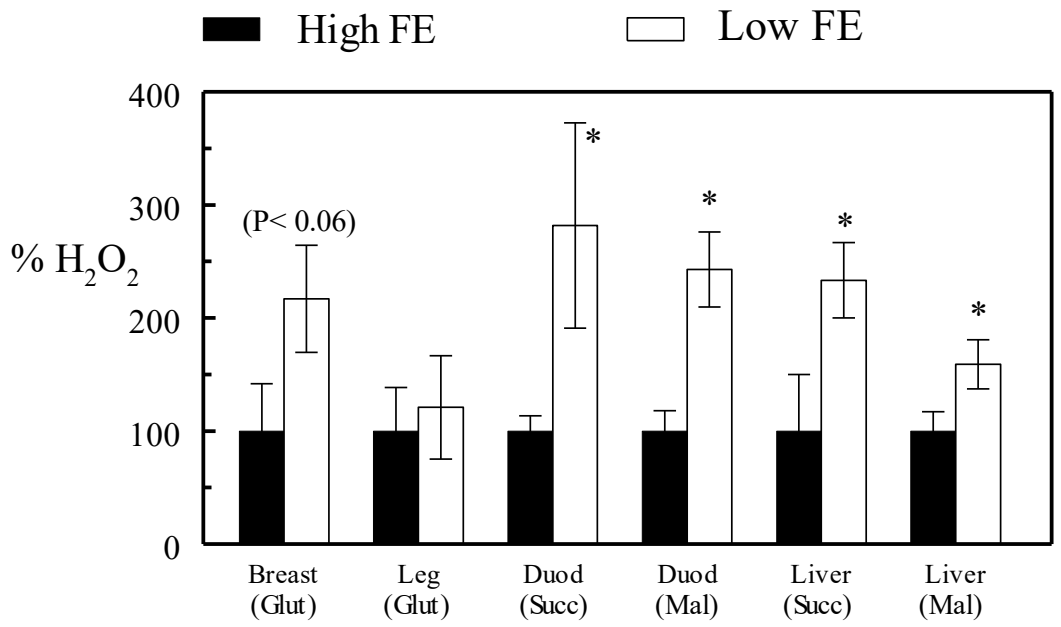


FIGURE 2. Relative levels of basal hydrogen peroxide (H_2O_2) production in mitochondria obtained from broilers with high feed efficiency (FE, solid bars) and low FE (open bars). The data was obtained from breast and leg muscle (Bottje et al., 2002), from duodenum (Ojano-Dirain et al., 2004) and liver (Iqbal et al., 2005) (See Figure 6). Comparisons were made between low FE mitochondrial H_2O_2 production expressed as a percent of values obtained in high FE mitochondria. Energy sources used in these studies shown in parentheses below each tissue were glutamate (Glut), succinate (Succ) and malate (Mal). Each of the values represent the mean $\pm$ SE of 5 to 8 observations.

Figure 3

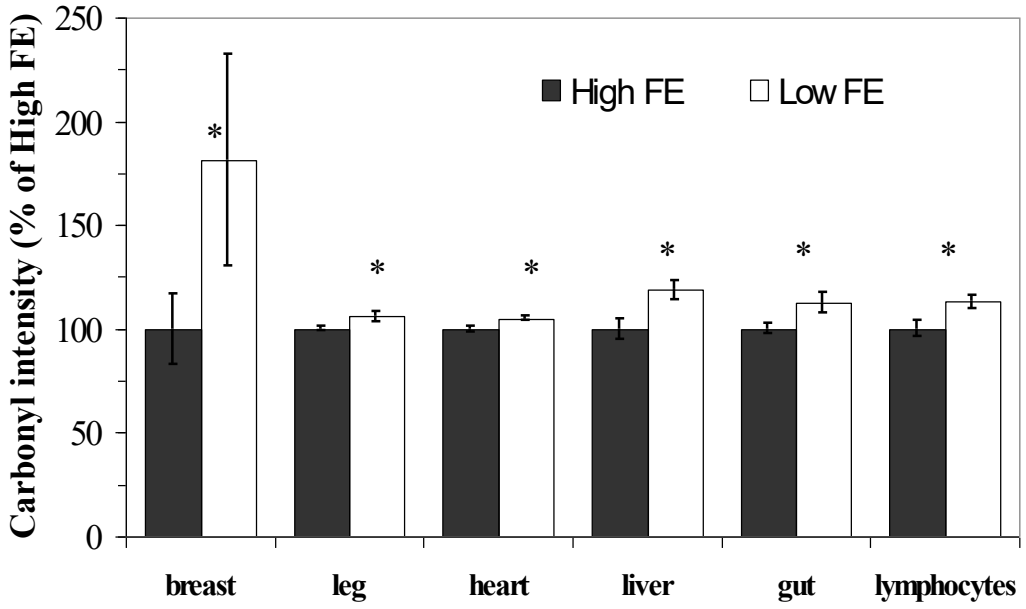


Figure 3. Relative amounts of protein carbonyls in breast muscle and upper duodenum (gut) mitochondria and in leg, heart, liverand lymphocytes obtained from broilers with High (solid bars) and Low (open bars) feed efficiency. Each bar represents the mean ± SE of 5 to 8 observations.

* Protein carbonyl levels in Low FE mitochondria or tissue are higher than High FE values ($P \leq 0.05$).

ENTERIC PATHOGENS ASSOCIATED WITH POULTRY PROCESSING

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Introduction

The United States is the world's largest producer and exporter of poultry meat (American Meat Institute, 2004). Each year in the U.S. over 8,700,000,000 chickens and 2,710,000 turkeys are processed in commercial facilities. Furthermore, chicken has become the most frequently consumed meat in the U. S. with a per capita consumption of 82.0 pounds per person reported in 2002, which is a substantial increase from 40.3 pounds per person that was consumed in 1970. Additionally, while 8.1 pounds of turkey meat were consumed per person in 1970, consumption increased to 17.7 pounds per person consumed in 2002.

However, contaminated poultry products can serve as sources of human foodborne illnesses. Each year, approximately 10% of the reported cases of outbreaks of human foodborne illnesses are related to contaminated poultry (Bryan, 1988). Annually, these poultry-related foodborne outbreaks are responsible for between 6 to 81 million cases of human foodborne illnesses; approximately 9,000 deaths; and financial losses of $5.6 to 9.4 billion in medical costs, lost wages, and reduced production (Mead, 1999). Therefore, foodborne pathogens associated with poultry processing continue to be a major food safety issue with regulatory agencies, scientific researchers, poultry processors, and consumers.

Bacteria associated with poultry processing

Campylobacter. *Campylobacter* is a Gram-negative, spiral shaped bacterium with fastidious growth requirements (Keener, et al., 2004). *Campylobacter* also possess characteristic rapid, darting motility, and the bacterium requires microaerophilic conditions

for growth. The status of *Campylobacter* as a human pathogen was first recognized in 1957 (King, 1957), and the microorganism's role as a common foodborne pathogen was recognized in 1977 (Skirrow, 1982). Currently, *Campylobacter* has been identified worldwide as the leading cause of human, bacterial foodborne illnesses (Mead, et al., 1999). Sources of campylobacteriosis include contaminated water, raw milk, and raw or undercooked poultry (Keener, et al., 2004).

Campylobacter strains most frequently isolated from humans are the same strains that are also most commonly isolated from poultry (Bryan and Doyle, 1995), and *Campylobacter jejuni* is the species responsible for 99% of the cases of campylobacteriosis (Friedman, 2000). Additionally, employment in poultry processing plants has been identified as a significant factor in contracting campylobacteriosis (Grados, et. al, 1973). Foodborne *Campylobacter* infections have been reported to be responsible for approximately 2.4 million illnesses, 150,000 physician visits, 13,174 hospitalizations, and 124 deaths annually (Mead, et al., 1999). Signs and symptoms of campylobacteriosis include abdominal pain, fever, nausea, and diarrhea. The neurological disorder, Guillain-Barré syndrome, has also been associated with campylobacteriosis.

Campylobacter primarily colonizes the ceca and colon of live poultry, and the bacterium can be spread to the skin of the carcass during processing (Berrang et al., 2000). The level of contamination of carcasses by *Campylobacter* decreases during processing, but the bacterium can still be recovered from fully processed carcasses (Hinton et al., 2004). Some researchers report that *Campylobacter* has been detected in 98% of retail poultry (Stern and Line, 1992), while other reports indicate that recovery of this pathogen from various poultry products range from 0-100% (Bryan and Doyle, 1995).

Salmonella. *Salmonella* is a Gram negative, motile, rod shaped bacterium with facultative atmospheric requirements. *Salmonella* has a long history as a human pathogen, and this bacterium is still recognized as a major human foodborne pathogen around the world. Foods commonly contaminated with *Salmonella* include raw milk, red meats, eggs, and poultry. Contaminated poultry products are the most common source of these foodborne infections, however (Baird-Parker, 1990). Over 2000 serotypes of this bacterium have been identified (Bryan and Doyle, 1995), but the serotypes *Salmonella* Enteritidis and *Salmonella* Typhimurium are frequently isolated from cases of human

foodborne illness associated with contaminated eggs and poultry meat. Each year, approximately 40,000 to 50,000 cases of salmonellosis and 600 deaths due to *Salmonella* infections are associated with contaminated food products. Signs and symptoms of salmonellosis include fever, headache, nausea and vomiting, abdominal pain, and diarrhea (Bryan and Doyle, 1995).

Salmonella primarily colonizes the ceca and large intestines of live broilers although resistance to colonization by this pathogen increases as broilers age (Brownell, et al., 1970). When broilers colonized by *Salmonella* enter processing facilities, cross contamination can lead to the spread of the organisms to other carcasses and processing equipment. *Salmonella* on contaminated carcasses can survive processing operations; therefore, this pathogen can also be recovered from fully processed carcasses (Knivett, 1971).

Escherichia coli. *E. coli* is a Gram-negative, motile, rod shaped bacterium with facultative oxygen requirements. The organism is a member of the group of coliform bacteria, which consists of Gram-negative rod-shaped bacteria that produce gas from glucose and ferment lactose to acid and gas within 48 h at 35ºC. Coliforms are classified as indicator microorganisms because they are components of the native microflora of the intestinal tract of animals; therefore, their presence is sometimes used as a measure of fecal contamination. Since some pathogenic bacteria are also associated with fecal material, the presence of coliforms can potentially indicate the presence of pathogenic bacteria. Most coliforms are non-pathogenic; however, and since coliforms can be isolated from the environment, as well as from the intestinal tract, the presence of these bacteria does not always indicate that pathogens are present (Greenberg and Hunt, 1985). Nonetheless, large numbers of these bacteria are undesirable in food products because they indicate unsanitary processing practices (Jay, 1978).

Poultry Processing Operations

Commercial poultry processing operations generally include scalding, defeathering (picking), evisceration, and chilling. Live poultry entering the processing facility carry microorganisms on their feathers, feet, and skin and in their intestinal tract (Kotula and Pandya, 1995). Between 80 to 100% of broilers entering processing plants are

contaminated by *Campylobacter*, and 60 to 90% of these chickens have been reported to carry *Salmonella*. Generally, processing decreases the level of contamination of broiler carcasses (Izat et. al, 1988), but individual processing operations may increase or decrease the level of contamination of the carcasses (Hinton, et al., 2004a).

Scalding. After broilers are killed the carcasses are scalded by immersion in scald tanks filled with hot (50-60°C) water (Cason, et al., 1999). Scalding opens feather follicles on the carcasses; thereby, increasing the efficiency of mechanical defeathering. The level of scald water contamination is directly related to the level of contamination of carcasses entering the scald tank. During scalding operations, the concentration of microbial contaminants in the scald water increases, then reaches equilibrium (Mulder, 1978), as microorganisms from the carcasses are killed by hot water, float away in water overflow, or remain in the scalder (Bryan and Doyle, 1995). Between 5,900 to 17,000 cfu/ml have been isolated from scald water at the end of a processing shift (Walker and Ayres, 1959). Scalding operations have been shown to reduce the number of aerobes, *E. coli*, and *Campylobacter* (Cason, et. al 1999) on carcasses during processing.

Counterflow scalders are a special type of scalding system that operates by adding clean water near the point where carcasses exit a series of scalders; therefore, the carcasses and water move in opposite directions through the series of tanks. In a study that examined bacterial contamination in a 3 tank counterflow system, significantly fewer coliforms, *E. coli*, *Campylobacter*, and *Salmonella* were isolated from the final tank than from the first tank (Cason and Hinton, 2006).

Defeathering. Scalded carcasses are passed through mechanical defeathering machines for feather removal. These machines can be major sites of cross contamination (Hinton, et al., 2004b) because bacteria can form biofilms on the surface of rubber fingers. Since these fingers can be difficult to clean and disinfect, bacteria can be passed from carcass to carcass as carcasses are moved through the machine. Additional carcass contamination may result from the physical force on the carcasses as picker fingers press against the carcass during defeathering and force fecal material through the cloaca and onto the skin of the carcass (Berrang and Dickens, 2000).

Evisceration. Modern processing facilities use automated, mechanical devices during evisceration to remove internal organs from carcasses after defeathering. Cross

contamination between carcasses, evisceration equipment, and water can also occur during this operation. Approximately 78% of broilers entering eviscerators carry *Campylobacter* on skin, while 48% of carcasses carry this pathogen in their crops, and 94% of the carcasses carry the bacterium in their intestinal tracts (Jeffery, et al., 2001). Causes of carcass cross contamination during evisceration include the rupture of crops and intestines by personnel and machines and the mechanical force that pushes fecal material out of the cloaca (Mead, 1989). Each of these actions can lead to increased contamination of the carcass by fecal microorganisms.

Washing. In 1978, the use of carcass washers in poultry processing lines was adopted (Kenner, et al., 2004). These machines are employed to wash away blood and fecal materials present on carcasses after evisceration and before the carcasses enter chill tanks. Between 20 to 50 ppm of chlorine may be added to wash water to decrease microbial contamination. Types of washers used include brush washers that scrub carcasses with rubber fingers while washing with water, cabinet washers that wash carcasses with a series of spray nozzles within an enclosed cabinet, and inside-outside washers that rotate the carcass while a series of nozzles wash the outside as water sprayed from an internal probe washes inside the carcass cavity. Washers can use between 25 to 50 gallons of water per minute or up to 9 liters of water per carcass. The cost of washing carcasses has been estimated to cost between $500 thousand to $1 million/year (Jackson, et al. 1999).

Chilling. After evisceration, commercial poultry processors rapidly chill carcasses to prevent bacterial growth. Processors may employ a variety of chilling methods, and the level of cross contamination may be related to the type of chilling device used during processing. Immersion chilling involves submerging and agitating carcasses in mixtures of ice and water. Although, cold temperatures of chiller water can inhibit microbial growth, microorganisms washed from carcasses in immersion chillers can contaminate other carcasses in the chiller during this process (Mead, 1982). Chiller water may be chlorinated with 20 to 50 ppm chlorine to control cross contamination (Russell and Keener, 2007), but chlorine has little effect on microorganisms that remain attached to the carcass. Chlorine is effective in killing microorganisms floating in chiller water, however. Spray chillers are a type of chiller that cools carcasses by spraying chilled water onto carcasses as they pass by on a conveyor belt. Air chillers operate in a similar fashion as spray chillers, but cold air is

blown onto passing carcasses instead of water (Sanchez et al, 2002). Since there is little or no carcass-to-carcass contact during spray chilling or air chilling, cross contamination is reduced.

USDA-FSIS Pathogen Reduction/HACCP ruling. The United States Department of Agriculture (USDA)- Food Safety Inspection Service (FSIS) Pathogen Reduction/HACCP ruling was passed in 1996 in an effort to decrease microbial contamination in poultry and meat slaughtering facilities. Hazard Analysis and Critical Control Points (HACCP) includes focusing on areas in processing operations where opportunities for contamination are prevalent and incorporating processing controls designed to reduce or prevent food safety hazards. The ruling requires facilities to implement a written plan (sanitation standard operating procedures) that outlines how sanitary conditions will be maintained within the facility during processing. Each facility is responsible for monitoring processing operations for the level of generic *E. coli* present to provide data that verifies the efficiency of controls for preventing fecal contamination. The FSIS is responsible for sampling processing operations for *Salmonella* within processing facilities in order to insure that facilities meet performance standards set by the FSIS. Additionally, a zero tolerance level for visible feces on carcasses before entering chilling operations was implemented. Visible feces may be removed from the carcass by trimming or washing or by on- or off-line processing.

Sanitizers. Sanitizers used in poultry processing operations must be efficient; decrease the level of microbial contamination; be safe for processing workers and consumers; and have little or no negative effect on the appearance, taste, odors, or quality of processed carcasses (Scientific Committee on Veterinary Measures Relating to Public Health, 2004). Physical sanitizers include cold water, hot water, pressure, and steam. Chemical sanitizers include chlorine, chlorine dioxide, trisodium phosphate (TSP), organic acids/bases, and inorganic acids/bases. Chlorine is the most widely used sanitizer in commercial poultry processing facilities because it has a broad antimicrobial spectrum and is relatively inexpensive. However, the use of chlorine in processing has been prohibited in Europe and Canada because of the potential for the formation of carcinogenic compounds.

Conclusion

The consumption of poultry products continues to increase as health conscious consumers seek low fat, nutritious diets. Although it is the responsibility of the consumer to insure that poultry products are stored and prepared properly, researchers and poultry processors must continue to search for methods to reduce microbial contamination that occurs during poultry processing. Decreasing contamination of poultry carcasses during processing will decrease the number of foodborne illnesses associated with poultry consumption and insure that consumers will have access to a wholesome, safe product.

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Effect of Heat Stress on Production Parameters and Immune Responses

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Introduction

Modern commercial poultry have the ability to grow to a large size in a short amount of time due to intensive genetic selection. However, this selection may be at the expense of other traits, including tolerance of heat (Washburn, 1980; Geraert, 1993). When discussing heat stress and heat tolerance, it is important to keep in mind that in different parts of the world, heat stress may mean very different things. In a cool climate, chickens may feel heat stressed at 25C, but in a hot climate, heat stress may not become a problem until temperatures reach 32C. The duration of heat stress can be very short, such as during transport, or may occur in a cyclic pattern with cooler temperatures at night and very hot temperatures during the day (Balnave, 2004). In fact, even heat stress during transport of chicks for as short as one hour can depress the growth rate in chicks for up to 16 days (Ernst et al., 1984). Although heat stress can occur in different ways, it is almost always detrimental to the health and performance of poultry. This paper will review the effects of the heat stress on chickens, and also highlight research which provides some potential solutions.

Physiological Responses to Heat Stress

One of the first responses to heat stress, and one which can indicate the extent to which a bird is stressed is an increase in body temperature (Cooper and Washburn, 1998). An average body temperature for chickens is 40.5 to 41.5 C. An increase in body temperature of 1 to 2 C can occur as stored heat in birds, after which the heat must be released from the body or it will begin to damage the bird and will eventually cause death (Etches et al., 1995). Observable changes in the behavior of chickens are the first visible indications of heat stress. The most obvious behavior is panting, in which the chickens will cool themselves by evaporation of water from the nasal passages and upper respiratory tract (Etches et al., 1995). In one study, it was found that hens could increase their rate of water evaporation by panting from 5 to 18 g/hr (Lee et al, 1945). Paradoxically though, panting also has adverse effects. Panting can cause alkalosis, which is a result of increased plasma pH. Briefly, panting causes birds to lose more CO_2 from the lungs, so that there is less CO_2 and bicarbonate in the blood, which increases the plasma pH (Richards, 1970). Poor eggshell quality results from the reduction of available bicarbonate (Mongin, 1968). Other behavioral changes due to heat stress are: less time spent walking and standing, less feed consumption, higher water intake, and less social behavior (Etches et al., 1995). Obviously, these behavioral changes will result in poor performance as body weight is rapidly lost. Heat stress has a negative correlation with feed consumption and feed conversion ratio as well (Cooper and Washburn, 1998). Other physiological indications of

heat stress include decreased hematocrit levels, increase in heterophil/ lymphocyte ratios, and an increase in tonic immobility scores which are an indication of fearfulness (Altan et al., 2003). Other studies also report an increase in superoxide radicals in skeletal muscle mitochondria of broilers (but not layers) subjected to 18 h of heat stress on day 16. These reactive oxygen species result from the 'leaking' of electrons from the electron transport chain in the mitochondria, which then react with oxygen (Mujahid et al., 2005).

Besides affecting performance, heat stress can adversely affect the immune system in chickens, though the mechanism is not well understood. Heat stress of laying hens from 5 to 31 weeks of age resulted in increased mortality and decreased egg production. In this experiment heterophil/ lymphocyte ratios were also significantly increased, and antibody titers to sheep red blood cells were decreased (Mashaly et al., 2004). Heat stress also was found to increase bacteremia and mortality in chickens who received an oral challenge with a pathogenic *E. coli*, potentially due to increased penetration of the bacteria into the bloodstream from the gastrointestinal tract (Leitner and Heller, 1992).

Not only does heat stress effect peformance, production and immunity, it also has adverse effects on fertility. Heat stress decreased sperm penetration and egg fertility in broiler breeder hens receiving sperm from heat stressed males (McDaniel et al., 1995). *In vitro* studies with semen indicate that heat stress significantly decreased sperm viability and sperm quality index scores (Karaca et al., 2002).

Acclimatization and Thermal Conditioning in Broilers

Many solutions for improving performance during heat stress are aimed at maintaining a lower body temperature, which will in turn prevent the lethargic behaviors described above and maintain a higher level of performance. Acclimatization is a method explored in which the temperature is raised gradually over time, which may allow the birds to adjust gradually to hot temperatures. In one study, it was found that the body temperature of chickens increased when ambient temperatures reached 30C under normal conditions. When they were acclimatized for 3 to 5 days, their body temperature did not increase until temperatures reached 33C (Boone and Hughes, 1971). Though this research is encouraging, in many countries, poultry will experience temperatures well above 33C. Also, heat stress is often unplanned, that is, there is not an opportunity to anticipate the stress and acclimate birds accordingly.

An alternate solution might be the use of thermal conditioning or feed restriction at a young age to increase chicks resistance to a heat stressor in the future. De Basilio et al. (2003) found that thermal conditioning occurring for 24 hours on day 5 significantly reduced the body temperature of broilers at 7 d of age. Arjona et al. (1988) also found that the use of early thermal conditioning (35 to 37.8C for 24 h on day 5) significantly reduced mortality during a heat challenge at the same temperature for 8 h on days 44 and 45. In 2001, Yahav and Mc Murtry (2001) investigated the optimal timing and duration of early thermal conditioning in broiler chicks, and they found that thermal conditioning on day 3 for 24 hours yielded the best results in regards to body weight and mortality. Use of longer

periods of thermal conditioning reduced compensatory growth, and conditioning on day 3 yielded the lowest mortality of the treatments evaluated. When comparing one day (d5 of age) of thermal conditioning to two days of thermal conditioning (d5 and d7), Yahav and Hurwitz (1996) found that the chickens receiving two days of thermal conditioning did not achieve complete growth compensation during the experiment. Heat challenge occurred on day 42 at 35C. However, with either form of conditioning, the mortality was significantly lower than control chicks, and heart weight and hematocrit were also significantly lower.

Using a different protocol for thermal conditioning, Liew et al. (2003) found no difference in mortality following heat challenge. In these studies, the thermal conditioning occurred from day 1 to day 21, and consisted of 36C for 1 hour a day. Additionally, 60% feed restriction (chicks are allowed to eat only 60% of the amount the controls consume) or a combination of both thermal conditioning and feed restriction were compared. Heat challenge was administered from day 36 to 50 for 2h each day at 38C. Additionally, a challenge with a 10X dose of IBD vaccine was administered at day 37, during the heat challenge period. The authors found no differences in body weight or IBD antibody titer, but did have significantly less mortality in the group receiving both feed restriction and thermal conditioning treatments. Additionally, this group had significantly higher HSP 70 levels, which was also correlated with significantly lower bursal histological scores. In another study, Yahav et al (1997) found that thermotolerant chicks in fact had lower levels of HSP, which indicates that the induction of HSP is not a part of thermotolerance, but simply a response in the bird to higher temperatures.

Zulkifli and coworkers found in a feed restriction experiment that allowing broiler chicks to receive only 60% of the feed that control chicks ate on days 4, 5, and 6 allowed them to have a higher body weight than control chicks following a heat stress at for 2h at 38C from day 35 to 41. In this study the mortality during heat stress was also significantly lower in the 60% feed restricted group. There was 0% mortality in this group, compared to 19% in the controls (non-feed restricted) Additionally, titers for Newcastle Disease following vaccination were significantly decreased in controls following heat stress, but were unchanged in feed restricted chickens. (Zulkifli et al., 2000a). Yahav and Plavnik (1999) also compared feed restriction (to allow only 50% of normal growth rate from d7 to d14) with a thermal conditioning at 36C for 24 h at day 5. A third treatment consisted of both the feed restriction and thermal conditioning. On day 42 the birds were heat stressed at 35C and 20-30% relative humidity for 6 hours. Mortality in all three treatments was significantly lower than controls in the first experiment, and all but the feed restricted alone chickens had lower mortality in the second experiment. Although there was some decreased growth due to the early treatments, all body weights were the same by 35 d of age.

De Basilio et al. (2001) compared the use of thermal conditioning at day 5 for 24 h to the use of a dual feeding program where the chickens received a low protein and high

energy diet during the day from day 19 to day 21, but a high protein and low energy diet at night. A heat challenge was administered on day 34 of 36C for 7 hours. Birds who experienced thermal conditioning or dual feeding had significantly less mortality compared to un-treated control chickens. The body temperature of the broilers who survived the heat challenge was also significantly lower in both treatments. The investigators also observed a correlation between body temperature at day 32 (prior to heat challenge) and mortality. Chickens who had a body temperature of greater than 42.2C had 75% mortality, while chickens with body temperatures of less than 41.5C had only 3% mortality.

Feed and Water Supplements During Heat Stress

Bartlett and Smith found in 2003 that formulation of a diet with 181mg/kg zinc increased the total IgM and IgG in the primary immune response following heat stress compared to diets with lower levels of zinc. The high zinc diet also resulted in increased numbers of macrophages. The hypothesis was that the reduction in feed consumption during heat stress resulted in a zinc deficiency. However, in this experiment all chicks who were heat shocked had significantly lower body weight, feed conversion, and feed intake.

Adminstration of a 0.6% KCl solution increased water intake during heat stress, and decreased accumulation of ions in the blood associated with heat stress (Ait-Boulahsen et al, 1995). Carpenter et al (1992) found that use of nipple drinkers with a higher water flow rate (2.3 mL/s compared with 0.4 mL/s) decreased mortality in heat stressed broilers. However, they also found significantly increased litter moisture in pens where high-flow drinkers were used. Ferket and Qureshi (1991) explored the addition of vitamins A, D, E, and B with or without electrolytes in the drinking water of heat stressed broilers. The supplements were added to the drinking water on days 16 to 21 and 38 to 43. The heat stress was administered during the last 72 hours of water treatments, and was at a temperature of 35C. Addition of the vitamins alone significantly increased feed conversion (5.6%) and body weight gain (6.7%). This treatment also significantly increased the levels of IgG following the second vaccine administration, and the numbers of macrophages elicited with sephadex (though there were no differences in macrophage adherence or phagocytic activity). Addition of ascorbic acid (vitamin C) (0 to 1,000ppm) and or acetylsalicylic acid (0 to 500 ppm) to the feed of chickens who were heat stressed in cyclic conditions (24 to 35 C) did not improve weight or feed efficiency compared with stressed control broilers at 21 days (Stilborn et al., 1988).

Other novel approaches to reducing heat stress are cooled perches and probiotics. Water cooled perches used in a broiler trial also increased feed efficiency, feed consumption, and body weight, when compared to non-cooled perches during chronic heat stress (32.6C for 4 weeks, beginning at 16d of age) (Reilly et al, 1991). One study evaluated the use of a probiotic culture (12 strains of *Lactobacilli*) or subtherapeutic levels of oxytetracycline in the feed during heat stress. Heat challenge was applied from day 21 to 42 for 3h each day at 36C. The chicks receiving probiotics had significantly higher body weight, lower feed conversion ratio, and higher antibody production to Newcastle disease

vaccine following heat challenge than control or oxytetracycline treated chicks. There were no differences in mortality between groups (Zulkifli et al, 2000b).

Effects of Heat Stress on Laying Chickens

Heat stress in laying chickens is of concern due to the detrimental effects of heat stress on both egg production and egg quality such as decrease in eggshell weight and thickness, decrease in specific gravity, and increase in breakage (Mashaly et al., 2004; Koelkebeck et al., 1993; and Yahav et al., 2000). Research has evaluated potential ways to improve production and quality including supplementation of the diet of layers before or during heat stress. In 1984, Emery et al. found that the reduction in egg size and shell thickness in heat stressed hens is not due to consumption of less feed alone. In this experiment, non-heat stressed hens were pair fed according to the consumption of heat stressed hens, and produced significantly heavier eggs with increased shell thickness. Further, amino acid digestibility is not decreased by heat stress (Koelkebeck et al., 1998).

Vitamin Supplements for Laying Chickens

Though the effects of heat stress in hens is not linked to amount of feed or amino acid digestion, addition of selected vitamins have proven beneficial for heat stressed hens. Gross (1988) found the addition of 350 mg/kg of ascorbic acid (vitamin C) to the layer feed or one day at 7 wk of age significantly decreased mortality from 40% to 0 when subjected to heat stress for 2 hr the next day. Bollengier-Lee et al. (1998) found that the addition of 500mg/kg vitamin E increased egg production in hens exposed to chronic heat stress at 32C from either 24 to 28 weeks or 32 to 36 weeks. Further studies found that an optimal concentration of 250mg/kg vitamin E supplementation before, during, and after heat stress significantly increased egg production compared with non-supplemented controls in a chronic heat stress model (32C from weeks 26 to 30) (Bollengier-Lee et al, 1999). Another study explored the effects of supplemental vitamin E at concentrations of 25, 45 or 65 IU/ kg feed. Addition of 65 IU significantly increased egg mass and Haugh units, and 45 or 65 IU increased egg yolk mass. Lymphocyte proliferation measured *in vivo* was also increased from hens who receiving vitamin E supplementation (Puthpongsiriporn et al, 2001). Alternatively, Lin et al. (2002) evaluated the supplementation of layer diets with 9,000 IU/kg vitamin A. In one experiment vitamin A supplementation significantly increased both feed intake and laying rate, however did not affect antibody titers to Newcastle disease vaccine. In a second experiment, supplementation with 6,000 or 9,000 IU/kg of vitamin A significantly increased egg weight but not feed intake or laying rate. Hansen et al. (2004) found the circulating levels of plasma estradiol and calcium absorbed by duodenal cells were significantly reduced. Administration of estrogen to hens through implants prior to heat shock significantly increased plasma estradiol regardless of temperature. Also supplementation of the diet with 22,000 IU/kg vitamin D in the feed for 2 weeks before 24 hours of heat challenge increase calcium absorption by 3 fold.

Cooling of Laying Chickens

Other potential methods for management of heat stress in hens involve cooling of the birds. Blood flow through the comb, wattles, and shanks can dissipate heat to the environment, and explain why one behavior in heat stressed chickens is the dunking of the head in the waterer if possible (Whittow, 1986). While some layer houses include equipment for spraying a fine mist of water over the birds during hot hours, Wolfensen et al. (2001) tried a different method for wetting the birds. Their system allowed them to wet the ventral (underside) of the hens instead of the dorsal side which is wet with misting systems. Due to the differences in the feathers and higher areas of apteria on the breast and abdomen of birds, there is more skin available for evaporative cooling. When comparing the two systems, they found no change in egg weight with ventral cooling, compared to an 11% decrease in egg weight with dorsal cooling. Although heat stressed hens receiving either treatment experienced less feed intake and a decrease in egg shell index, the decreases were less pronounced in hens receiving the ventral cooling technique.

Conclusions

Heat stress has negative effects on all aspects of poultry production, which are economically significant. Additionally, some studies have found a link between heat stress and increased heterophil/ lymphocyte ratio or lower antibody production. Modern poultry bred for fast growth are increasingly susceptible to stress at high temperatures. In broilers, the use of thermal conditioning, water-cooled perches, and supplementation with probiotics have all been found to reduce the effects of experimental heat stress. In laying hens, vitamin supplementation and cooling with water have been found beneficial.

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Isolation and Prevention of *Bordetella avium* in Commercial turkeys

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I. Introduction

Bordetella avium is the causative agent for bordetellosis in birds. It is a small, gram-negative, non-fermentative, motile, strictly aerobic bacillus that colonizes the trachea of chickens, turkeys and other poultry. This bacterium was first isolated from young turkeys in 1967 and was officially named *Bordetella avium* in 1984. Studies have also shown that infection by this bacterium is not limited to poultry; other birds can be carriers though they may not develop symptoms of disease. The fact that there are many carriers of this bacterium explains the frequent outbreaks of the disease. The infection is not lethal, but infected birds are susceptible to secondary infections, which can lead to mortality. *B. avium* outbreaks are responsible for severe economic losses in poultry-producing regions of the world (Skeeles and Arp, 1997). This disease is worldwide and some speculate that it is being carried within 80% of caged turkeys.

B. avium commences a path of infection by colonizing the ciliated epithelium of the birds tracheal mucosa. As the disease progresses, it will destroy this outer layer of tissue along the respiratory tract and while doing so produce toxins that can affect other groups of tissue in the body. This infection will also predispose the birds to other infectious diseases. *B. avium* acts quite similar to its human counterpart – Bordetella pertussis (whooping cough) however, there is no evidence that *B. avium* can infect humans.

Most research to date on the effects of *B. avium* has focused around the turkey because of the economic impact of this disease on the poultry industry. Research on turkeys, which is well documented (Skeeles and Arp, 1997).

II. Signs of the disease

B. avium does not appear to be capable of establishing a disease state within adult birds. Bordetellosis has a severe effect on the young turkeys (4-5 weeks of age). Symptoms of this disease that we have observed specific only to the young and unweaned birds are as follows:

- Sneezing with significant mucoid exudates (serious nasal discharge)
- Lethargic behavior
- The beak gradually closes over a period of 12-24 hours to a point of being "locked" shut (temporomandibular rigidity or "lock jaw" syndrome)
- The birds appearance becomes pasty and pale
- Dehydration and weight loss becomes apparent
- Constant begging for food
- Protruding eyes that appear half shut. This is due to swelling of the suborbital chamber of the infraorbital sinus, which forces the lower eyelid upward.
- Eyes are glassy looking
- The head appears swollen due to inflammation of the skeletal (voluntary) muscles adjacent to the inflammed nasal cavity and infraorbital sinus. Inflammation of the skeletal muscle responsible for opening the beak creates the "lock-jaw" condition.

We have observed these symptoms of the disease occurring as young as 3 days old and as late as 4 weeks old. If the birds are breeding and producing chicks with the above mentioned signs then the adult pair should be considered carriers of the disease until proven otherwise. The only sure way to know if an adult is a carrier of *B. avium* is to have it tested by a qualified laboratory.

III. Morbidity and Mortality

Morbidity (sickness) may or may not occur when the young birds are directly exposed to *B. avium*. Some newborn turkeys will survive being raised in an infected clutch without any signs of the suggesting a resistance to the disease. This is supported by the study performed by Clubb *et al..* in which *B. avium* could be isolated from 30% of asymptomatic chicks within an infected aviary. These asymptomatic carriers can infect other chicks within the aviary. In studies with young turkeys morbidity was on the range of 80 to 100%.

In studies of young turkeys, the mortality (death) rate from bordetellosis was normally less than 10%, however in cases of high mortality (>40%) *Escherichia coli* was also isolated. There was also a study (Hinz *et al.*) that examined an outbreak of *B. avium* in combination with *Chlamydia psittaci* in several turkey flocks. Mortality in these affected flocks ranged from 7 – 20%. The high mortality was attributed to secondary infections from *Klebsiella pneumoniae, E. coli*, and *Pseudomonas fluoreszenz*.

Similar to the study performed by Hinz *et al.*, we experienced an outbreak in our flock of *B. avium* in combination with *Chlamydia psittaci* (Psittacosis). Upper respiratory swabs taken from infected turkeys produced cultures of

both *Klebsiella* and *Pseudomonas* on agar plates. Since *B. avium* produces a toxin (tracheal cytotoxin (TCT)) that damages the ciliated epithelial cells of the respiratory tract, the birds are unable to adequately clear these opportunistic bacterium from its lungs. Mortality among our adult turkeys was 3%. This low mortality may have been attributed to quick recognition of the psittacosis problem once it became active followed by prompt treatment.

The severity of bordetellosis varies with different species of birds. Studies have shown that the disease is less severe in chickens than in turkeys. Our results would indicate that turkeys experience symptoms similar to turkeys but suffer a higher mortality rate from bordetellosis than any other bird so far studied and reported.

The histopathology of bordetellosis within turkeys is well documented. The bacteria first colonizes the ciliated epithelium on the nasal mucosa, from here it works it way into the trachea then into the primary bronchi within 7 to 10 days. *B. avium* has only been found attached to cilia and not to any other type of cell. During the third and fourth week of the disease, the tracheal mucosa becomes distorted by folds and abnormal epithelial growth. The epithelium returns to normal after the disease has run its course, typically 4 to 6 weeks from the onset of the disease. Also accompanying this respiratory lining problem is softening and distortion of the tracheal cartilage. This condition will create folds that can accumulate mucoid exudate and lead to death by suffocation. This condition of the tracheal cartilage will persist for at least 53 days after infection. The turkey's immunological system will develop antibodies to the bacteria and eventually prevent the bacteria from attaching to newly developed ciliated epithelium. This immunological response will diminish over the next 4 to 8 weeks allowing any residual *B. avium* in the nasal cavity or sinus to once again expand and produce another case of bordetellosis. We have observed a couple of turkeys (6 to 9 months of age) in our flock that had persistent re-occurrences of upper respiratory infections until treated for bordetellosis and psittacosis. Although we are not sure which pathogen continued the infection (i.e., *B. avium* or *Chlamydia psittaci*), it does demonstrate the difficulty within some turkeys to be able to rid respiratory infection on their own.

In a limited study by Clubb *et al.* directly infecting 2 1/2 week old cockatiel chicks with *B. avium* the following sequence of events was observed:

- Sneezing after one day of infection
- Nares erythemic after the second day of infection
- By the fourth day of infection the chick refuses food

- By day five suborbital swelling and the jaw starts to lock shut

IV. Disease Transmission

B. avium is transmitted through close contact with an infected bird or through exposure to litter or water contaminated by infected birds. Current studies indicate that aerosol transmission is unlikely since healthy birds in cages next to infected birds do not contract the disease. Parenting turkeys carrying the disease will pass it to their young after they have hatched from the egg (unlike psittacosis). The disease appears to develop in the young cockatiel within 3 days of exposure to the carrier parent. We have found that newborn turkeys are very susceptible to bordetellosis.

V. Bacterium Susceptibility to Environment and Chemicals

One study performed within turkey houses has shown that *B. avium* survives best under the conditions of low temperature, low humidity and neutral pH. The bacterium was able to survive for 25 – 33 days within feces at 10 C and relative humidity 32 – 58%. When the temperature was raised to 40 C survival of the bacteria was less than two days. Another study reported survival of the bacteria for at least 6 months in undisturbed damp litter.

B. avium appears to be vulnerable to most commonly used disinfectants.

VI. Treatment

Treatment with various antibiotics has not been very successful in treating infected flocks. Susceptibility tests *in vitro* indicate that many gram-negative and broad-spectrum antibiotics should work but only spectinomycin appears to control the disease in the field. Minimization of exposure is the best to minimize adverse effects (Jordan editor Poultry Diseases.

Since *B. avium* is usually accompanied by psittacosis a tissue culture from necropsy of a recent death should be performed to determine if psittacosis is present. In our case, psittacosis was present, therefore after the 13 day treatment we proceeded with a psittacosis treatment for our birds. Had the results been negative for psittacosis we would have still proceeded with the psittacosis treatment to be on the safe side.

Different strains of *B. avium* have been identified in turkeys. The differences exist in toxin production, adherence to tracheal mucosa, plasmid profiles, antibiotic sensitivity, pathogenicity and colony morphology. It would be reasonable to assume that various strains of *B. avium* infect turkeys; therefore, treatment results may vary. Attempting to treat bordetellosis by

testing and eliminating those turkeys that culture positive for the disease will most likely result in failure to eradicate the disease from the aviary. Since not all chicks symptomatic for bordetellosis show positive for *B. avium* on a culture test, one could assume the same for carriers of *B. avium*. Treating the entire flock for the disease is the best approach.

VII. Prevention

The best method of prevention is to be very careful of the new birds you introduce to your flock and be mindful if you were around other birds/aviaries that could potentially be contaminated with the disease. A disinfectant foot bath, change of clothing and even a shower is recommended to prevent spread from one flock to another. Bordetellosis is disheartening in what it does to cockatiel chicks and its treatment for the carriers of the disease is laborious and lengthy for the owner. Bird keepers have the option of quarantining new birds and treating them for carrier diseases (i.e., bordetellosis and psittacosis) before introduction to their aviary or introducing them to the aviary and hoping these diseases are not present to infect their entire flock.

VIII. Vaccination

In the United States there is a major problem with Bordetellosis (Turkey Coryza). Certain commercial flocks may always beak with the disease. Immunization with a commercial available vaccine was unable to decrease the incidence of Turkey Corza disease. Recently, our laboratory have isolated and identified an autogenous strain of *B. avium* from one such flock that consistently broke with Bordetellosis. We treated a group of naive turkeys with this strain of *B. avium*. We were unable to detect an immune response using a commercial available assay for *B. avium*. We then developed an enzyme-linked immunochemical assay (ELISA) specific for this strain in order to determine if there was an immune response to the bacteria. We found that birds immunized with the autogenous strain of *B. avium* tested with the ELISA designed for that strain had a good antibody response in contrast to the results using the commercially available assay for *B. avium.*

A commercial flock that consistently broke with Bordetellosis with significant losses was used for a field trial of the autogenous vaccine. Birds from this flock were vaccinated with the autogenous strain of *B. avium*. This flock that consistently broke with Bordetellosis did not break with the disease following vaccination. The antibody response was even greater than that observed with the naive birds treated under controlled conditions at our laboratory indicating that these birds may have had an amnestic response due to a continual exposure to *B. avium* at the house. This secondary response to the continual exposure to *B. avium*, appears to further protect

the flock from the disease. With continued use of vaccination the devastation caused by *Bordetella avium* may be minimized.

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Managing Broiler Breeders for Eggs, Chicks, and Broiler Performance

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Abstract Research has established the principle of a minimum cumulative nutrient requirement during rearing prior to photostimulation of broiler parent stock (broiler breeders). In general, the minimum intake during rearing should provide approximately 23,000 kcal ME and 1,200 g CP for females and 29,600 kcal ME and 1,475 g CP for males. However, these cumulative targets probably should not be reached later than 21 wk of age (147 d) and preferably at least one week before photostimulation to insure that smaller birds in the flock have sufficient nutrients to respond to photostimulation and reproduce properly. The form (weekly changes) of the feeding program that allows the birds to reach these targets has been shown to be important in controlling BW, carcass composition, reproductive development, livability, and subsequent nutrient requirements during the breeding period. Further, the feeding program, especially for males, must be positioned relative to the existing BW as daily maintenance requirements have been shown to be much greater for males than for females. If males received less than relatively adequate nutrition they were found to exhibit reduced fertility, increased mortality, and reduced broiler progeny performance. The present data suggest that a decline in fertility due to male management will be accompanied by a decline in broiler progeny performance as the largest males generally reduce mating in such circumstances. Females also contribute to broiler performance in that the consumption of more feed during rearing was found to increase broiler progeny performance during the early laying period.

Introduction

Many broiler breeders have been reared since we first began to explore the idea that something other than just body weight (BW) and uniformity, both long standing management paradigms, were involved in the proper rearing of broiler breeders (broiler parent stock). The results of our original work was first discussed at the NCSU Broiler Breeder Workshop in 1995 under the umbrella that we called the *Concept of Minimum Cumulative Nutrition* and was first published as a full paper in a scientific journal two years later (Walsh and Brake, 1997). The generalities of this concept have been widely discussed at scientific and technical meetings since 1995 and have been adapted by many broiler breeder managers and commonly available technical manuals.

It has been rewarding to see such a lively discussion among our industry clientele of university-based research but it has become apparent that the focus of such discussion has shifted away from the original comprehensive concept that emphasized the form of the feeding program (Walsh and Brake, 1999), long-term relative consistency (Peak, 1996), and age at photostimulation in conjunction with the cumulative nutrition targets (Walsh and Brake, 1997). Many managers and technical advisors seem to have placed emphasis

only on the cumulative metabolizable energy (ME) and crude protein (CP) targets as they have struggled to balance this concept with the paradigms of BW targets and uniformity. It has become apparent that an absence of appreciation for the total concept may lead to problems with persistency of lay and hatchability as well as common problems such as podiodermititis. Further, it has become increasingly apparent that the feeding program can affect breeder carcass conformation in such a manner as to affect egg weight, livability, feed consumption, fertility, and broiler progeny performance.

This manuscript will review key aspects of the original research and concept and emphasize essential points of management that require careful attention some ten years after the concept was first discussed. Further, the beneficial effects on subsequent broiler progeny performance of a lifelong programmed and consistent approach to broiler breeder feeding management will be discussed.

Genetics, Nutrition, and Reproduction

Poultry breeding remains largely based on classical quantitative genetics, i.e. the best male birds are mated with the best female birds, more or less. In essence, pedigree broiler candidates have continued to be full-fed high density diets to allow individuals that have the greatest ability to utilize CP and ME to grow fast, convert feed efficiently, and produce good carcass yield to become apparent by their performance at a typical broiler killing age. Selection on high density diets apparently necessitates high density diets in order for the broiler progeny to fully express their genetic potential. As demonstrated by Lilburn et al. (1992), the parent stock also must not be deprived of sufficient nutrition during rearing if reproduction is to be optimal as birds selected on high density diets may not be able to perform consistently well (growth and reproduction) on low density diets such as may be used during rearing of broiler breeders. This is the fundamental reason that less than adequate nutrition during rearing can lead to poor reproductive performance.

Interaction of Genetics, Cumulative Nutrition, Feeding Program, Environmental Temperature, and Lighting Program

The very important interaction between climate, photostimulation, and nutrition can be illustrated by examining the seasonality of broiler breeder reproduction in temperate climates. The differences between "in-season" and "out-of-season" breeders have historically been attributed to daylength. However, our increased knowledge about the interaction between daylength and seasonal differences in temperature, feeding program, and cumulative nutrition has provided an alternative explanation of this seasonality. "In-season" broiler breeders have been generally the better performing birds in a temperate climate, even when the flocks have been reared in black-out houses. These "in-season" flocks have typically hatched during warm periods of the year when daylengths were also long. Daylength and temperature have both declined during the rearing period in such cases. As broiler breeders have typically been fed to achieve a standard BW, the cool weather at the end of the rearing period of "in-season" flocks has generally dictated that more feed be fed. Thus, the cumulative nutrition has been greater for such "in-season" breeders if photostimulation was not too early. In contrast, "out-of-season" flocks have

been hatched in cooler seasons and have been typically reared while both daylength and temperatures were increasing. As broiler breeder flocks have approached the age of photostimulation in warmer temperatures they have generally required less feed to achieve the standard BW and have thus accumulated less nutrition at the point of photostimulation. This latter scenario has typically always been the case in hot tropical countries, especially when black-out rearing was employed. An increased target BW has often been used as a "treatment" for "out-of-season" flocks because, as we now have learned, a heavier BW effectively increased the cumulative nutrition. Thus, minimum cumulative nutrition does not change seasonally but BW does change in a manner that reflects temperature and light intensity (bird activity).

It has become obvious that broiler breeders are now near what may be the minimum cumulative nutrient intakes at 140 d when grown to the typically specified target BW, i.e. there is very little, if any, surplus cumulative nutrition in our birds. A review of NC State University broiler breeder research flock data revealed that 1988 broiler breeder females could be grown to a 140 d BW of ~2.0 kg with ~28,000 kcal cumulative ME while 1998 females could achieve the same BW with as little as 20,000 kcal cumulative ME, which we know to be less than adequate (Table 1). This change was probably due to the remarkable genetic progress made in broiler feed conversion. With improved feed conversion it may simply take longer to accumulate the necessary nutrition for a proper response to photostimulation if typical BW standards were to be achieved. The alternative would be to allow BW to increase and/or change the nutritional and/or feeding profile(s).

I wrote some time ago that if the current genetic trend toward improved feed efficiency continued broiler breeders would have to be photostimulated much later and/or grown to a slightly higher BW in order to accumulate sufficient nutrition for proper responsiveness to photostimulation. I now believe that we can only delay photostimulation for a relatively short time due to the economic need to produce eggs in a timely manner. Thus, a higher BW at 20 wk of age may be inevitable. The question of most concern at the present time would be how to achieve a higher BW in a logical and productive manner.

At this point it should be stated that photostimulation somehow changes broiler breeders from nutrient-accumulating organisms to nutrient-expending organisms. The act of photostimulation can obviously interrupt the process of nutrient accumulation and create long-term fertility problems (de Reviers, 1977; de Reviers, 1980; de Reviers and Williams, 1984; de Reviers and Seigneurin, 1990). This scenario would be expected to be most true for birds with low BW (not yet accumulated sufficient nutrition). This would most likely be the reason that good BW uniformity has often been found to aid reproductive performance. However, another means to achieve the same goal is to simply wait one week beyond achieving the minimum cumulative nutrition targets before photostimulation.

Management of Females for Egg Production

During the last 15 years, our laboratory has examined the relationship between cumulative nutrition during the rearing period and subsequent female reproductive performance. Four flocks of broiler breeders of the same strain were compared in an early study (Table 1;

Peak and Brake, 1994). Photostimulation was at 141 d (20 wk) of age. Table 1 shows the cumulative CP, ME, BW at 140 d, and eggs per hen housed. The groups were fed the same diet during rearing but the feed was allocated differently each week during the rearing period (Figure 1) to achieve the cumulative differences. When the birds were photostimulated at less than ~22,000 kcal cumulative ME and ~1200 g CP there was a reduction in eggs per hen of ~15. This first suggested to us that there was a ***minimum*** nutrient intake, irrespective of BW, required to obtain acceptable levels of egg production. It should be remembered that photostimulation 7 d later would have added approximately 2000 kcal ME and 100 g CP to each of the cumulative totals shown in Table 1. Subsequent evaluation has suggested a role for the form of the feeding program (Figure 1) as well.

Table 1. The relationship between cumulative nutrient intake to photostimulation[1] at 20 wk of age and egg production

Breeder Flock[2]	Cumulative @ 20 wk		BW[3]	Eggs per hen housed (25-64 wk)
	ME	CP		
	(kcal/bird)	(g/bird)	(kg)	(n)
BB-1	25397	1397	2.06	159.8
BB-2	22207	1221	1.86	164.6
BB-3	20792	1144	1.98	149.4
BB-4	18985	1044	1.87	149.7

[1] Photostimulation was at 141 d of age.
[2] Each group was comprised of 2400 birds.
[3] All birds weighed at 140 d of age.

The feeding programs shown in Figure 1 below reflect four different approaches to rearing the broiler breeder pullets shown in Table 1. A good way to examine feeding programs is to compare the feed intake at 15 wk of age as this will reflect both early restriction as well as the rate of weekly feed increases during late rearing. The program for the BB-1 flock had more than adequate feed throughout rearing while the feeding programs for the other three flocks exhibited what could be considered excessive feed restriction at 15 wk of age followed by a fairly wide range of weekly feed increases thereafter. The BB-3 flock was most interesting in that the feed allocation was lowest at 15 wk, in an effort to control BW, and was followed by a rapid increase in feed allocation sufficient to achieve a BW somewhat similar to that of BB-1. However, reproductive performance was obviously poor in spite of achieving the standard target BW. Such feeding programs tend to produce foot pad problems, poor feathering, and higher than normal mortality. The BB-2 and BB-4 flocks achieved similar BW at 20 wk but a considerable difference in cumulative nutrition that was reflected in egg production in a dose-related manner.

Feeding Programs For Egg Production

In the USA and elsewhere in the world a good number of females continue to be reared mixed with males. In an experiment to investigate this procedure males were full-fed an 18% CP diet until mixed with females at 2, 4, 6, or 8 wk of age while the females received

an 18% CP diet for 1 wk followed by a 15% CP diet to photostimulation. The male and female feeding programs are shown in Figure 2.

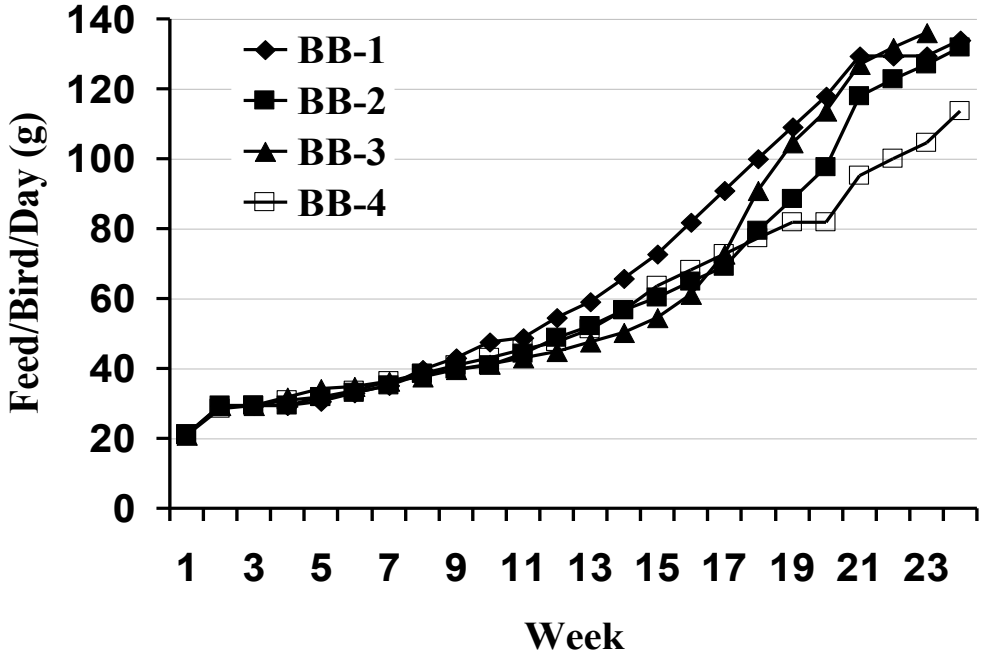


Figure 1. Feeding programs for the four flocks shown in Table 1. As a point of reference, the female feed allocation at 15 wk of age for BB-1, BB-2, BB-3, and BB-4 was 73, 60, 55, and 64 g on a daily basis, respectively.

All males and all females were weighed every 2 wk. Female BW was virtually identical across male treatments. The male BW reflected a dose response to increased amounts of feed prior to mixing with 8 wk makes reaching 2 kg before mixing and the 4 wk males approximating primary breeder company recommended target BW standards (current at that time) throughout rearing. Cumulative fertility was 66.9, 68.5, 76.6, and 85.2% for the 2, 4, 6, and 8 wk males, respectively. These fertility numbers were lower than optimum because males and females were fed together after 21 wk of age to exaggerate the effect of cumulative nutrition during rearing and to allow the males to be exposed to decreasing feed allocation after 35 wk of age, when the female feed was decreased. In spite of this, some of the pens with the 8-wk mixed males exhibited fertility in excess of 90% at 64 wk of age without sex-separate feeding during the production period.

The actual feed intake of the males mixed with females at 6 wk of age (as an example) and that of the females can be estimated using the formulas of Combs (1968) from the BW taken from all birds every 2 wk. These data showed that the real pattern of female feed consumption (Figure 3) differed significantly from the programmed pattern, especially after 14 wk of age. This must be extremely important as females grown sex-separate on the programmed feed amount laid fewer eggs per hen. These data (and field experience) suggest that large feed increases late in rearing (in black-out where there would be little

reproductive development) for pullets resulted in excessive "fleshing" (breast meat development). The excess breast meat may have increased maintenance requirements and inhibited reproductive development.

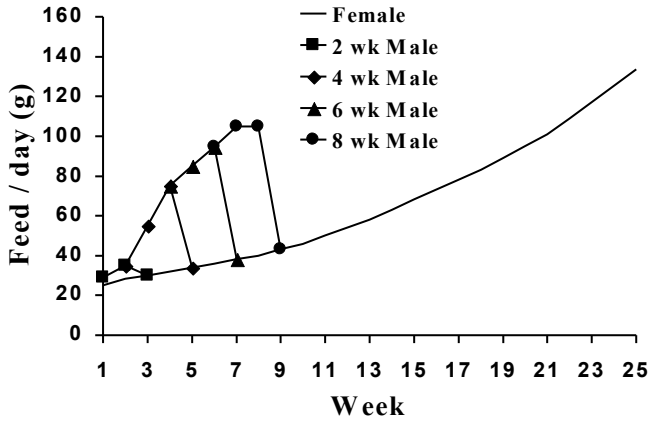


Figure 2. Feed consumption of males reared separate to 2, 4, 6 or 8 wk of age and feeding program of the females that they were mixed with at the indicated ages (Adapted from Peak *et al.,* 1998). As points of reference the targeted daily feed amounts for females at 15 and 20 wk of age were 68 and 95 g per female, respectively, with 7 g per wk increases after 20 wk.

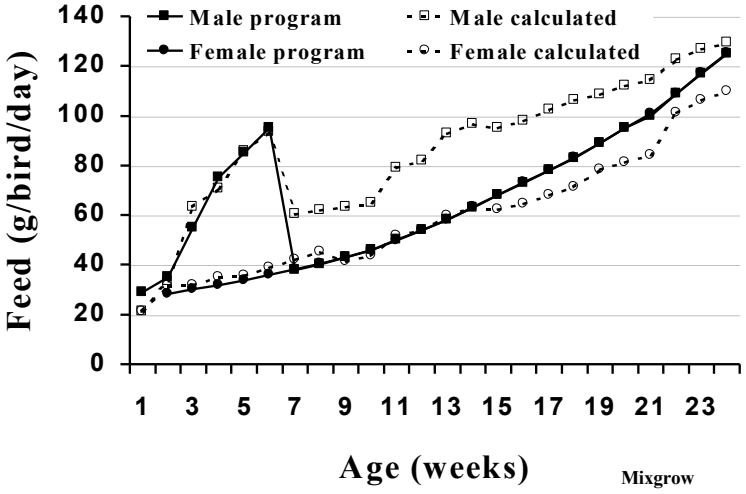


Figure 3. Calculated actual and programmed feeding of males and females mixed at 6 wk of age. Programmed feeding was the amount of feed fed as shown in Figure 2 and the calculated feed consumed was based upon calculations from actual BW of males and females based upon the formulas of Combs (1968).

This may be why heavy breasts relative to fat pad developed when feed increases were too rapid. Birds with excess breast meat relative to fat pad tend to exhibit a reduced appetite in

hot weather (even in tunnel-ventilated and evaporatively cooled houses), increased susceptibility to heat stress, poor peak egg production, and poor persistency. A conservative feeding approach with regards to weekly feed increases both before and after photostimulation would be advisable with females. These data further suggested that if slower feed increases would be needed late in rearing to control body conformation while still achieving adequate cumulative nutrient intakes then it was apparent that feed intakes early in rearing had to be adequate. As discussed for Figure 1 the weekly feed allocation at 15 wk of age may be a good indicator to study.

In a manner similar to the need to modulate any large increases in feed intake, diets should be formulated to minimize abrupt changes in composition that will create situations that would be similar to abrupt changes in the feeding rate. A single dietary ME for all diets is recommended to assist production managers maintain consistent feed increases. Similarly, modern broiler breeders may respond robustly to abrupt changes in CP with an unexpected increase in BW. A smooth transition among starter-grower-breeder diets should be considered during feed formulation. It is suggested that total lysine levels be ~5% of CP and methionine + cystine be ~0.60-0.63% of the diet for most feeds. A 15% CP diet with ~0.75% total lysine should be sufficient to support egg production without producing excessive amounts of breast meat. A similar level of CP and amino acids should be minimally sufficient for rearing diets. It is also important to have sufficient micro-nutrients in the both rearing and breeder feeds. The females must have these micro-nutrients to deal effectively with the stress of restricted feeding during rearing and be able to transfer sufficient nutrients to the embryo through the yolk sac during breeding.

Management of Females for Fertility

Fertility problems have been generally associated with the male (Hocking, 1990; Mauldin, 1992) but the female has been shown to be an issue as well (McDaniel *et al.*, 1981; Lopez and Leeson, 1995). The fact that cumulative CP nutrition at photostimulation can have a significant effect on female fertility has been clearly defined (Walsh, 1996; Walsh and Brake, 1997, 1999). The female has been shown to contribute to fertility through mating receptivity and spermatozoal storage in special spermatozoal host glands in the oviduct (VanKrey and Siegel, 1974).

Figure 4 shows the cumulative fertility for several female experimental groups from 28 to 64 wk of age along with the fertility for the last 8 wk of production (57 to 64 wk of age). All males were managed in a similar manner across all female experimental groups. It was also important to note that the effects of nutrition and management during rearing and the early breeding period have often only been observed very late in the breeding period. From Figure 4 it was clear that there was a ***minimum*** cumulative CP intake of ~1200 g CP or greater at photostimulation for females, irrespective of BW. This projected minimum assumed that the total lysine, on a corn-soy-based diet, was 5% of CP and methionine + cystine was 83% of lysine. These data showed that a minimum cumulative protein intake needed to be achieved along with the minimum cumulative ME intake with a feeding program that created the proper body composition as well as BW and uniformity.

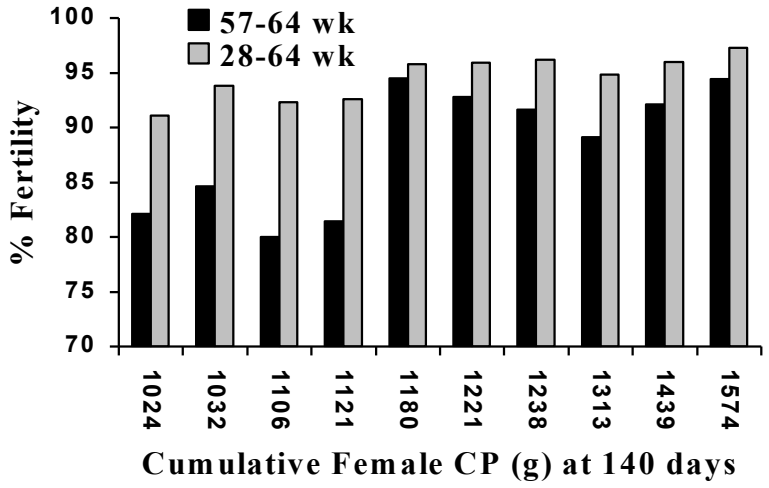


Figure 4. Graphic summation of data that demonstrates the effect of cumulative intake of CP prior to photostimulation on overall fertility as well as fertility during the last 8 wk of the production cycle (Adapted from Walsh, 1996, and Brake et al., 1998).

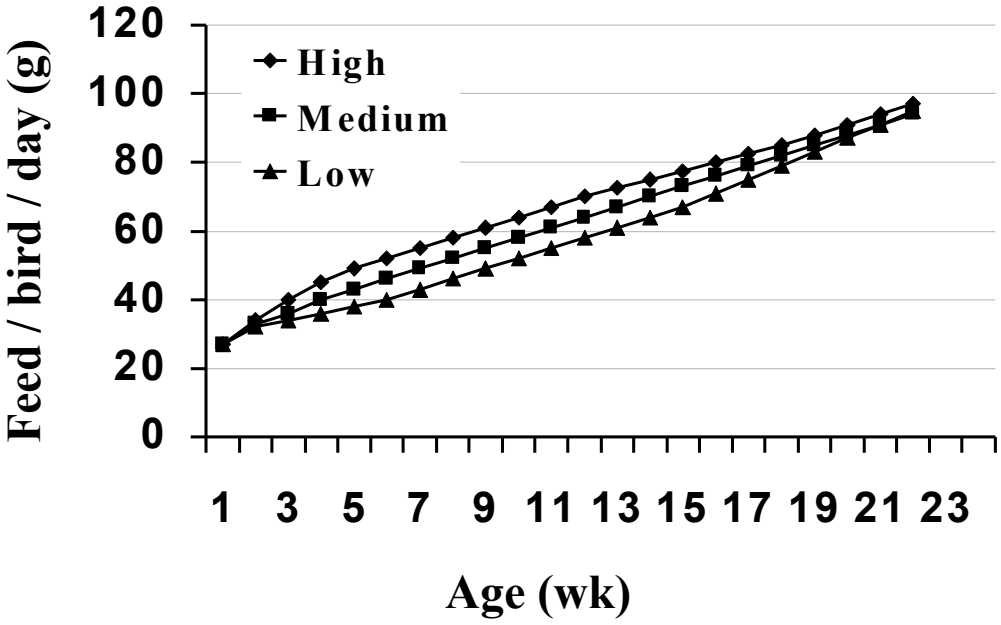
Effects of Female Broiler Breeder Nutrition and Management on Broiler Progeny

The results of the broiler progeny have long been recognized as being as important as fertility and egg production as outcomes of good broiler breeder management but data to clearly relate broiler breeder management to broiler performance have been scarce. To evaluate vertical effects of cumulative nutrition during the pullet rearing period on performance of broiler offspring, broiler trials were conducted using chicks hatched from broiler breeders reared on three graded levels of cumulative CP and ME intakes (High (27,780 kcal ME and 1,485 g CP), Medium (26,020 kcal ME and 1,391 g CP), and Low (24,240 kcal ME and 1,296 g CP)) to 22 wk (154 d) of age. The three pullet groups were fed the same starter and grower diets with cumulative differences achieved by varying the volume fed as shown in Figure 5. Males were grown sex-separate on the starter diet to a cumulative nutrient intake of about 30,000 kcal ME and 1600 g CP as determined by our laboratory to be satisfactory (Peak, 1996; 2001). A single breeder laying diet and identical management practices were applied to breeder hens. Males and females were fed sex-separately the same breeder laying diet during the laying period. The broiler breeders were from a Cobb 500 package with approximately 2000 birds in each flock with four replicate pens for each cumulative nutrition treatment. The breeder facility was a two-thirds slat design with curtains and fans for ventilation. Breeders were moved from a black-out rearing (8 h of light) facility to the laying facility and photostimulated (14 h of light) at 22 wk of age.

Although this experiment was replicated three times with similar results only one of the broiler breeder flocks will be examined herein. As points of reference the daily feed amounts at 15 wk of age were 67, 73, and 77.5 g per female for the Low, Medium, and

High programs, respectively. This range of feed allocation would be expected to cover the normal feeding range although feed allocations less than 67 g are often observed at 15 wk of age.

Figure 5. Pullet feeding program used to produce three graded levels of cumulative CP and ME intakes (High (27,780 kcal ME and 1,485 g CP), Medium (26,020 kcal ME and 1,391 g CP), and Low (24,240 kcal ME and 1,296 g CP)) to 22 wk of age.



All pullets were weighed at 22 wk of age to confirm treatment effects and the Low, Medium, and High treatments weighed 2,450 g, 2,550 g, and 2,660 g, respectively. Although pullet BW did differ during rearing, these differences gradually disappeared during the laying period (Figure 6). Sample BW taken from 20 hens per pen near the time of each egg collection for the two broiler trials are shown in Table 2.

Figure 6. Female BW during the rearing and laying periods resulting from the Low, Medium, and High cumulative feeding programs during rearing.

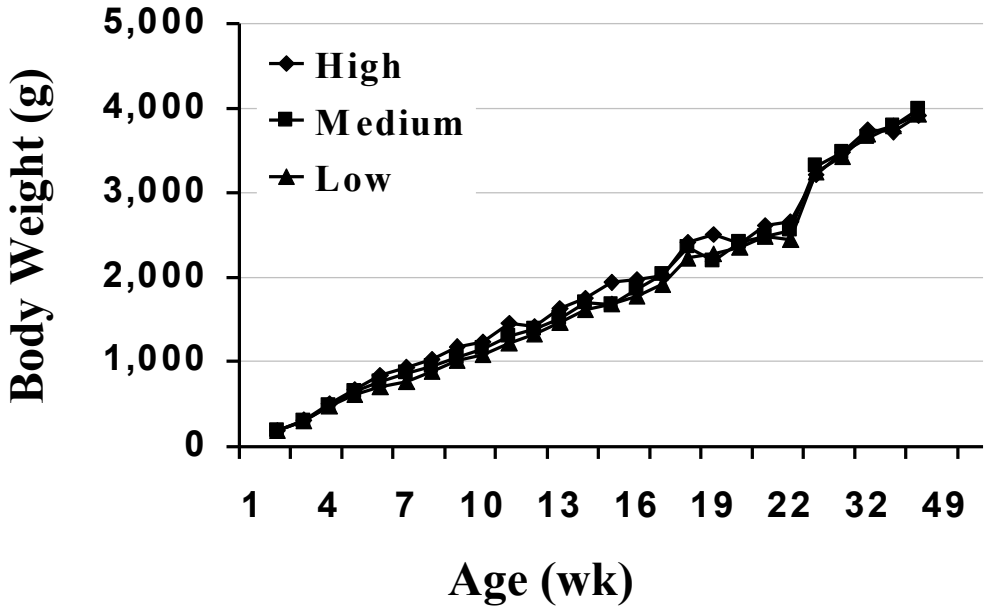


Table 2. Effect of cumulative pullet nutrition during the rearing period on subsequent breeder hen weight[1]

Broiler Trial	Breeder Age	Cumulative Pullet Nutrition High	Medium	Low
	(wk)	(kg)		
1	28	3.62	3.56	3.45
2	40	3.96	3.96	3.89

[1]Hen BW taken from a random sample of 20 hens from each of twelve 200-hen pens at the ages shown.

Two broiler trials evaluated chicks hatched at 28 and 39 wk of age, respectively, two ages that were normally expected to produce chicks of different quality and of different broiler performance. Broilers were fed broiler starter crumbles to 21 d of age. There was no consistent effect of cumulative pullet nutrition on any reproductive variable measured. Egg production, fertility, and fertile hatchability were very similar while percentage shell was not significantly affected (data not shown). Egg weight (Table 3) was not significantly affected.

Table 3. Effect of cumulative pullet nutrition during the rearing period on subsequent egg weight

Breeder Age	Cumulative Pullet Nutrition[1]			P =
	High	Medium	Low	
(wk)	(g)			
28	55.26	57.23	54.49	0.07
34	63.79	63.19	62.71	0.18
40	67.38	66.95	66.69	0.58

[1]Breeders fed three graded levels of cumulative CP and ME intakes (High (27,780 kcal ME and 1,485 g CP), Medium (26,020 kcal ME and 1,391 g CP), and Low (24,240 kcal ME and 1,296 g CP)) to 22 wk of age.

There were no consistent significant effects of plane of pullet cumulative rearing nutrition on broiler feed conversion or mortality to 21 d of age (data not shown). It was interesting to note that the male BW at 21 d of age was in the 900 g range, irrespective of breeder flock age (Table 4). This was exceptional early broiler growth by most standards and the fact that the increased BW observed in the male was evidenced in the presence of such good growth performance from the control (Low) group was remarkable.

Table 4. Effect of cumulative pullet nutrition during the rearing period on subsequent 21-d broiler BW

Broiler Trial	Breeder Age	Broiler Sex	Cumulative Pullet Nutrition[1]			P =
			High	Medium	Low	
	(wk)		(g)			
1	28	Male	946	935	901	0.06
1	28	Female	896	873	859	0.03
2	39	Male	953	924	917	0.31
2	39	Female	870	886	871	0.57

[1]Breeders fed three graded levels of cumulative CP (CP) and metabolizable energy (ME) intakes (High (27,780 kcal ME and 1,485 g CP), Medium (26,020 kcal ME and 1,391 g CP), and Low (27,780 kcal ME and 1,296 g CP)) to 22 wk of age.

Effects of Male Broiler Breeder Nutrition and Management on Broiler Progeny

As problems with broiler performance during the early stages of the production cycle of each broiler breeder flock are common and the data shown above demonstrated how this

could be ameliorated with changes in female rearing management it was deemed important to turn our attention to how the management of males could also affect the critical early stages of the breeder laying cycle. In a series of experiments with Ross 344 males not detailed herein we have determined that 29,600 kcal ME and 1,475 g CP to 21 wk of age was near the minimum cumulative nutrient intake for separate grown males in black-out rearing conditions (Romero-Sanchez et al., 2007abc). In Figure 7 the BW data from a study that compared this minimum cumulative intake (LoDiet) with a slightly higher plane of rearing nutrition (HiDiet) were compared. It should be noted that differences in BW disappeared as soon as all the males began to be provided the same daily feed allocation but the transition to the same feed allocation did cause a transient plateau in BW for the HiDiet males. This was most likely due to less than adequate feed to support the higher BW as detailed in Figure 8. As also shown in Figure 8, males were identified as being in the Light or Heavy 50% of each group but continued to be mixed together and fed with their respective females from photostimulation at 21 wk of age. The feeding program and nutrition was the same for all of the birds following the transition period. Thus, the largest males were relatively underfed compared to their smaller pen mates in each pen. This effect can be clearly observed by a close examination of Figure 8. The Heavy 50% of the males from the HiDiet group (closed squares) failed to gain BW consistently from 22-32 wk of age while the Heavy 50% of the LoDiet group (closed circles) were more consistent in their BW gain. Both groups of Light 50% males exhibited consistent BW gain as well. This failure to gain BW in a consistent manner was most likely the cause for the lower fertility of the HiDiet group (Figure 9). Fertility has been found to be largely a function of the number of males mating in a flock. In the case of the HiDiet group the larger males were most likely not mating, as evidenced by a failure to gain BW, and this resulted in lower broiler BW. The logic for this explanation lies in the simple fact that the Heavy 50% of the males has to be genetically similar in both the LoDiet and HiDiet groups so that when the largest males do not mate the largest broilers will not be produced.

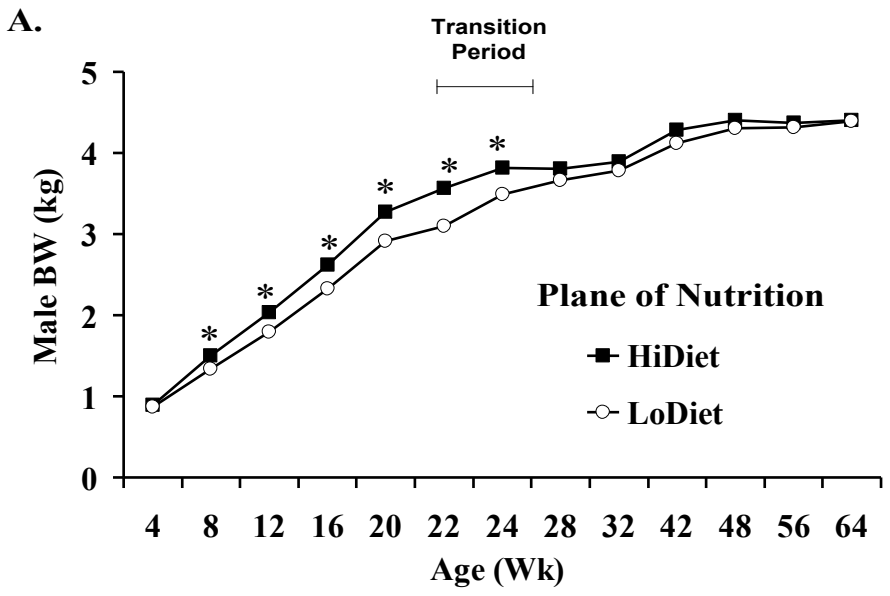


Figure 7. Ross 344 broiler breeder male BW when reared on two planes of

cumulative nutrient intake that provided either the minimum 29,600 kcal ME and 1,475 g CP to 21 wk of age on the LoDiet program or 33,500 kcal ME and 1,730 g CP to 21 wk of age on the HiDiet program.

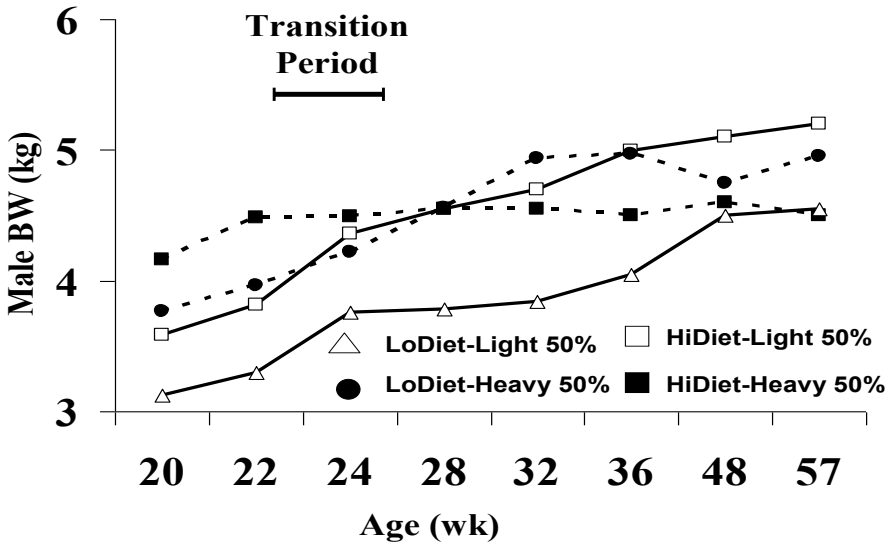


Figure 8. Ross 344 broiler breeder male BW when reared on two planes of cumulative nutrient to 21 wk of age (LoDiet or HiDiet program) but fed the same thereafter. During the transition period the two rearing diets were blended together to reach a common diet and feeding program for the breeding period. Individual males were identified as being in either the Light or Heavy 50% of each pen from 21 wk of age.

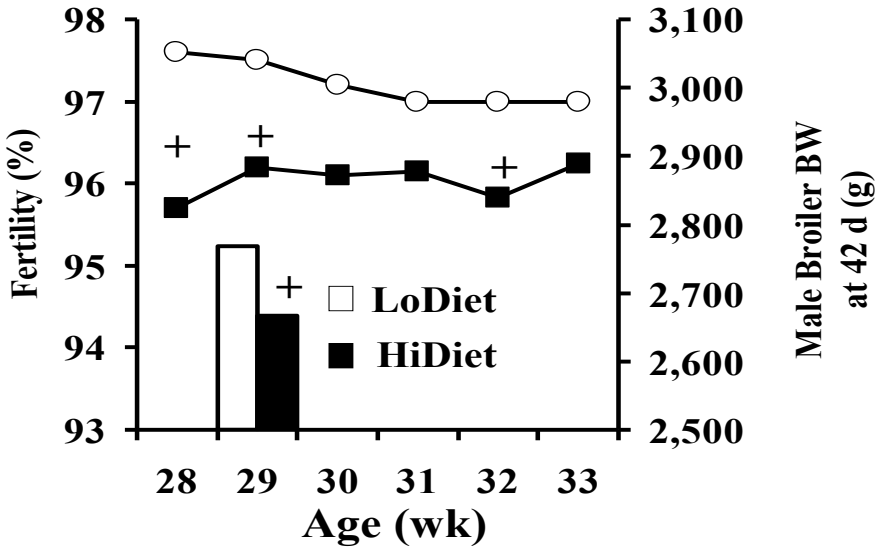


Figure 9. Fertility from 28 to 33 wk of age for the LoDiet and HiDiet groups. Eggs were collected from the flock at 29 wk of age and the broilers reared to 42

d of age to show the effect of breeder feeding program and fertility on broiler progeny performance.

In fact, further studies in our laboratory (Romero-Sanchez et al., 2007abc) have shown that any time that fertility declined in a broiler breeder flock due to underfeeding of males that there was an associated decrease in broiler progeny performance. This was similar to the reports of Attia et al. (1993; 1995) that showed improved broiler performance due to a greater daily ME allocation in Ross 344 broiler breeder males. The conclusion appears to be simple. The broiler breeder males that produce the progeny with the greatest genetic potential are the largest broiler breeder males and they have to fed in a programmed manner that achieves early sexual maturity and persistent fertility.

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APLICANDO A TECNOLOGIA PARA O DIAGNÓSTICO E CONTROLE DE MICOTOXINAS: EXPERIÊNCIA BRASILEIRA

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RESUMO

As micotoxinas são substâncias tóxicas resultantes do metabolismo secundário de diversas linhagens de fungos filamentosos. São de ocorrência universal, porém predominam em climas tropicais e subtropicais, onde o desenvolvimento fúngico é favorecido pela umidade e temperatura. Para que seus efeitos no organismo animal possam ser minimizados, é importante adotar práticas de controle e gerenciamento destas substâncias. Para tanto, é importante que algumas atividades críticas sejam normatizadas, como processo de amostragem correta, métodos de diagnósticos eficientes, ferramentas de controle dos resultados obtidos e só então a tomada de decisão. Quando as micotoxinas já estiverem presentes nos alimentos, os objetivos devem ser de minimizar os efeitos destes contaminantes. A utilização de aditivos anti-micotoxinas é uma técnica eficiente para diminuir a absorção das micotoxinas no trato gastrointestinal das aves. O profissional deve ter segurança na determinação da dieta de menor risco às intoxicações dos animais, aliado à relação custo/benefício ideal, permitindo dessa forma a otimização da produtividade do rebanho.

1. PRINCIPAIS MICOTOXINAS DE IMPORTÂNCIA NA AVICULTURA

Na Tabela 1 estão relacionadas as micotoxinas de maior impacto na produção avícola, bem como os fungos que produzem cada uma delas e as condições que favorecem a formação destes compostos.

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Tabela 1 – Principais micotoxinas, fungos produtores, alimentos mais contaminados e condições de ocorrência em avicultura.

Micotoxina	Fungos que mais produzem	Alimentos mais propensos à contaminação	Fator desencadeante da contaminação
Aflatoxinas	*Aspergillus flavus* e *A. parasiticus*	Amendoim, castanhas, nozes, milho e cereais em geral	Armazenamento em condições inadequadas
Ácido Ciclopiazônico	*Aspergillus flavus*	Milho e amendoim	Armazenamento em condições inadequadas
Tricotecenos	*Fusarium sp.*	Milho e cereais de inverno	Temperatura baixa, alta umidade e problemas de armazenamento
Fumonisinas	*Fusarium sp.*	Milho e cereais de inverno	Estação seca seguida de alta umidade e temperaturas moderadas
Ocratoxina A	*Aspergillus alutaceus* e *Penicilium sp*	Milho, café e grãos estocados	Deficiências no armazenamento

1.1. Aflatoxinas

Aflatoxinas são metabólitos secundários, produzidos por fungos do gênero *Aspergillus*, sobretudo *A. flavus* e *A. parasiticus*. Foram descobertas na década de 1960, após provocarem um surto (*Turkey X disease*) com alta letalidade em perus na Inglaterra. Neste surto, milhares de aves morreram após consumirem ração contendo torta de amendoim. O principal fungo encontrado na torta de amendoim foi o *Aspergillus flavus* dando o nome a essa toxina.

Em surtos de aflatoxicose, uma das características mais marcantes é a má absorção que se manifesta pela presença de partículas de ração mal digeridas nas excretas das aves. Está associada com esteatorréia ou excreção aumentada de lipídeos. A esteatorréia presente na aflatoxicose pode ser severa com incremento de até dez vezes do teor de gordura nas fezes. Em frangos de corte, a esteatorréia é acompanhada por uma diminuição nas atividades específicas e totais da lipase pancreática, principal enzima digestiva das gorduras, e pela diminuição dos sais biliares, necessários tanto para a digestão

como para a absorção de gorduras, levando a esteatose hepática (fígado gorduroso). Palidez das mucosas e pernas também se observa em frangos e poedeiras que recebem ração contaminada com aflatoxinas. Essa pigmentação deficiente parece ser resultado da menor absorção, diminuição no transporte e deposição tecidual dos carotenóides da dieta, sendo a aflatoxicose identificada como “síndrome da ave pálida”.

1.1.1. Efeito das aflatoxinas sobre a postura

O diagnóstico dos distúrbios causados pelas aflatoxinas sobre a produção de ovos é possível somente após alguns dias ou semanas. A presença de folículos pré-ovulatórios já formados antes do consumo da micotoxina no trato reprodutivo das aves justifica essa resposta tardia. A diminuição da produção de ovos é precedida pela redução nos níveis sangüíneos de proteínas e lipídeos. Poedeiras que consomem dieta contendo 5 ppm de aflatoxinas durante 4 semanas, podem apresentar redução na produção de ovos a partir do oitavo dia, atingindo queda na produção na ordem de 35%, uma semana após a retirada da micotoxina da dieta (Rosa et al., 2001).

Além de reduzir a produção de ovos, a aflatoxicose também induz a redução do tamanho dos ovos, bem como a redução proporcional no tamanho das gemas, devido aos prejuízos causados na síntese protéica e lipídica. Contudo, a deposição de cálcio na casca dos ovos por si só não é afetada. A resistência da casca aumenta quando aves consomem aflatoxinas devido à redução na casca desses ovos não ter a mesma proporção da redução que ocorre na clara e gema. Este aumento da espessura da casca pode afetar a eclodibilidade pela redução nas trocas gasosas entre o embrião e o ambiente.

A mortalidade embrionária em ovos de matrizes intoxicadas com aflatoxinas ocorre pelo fato de que essas substâncias, após serem biotransformadas no fígado, têm como um dos principais metabólitos a aflatoxina M_1 que é eliminada do organismo através da gema. Além disso, a própria aflatoxina B_1 e o aflatoxicol também podem ser encontrados na gema, a partir de 24 horas após a ingestão das aflatoxinas.

Em casos de aflatoxicose, os picos de mortalidade embrionária ocorrem no terço final da incubação. Isto se deve ao fato de que os metabólitos das aflatoxinas estão concentrados na gema, a qual é utilizada pelo embrião, como fonte energética, neste período do processo de incubação.

1.1.2. Efeito das aflatoxinas sobre a produção de perus

Nos últimos anos, o Brasil tem obtido um considerável incremento na produção e exportação de carnes e subprodutos de aves, que não frangos. Neste contexto, tem grande importância a produção de perus, que nos últimos 6 anos (2000 – 2005) teve um incremento de 140% (UBA, 2006). É universalmente conhecido o fato de que perus são mais sensíveis aos efeitos das aflatoxinas que frangos de corte, sem que, no entanto, se conhecesse o real impacto dessas micotoxinas no desenvolvimento dessas aves.

Estudos conduzidos no LAMIC/UFSM demonstraram que, durante os primeiros 42 dias, perus apresentam uma sensibilidade à intoxicação por aflatoxinas cerca de 4 a 6 vezes maior do que frangos (Rauber, 2006). Nesse estudo, os perus foram alimentados com dietas contendo de 0 a 1000 ppb de aflatoxinas (divididos em 7 grupos), sendo que o grupo que recebeu a maior dose, apresentou um ganho de peso cerca de 38% inferior ao grupo controle (Tabela 2). Outro dado importante é relacionado à mortalidade, que foi de 37%, enquanto que no grupo controle não houve mortalidade. A evolução do ganho de peso nos animais intoxicados nos diferentes grupos é inversamente proporcional à dose de aflatoxinas presente na dieta (R= –0,84 e P=0,00; $Peso_{42}$= 2.298,9 – 0,87*ppb de aflatoxinas). Comparativamente, frangos de corte alimentados com 3000 ppb de aflatoxinas, durante 42 dias apresentaram uma redução de 27% no ganho de peso (Giacomini et al., 2006).

Tabela 2 – Peso médio de perus de corte intoxicados com aflatoxinas em diferentes concentrações, durante 42 dias.

Aflatoxinas (ppb)	Peso 21 dias[1] (CV)[2]	Peso 42 dias[1] (CV)[2]
0	676,85[ab] (7,3)	2.239,90[a] (6,2)
20	686,65[a] (8,2)	2.281,75[a] (5,9)
50	696,55[a] (6,5)	2.270,00[a] (6,8)
100	671,31[ab] (7,1)	2.253,54[a] (5,1)
200	639,67[b] (10,2)	2.092,05[b] (7,3)
500	566,79[c] (13,0)	1.916,09[c] (10,0)
1000	414,25[d] (12,1)	1.378,09[d] (14,5)

[a – d] Médias nas colunas com letras diferentes, diferem estatisticamente pelo teste de Bonferroni (P≤0,05);
[1] Peso médio das aves (g);
[2] CV= Coeficiente de Variação (%).

1.1.3. Impacto das aflatoxinas no desempenho de diferentes linhagens de frangos de corte

Alguns estudos recentes (Giacomini et al., 2006; Mallmann et al., 2006) demonstram que existem graus de susceptibilidade individual entre animais da mesma espécie e mesmo sexo, frente à intoxicação por aflatoxinas. Mariani (1998) comprovou a diferença de susceptibilidade de frangos de corte às aflatoxinas conforme a idade dessas aves, indicando que aves mais jovens apresentam maiores danos no seu desenvolvimento em comparação às aves mais velhas. Além dessas constatações, existe no meio científico e industrial a suposição de que as diferentes linhagens comerciais de frangos de corte disponíveis no mercado nacional e internacional possam apresentar diferenças no que diz respeito à resistência às aflatoxinas presentes nos alimentos ingeridos por essas aves. Com base nisto, foi desenvolvido no LAMIC um experimento que constatou que, de fato, existem diferenças de desempenho entre as três principais linhagens de frangos de corte utilizadas no Brasil, quando essas são alimentadas com ração contaminada com aflatoxinas (Tabela 3).

Tabela 3 – Diminuição relativa de peso (DRP) de frangos de corte de três linhagens comerciais (X,Y e Z), intoxicados com 3 ppm de aflatoxinas, de 1 a 42 dias de idade.

Lin[1]	7 dias		14 dias		21 dias		28 dias		35 dias		42 dias	
	DRP[2,3]	CV[4]	DRP[2,3]	CV[4]	DRP[2,3]	CV[4]	DRP[2,3]	CV[4]	DRP[2,3]	CV[4]	DRP[2,3]	CV[4]
X	2,5[a]	10,1	19,6[ab]	15,0	27,3[ab]	15,5	24,3[b]	13,0	23,0[b]	12,8	19,8[b]	12,8
Y	4,3[a]	12,6	22,4[a]	17,5	29,8[a]	19,0	29,5[a]	19,1	27,9[a]	18,2	24,7[a]	17,1
Z	3,7[a]	8,0	17,4[b]	9,7	25,8[b]	10,5	25,8[b]	12,6	21,3[b]	11,7	19,8[b]	10,7

[1] Lin= Linhagem utilizada
[2] DRP= Diminuição Relativa de Peso (%), diferença de peso entre os animais intoxicados e não intoxicados da mesma linhagem.
[3] Médias na mesma coluna, com letras diferentes diferem significativamente pelo teste de Tukey (P≤0,05).
[4] CV= Coeficiente de Variação referente aos pesos absolutos das aves intoxicadas (%).

A linhagem Y, a partir de 14, seguindo até os 42 dias, apresenta diminuição relativa de peso, significativamente superior a pelo menos uma das outras linhagens utilizadas neste experimento. Além das diferenças nas perdas, outro dado importante é o coeficiente de variação (CV) dos pesos das aves nos grupos intoxicados, sendo que a linhagem Y apresentou o maior CV entre as linhagens avaliadas, em todos os períodos. Este resultado indica que lotes de aves dessa linhagem apresentam uma maior desuniformidade, quando alimentados com dietas contendo aflatoxinas.

1.2. Ácido Ciclopiazônico

Além de produzirem as aflatoxinas, algumas cepas de *Aspergillus flavus* também produzem o Ácido Ciclopiazônico (CPA). Essa micotoxina tem sido associada a alguns sinais clínicos apresentados pelas aves no primeiro quadro de aflatoxicose descrito (Turkey X disease). Não obstante disso, análises das amostras daquele episódio indicaram a presença dessa micotoxina (Kuilman-Wahls, 2002; Hoerr, 2003). O CPA ocorre naturalmente no milho e amendoim e, geralmente, sua presença está associada à presença das aflatoxinas (Vaamonde, 2003).

Os principais sinais clínicos da intoxicação por CPA incluem diminuição no ganho de peso, vômito e sinais neurológicos (opistótono, hiperestesia e convulsão) sendo, geralmente, fatal. Lesões incluem degeneração e necrose hepática, lesões hemorrágicas no miocárdio, proventrículo, moela e

baço (Kuilman-Wahls, 2002; Hoerr, 2003). Dentre as lesões citadas, a mais marcante é a presença de erosões na moela das aves intoxicadas (Hoerr, 2003).

1.3. Tricotecenos

As principais micotoxinas do grupo dos tricotecenos compreendem a Toxina T-2, Deoxinivalenol (DON ou vomitoxina), Diacetoxiscirpenol (DAS), produzidas por fungos de diversos gêneros, principalmente *Fusarium*.

Intoxicações crônicas envolvendo toxina T-2 ou DAS induzem redução no consumo de ração e ganho de peso, lesões orais, necrose dos tecidos linfóide, hematopoiético e mucosa oral, com eventuais distúrbios nervosos (posição anormal das asas, diminuição de reflexos), empenamento anormal e diminuição na espessura da casca dos ovos. Particularmente em poedeiras, as lesões orais ocorrem em aproximadamente 50% dos lotes quando essas aves são alimentadas com ração contendo 2 ppm da toxina T-2. Contudo, a toxina T-2 apresenta alta toxicidade para macrófagos de frangos, inibindo a sua capacidade fagocitária. Essa toxina também induz a formação de peróxidos a partir dos lipídeos, tendo como conseqüência a diminuição da concentração de vitamina E nas aves.

Outras aves, como perus e gansos, são mais sensíveis à toxina T-2 que frangos de corte. Em gansos, a partir de 0,1 mg/kg de peso vivo ocorre a queda na produção de ovos e os níveis de postura e eclodibilidade diminuem em 50%, quando foram administrados 300 mg de toxina T-2/kg de peso vivo.

As micotoxinas T-2 e DAS produzem lesões orais em frangos de corte quando presentes em níveis a partir de 1 ppm na ração. As aves apresentam diminuição do consumo alimentar, retardo no crescimento, alterações no quadro sanguíneo e neurotoxicidade. Também são observadas lesões orais em peruzinhos alimentados com ração contendo concentrações de 5 ppm da toxina T-2 e redução de ganho de peso com 10 ppm da mesma micotoxina. Numa comparação direta, concluiu-se que peruzinhos são mais sensíveis que frangos de corte. Contudo, gansos também são muito sensíveis para a toxina T-2,

podendo apresentar letalidade quando estiver presente em concentrações a partir de 3 ppm.

As lesões orais decorrentes da intoxicação por DAS, se traduzem em necrose da ponta da língua, geralmente em matrizes e poedeiras comercias. No entanto, essas lesões podem também aparecer em frangos de corte. Por outro lado, as lesões encontradas em casos de intoxicação pela Toxina T-2, comumente são erosões e/ou ulcerações no palato e na comissura do bico das aves intoxicadas. Essas lesões podem ser encontradas tanto em aves poedeiras (matrizes e comerciais), quanto em frangos de corte.

Estudos similares realizados com DON, entretanto, têm esclarecido que, com exceção de um decréscimo transitório nos níveis de hemoglobina, ou um levíssimo efeito na qualidade do ovo, não há evidência significativa de que essa toxina afete o desempenho de aves. As aves são capazes de tolerar concentrações relativamente altas de DON na dieta e um pouco menos em relação à toxina T-2 e DAS. Os níveis de DON normalmente encontrados em rações contaminadas (0,35 a 8,0 ppm), não apresentam indicações de algum problema sanitário perceptível com frangos. Concentrações de DON acima de 82,8 ppm foram administradas em poedeiras por 27 dias sem nenhum efeito sobre o desempenho e sem apresentar lesões nas aves. Outros estudos descreveram lesões muito leves e redução na qualidade de ovos em aves que receberam 18 ppm de DON na dieta.

Os tricotecenos geralmente não induzem aumento de mortalidade em aves que não sejam frangos, requerendo níveis de várias centenas de partes por milhão para resultar em mortalidade significativa. De forma semelhante, em surtos de toxicose, atribuídos a toxina T-2 que afetaram patos domésticos, gansos, eqüinos e suínos, somente houve mortalidade em gansos, sugerindo uma grande sensibilidade dessa ave.

1.4. Fumonisinas

As fumonisinas, um grupo de dezenas de micotoxinas, são produzidas por fungos dos gêneros *Alternaria* e *Fusarium*, principalmente pelo

F. moniliforme. As fumonisinas de maior ocorrência e importância toxicológica são B_1 e B_2.

Os níveis de contaminação em milhos de diferentes partes do mundo estão, normalmente abaixo de 5 ppm e cerca de um terço das amostras analisadas são contaminadas. As análises realizadas nos últimos 11 anos no LAMIC (1996 – 2007) constatam que cerca de 42% das amostras de milho e 45% das amostras de ração são contaminadas por fumonisinas.

Alguns trabalhos indicam que os níveis tóxicos de fumonisina estão acima de 80 ppm. Outros pesquisadores realizaram experimentos com doses extremamente altas de fumonisina (61 a 546 ppm) e encontraram efeitos nocivos dessa toxina sobre o desempenho de frangos de corte. No entanto, estudos conduzidos pelo Laboratório de Análises Micotoxicológicas – LAMIC comprovaram que doses inferiores a 50 ppm de fumonisina B_1 impactam negativamente no peso de frangos de corte até 21 dias, representando perdas de 4%. Níveis de 100 ppm determinaram perdas de até 12% no ganho de peso aos 21 dias. Essas perdas, em nível de campo podem ser ainda maiores, uma vez em condições experimentais o efeito das micotoxinas é, geralmente, atenuado pela eliminação de fatores estressantes.

Outro fator importante a ser considerado no que se refere às fumonisinas, é o fato de que os fungos que produzem essas micotoxinas produzem uma série de outros compostos toxígenos. Essas substâncias podem estar presentes na alimentação das aves e determinar perdas de desempenho ainda mais significativas.

Nas aves intoxicadas por fumonisinas, os sinais clínicos geralmente incluem: menor ganho de peso, mortalidade, diarréia, ascite, hidropericardite e palidez do miocárdio, edema e congestão renal, ulceração na mucosa oral em perus, aumento no peso relativo de fígado, proventrículo e moela (Hoerr, 2003).

A intoxicação com fumonisina pode ser monitorada por meio de parâmetros sanguíneos. Ocorre alteração na relação entre os níveis circulantes de esfingosina e esfinganina, que são precursores dos esfingolipídios, quando da intoxicação com fumonisinas.

1.5. Toxinas mais importantes no Brasil

A analise de micotoxinas no Brasil já se tornou uma prática rotineira. Muitas empresas avícolas realizam seus próprios controles nas matérias-primas através de programas de monitoramento. Os dados das análises de rotina realizadas pelo Lamic encontram-se na Tabela 4, mostrando a prevalência destes compostos na cadeia produtiva brasileira.

Tabela 4 – Principais micotoxinas encontradas no Brasil.

Micotoxina	Amostras Analisadas	Positividade (%)	Média (ppb)
Aflatoxinas	**95.767**	**40,0**	**10,7**
Zearalenona	**78.540**	**17,6**	**43,5**
Ocratoxina A	**22.068**	**2,7**	**0,5**
Deoxinivalenol	**20.342**	**37,8**	**241,6**
Fumonisinas	**20.521**	**51,4**	**997,8**
Toxina T2	**12.904**	**1,4**	**12,0**
Diacetoxiscirpenol	**1.260**	**7,5**	**6,2**
3-DON	**304**	**12,2**	**5,1**
15-DON	**301**	**11,0**	**5,0**

Pelos resultados de contaminação e positividade apresentados na Tabela 2, podemos concluir que as micotoxinas de maior importância para a produção avícola no território brasileiro são as aflatoxinas, seguidas pelas fumonisinas e o deoxinivalenol. Para essas três micotoxinas, a positividade ultrapassa 47%, ou seja, pouco menos da metade de todos os alimentos analisados no Brasil apresentam contaminação por estas substâncias. Além disso, a contaminação média observada também é elevada, levando-se em consideração as doses máximas recomendadas para aves, descritas na Tabela 5.

1.6. Limites máximos de micotoxinas recomendados para aves de produção

Baseado nas informações da literatura, bem como nos experimentos in vivo realizados pelo Laboratório de Análises Micotoxicológicas da Universidade Federal de Santa Maria (LAMIC/UFSM) em aliança com o Instituto SAMITEC (Instituto de Soluções Analíticas Microbiológicas e Tecnológicas) e na ocorrência das micotoxinas evidenciada nos últimos anos em mais de 100 mil amostras de

matérias-primas e rações enviadas ao LAMIC, foram estabelecidas recomendações com relação aos limites de segurança de micotoxinas para aves de produção. Estes limites estão apresentados na Tabela 3.

Tabela 5 – Limites de segurança de micotoxinas (ppb) recomendados para aves de produção.

	Afla	FB	DON	T-2	DAS
Frangos de Corte Fase Inicial	0	100	200	0	0
Frangos de Corte Fase Crescimento	2	500	500	50	200
Frangos de Corte Fase Final	5	500	1000	50	200
Poedeiras Comerciais	10	1000	1000	100	500
Matrizes	10	1000	1000	100	500

2. CONTROLE E GERENCIAMENTO DE MICOTOXINAS

Controle e gerenciamento de micotoxinas implica em um processo que possui uma série de atividades críticas. Tudo se inicia pela definição de um programa de monitoramento. Este programa pressupõe a determinação de um processo de amostragem, passa por uma gama de análises e controles e se encerra na tomada de uma decisão. Essa deve considerar o uso seguro da dieta na qual o risco da intoxicação por micotoxinas possa ser minimizado e que o custo/benefício seja exatamente quantificado, permitindo a maximização da produtividade do rebanho.

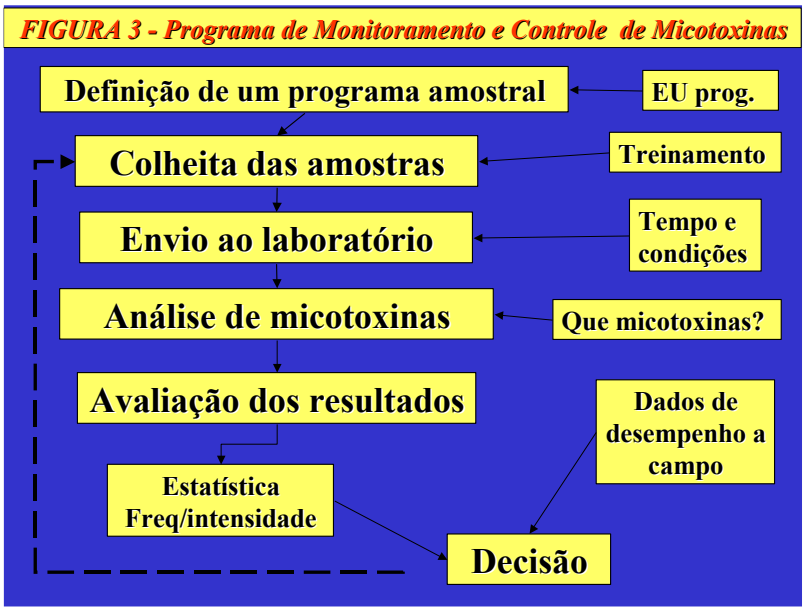
Teoricamente, o plano amostral deve levar em consideração alguns aspectos básicos, como:

a. Com que probabilidade, um lote com uma determinada concentração de micotoxinas é aceito ou rejeitado?
b. Qual a percentagem de erros nas classificações de lotes.
c. A concentração de uma determinada toxina em um lote aceito ou rejeitado.
d. Qual o custo do programa amostral?

2.1. PLANEJAMENTO DE UM PROGRAMA DE ANÁLISE DE MICOTOXINAS

Este planejamento requer o conhecimento das características da distribuição das micotoxinas. Os trabalhos de Whitaker et al. (1974) sobre a distribuição dos resultados das análises de micotoxinas em lotes são muito importantes. Nestes trabalhos, o conhecimento de curvas operacionais e da forma de interferirmos na diminuição do risco de amostragem são pressupostos fundamentais.

A forma mais prática, encontrada em nossas condições, para diminuição do risco de amostragem e com um compromisso entre custo/benefício encontra-se definida na Figura 3. O programa amostral empregado na maioria das indústrias assistidas pelo convênio entre LAMIC e o Instituto SAMITEC, ambos localizados em Santa Maria (RS, Brasil) é o recomendado pela Comissão de normas da União Européia (Amtsblatt der Europäische Gemeinschaften N L 102/1, TEIL II, 1976 e Futtermittelrecht mit Typenliste fur Einzel - und Mischfuttermitteln, 1994) e normatizado, posteriormente, pela ISO 6497:2002. Adaptações técnicas, para atender às dimensões da indústria de processamento de alimentos, foram realizadas para amostrar sempre as matérias-primas após serem moídas, possibilitando melhor exeqüibilidade e representatividade. A colheita das amostras deve ser realizada quando o material se encontra em movimento e moído, sendo realizada em intervalos de tempo pré-estabelecidos, dependendo da quantidade de toneladas produzidas por turno de produção. Assim, uma fábrica de rações que produz 100 T/dia, fará uma amostra coletiva mínima de 44,7 kg de material moído, segundo a equação $\sqrt{20xT}$. Este material será melhor colhido se amostrado dinamicamente, empregando-se o sistema "Furo na Rosca", onde é obtida uma amostra constante do fluxo do material, previamente moído, durante o processamento. Deste material deverá ser retirada uma amostra de no mínimo 1 kg destinada ao laboratório. Os responsáveis pela colheita do material, preferencialmente, deverão sempre estar envolvidos no processo e evidentemente treinados para tal. A participação do amostrador é fundamental para a operacionalização e bom andamento de um sistema de controle de micotoxinas.



O envio ao laboratório deverá ser o mais rápido possível, devendo o material ser acondicionado em embalagem resistente. Para áreas mais afastadas, recomenda-se o uso do serviço de transporte aéreo. É importante que o tempo de transporte não ultrapasse 48 horas.

A análise e principalmente a definição de quais as micotoxinas que devem entrar em um programa de controle somente poderão ser definidas pelo conhecimento da incidência das mesmas na área de origem do material a ser empregado na indústria. Em razão da necessidade da tomada de decisões rápidas nas agroindústrias, recomenda-se que a análise seja realizada o mais rápido possível.

Na avaliação dos resultados é necessário, inicialmente, estabelecer os limites de confiança para o sistema amostral. O procedimento usual inicia com a colheita de no mínimo 3 e no máximo 5 amostras por turno de produção. Os resultados são avaliados em sua amplitude, para com isto ajustar-se o número mínimo de amostras a serem analisadas. Após um período inicial, de aproximadamente 1 mês, é possível reduzir o número de amostras a até 1 por dia, dependendo da produção total e da variabilidade de contaminação do cereal empregado.

2.2. AMOSTRAGEM PARA PESQUISA DE MICOTOXINA.

Para melhorar a representatividade do resultado da análise de micotoxina, podem ser tomadas algumas medidas, como:

1. Amostragem com maior peso da amostra e número de pontos amostrados;
2. Sub-amostragem com o aumento de tamanho (peso) da sub-amostra ou pela diminuição do tamanho das partículas pela moagem;
3. Analisando um maior número de amostras.

Alguns cuidados básicos devem ser adotados para permitir uma amostragem eficiente:

- ✓ Colheita de amostra representativa, seguindo um plano amostral;
- ✓ A amostra deverá ser recolhida o mais próximo possível do local em que o animal intoxicado consumiu o alimento (comedouros);
- ✓ A colheita de sangue e órgãos permite uma análise retrospectiva de algumas contaminações, principalmente nas situações em que o alimento não se encontra mais disponível. Algumas micotoxicoses, como a ocratoxina A, são detectáveis, no sangue até 35 dias após a ingestão da toxina;
- ✓ Identificação do componente (ou componentes) na alimentação contaminada, nos casos em que se detectam micotoxinas na ração formulada;

2.3. DIAGNÓSTICO DE MICOTOXINAS

Atualmente, a metodologia mais específica, precisa e confiável é a obtida com o emprego de processos químicos. Esses procedimentos poderão ser tanto os dirigidos para a Cromatografia em Camada Delgada (TLC), quanto para a Cromatografia Líquida de Alta Resolução (HPLC). Recentemente, com o surgimento do HPLC acoplado à detecção por Espectrometria de Massa (LC/MS e LC/MSMS) além da Cromatografia Gasosa acoplada à MS (GC/MS), os

sistemas diagnósticos tendem a ser cada vez mais rápidos e precisos. Essas metodologias apresentam resultados semelhantes. Os testes de imunoensaio poderão ser empregados para triagem e, em casos excepcionais, para a semiquantificação. As avaliações químicas ainda constituem as metodologias internacionalmente mais aceitas e recomendadas para o diagnóstico de micotoxinas. O emprego de extração em fase sólida traz avanços, principalmente na padronização e automação das análises micotoxicológicas.

O sistema de análise empregado no monitoramento "on line" usa o HPLC acoplado a metodologias automatizadas de extração, purificação e derivação, bem como, sistemas de LC/MSMS. O tempo necessário para a análise desde a entrada da amostra no laboratório até a expedição do laudo final de análise não ultrapassa 48 horas para uma análise padrão, composta pelas principais micotoxinas e com limites quantificados em ppb.

2.4. DIMINUIÇÃO DOS EFEITOS TÓXICOS DAS MICOTOXINAS PELA UTILIZAÇÃO DE ADITIVOS ANTI-MICOTOXINAS (AAM)

Uma vez que as micotoxinas estejam formadas, qualquer esforço no sentido de prevenir o crescimento fúngico já é inútil. Um método largamente utilizado para o controle das micotoxicoses é o uso de materiais nutricionalmente inertes na dieta animal, a fim de diminuir a absorção das micotoxinas no trato gastrointestinal das aves. Essas substâncias eram chamadas de adsorventes de micotoxinas e, atualmente, são genericamente chamadas de Aditivos Anti-micotoxinas (AAM).

Apesar de existirem no mercado brasileiro um número significativo de produtos, Mallmann et al (2006) comprovam que apenas 50% dos produtos AAM adequadamente testados, apresentam potencial satisfatório para serem utilizados com essa finalidade. Não obstante, o fato de que boa parte dos produtos não atende às exigências para a utilização como AAM, fica, ainda, a incerteza de quanto e quando utilizar um produto comprovadamente eficaz. Essa resposta é obtida através do constante monitoramento das produções das fábricas de rações. De um modo geral, admite-se que todas as dietas para a fase pré-inicial devam

ter em sua formulação a inclusão de um AAM; as dietas preparadas para as demais fases, devem levar em consideração o Risco Micotoxinas para a inclusão ou não do AAM. Este índice (RM) leva em consideração a interação entre a contaminação média das amostras analisadas e a incidência média de cada micotoxina nessas amostras, o que serve de referência para a tomada de decisões com relação à utilização dos AAM.

3. CONCLUSÕES E RECOMENDAÇÕES

A presença de micotoxinas nas dietas fornecidas às aves pode determinar perdas consideráveis no sistema de produção avícola brasileiro. A considerável presença das micotoxinas nos principais componentes da dieta das aves determina que se adote um programa contínuo de controle, podendo este ser baseado no uso de AAM (adsorventes). Para a adoção de medidas de controle, faz-se necessário que se saiba com precisão a contaminação existente, tornando imprescindível a implementação de um programa de monitoramento das matérias-primas e/ou das rações destinadas ao consumo.

O controle futuro do problema das micotoxinas na economia pecuária, depende da implantação de políticas adequadas no âmbito do manejo agrícola, bem como dos sistemas de armazenagem, raízes do problema. Somente políticas nessas áreas, significarão resultados econômicos duradouros para a avicultura.

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Digestive Physiology and the Role of Microorganisms

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SUMMARY

The gastrointestinal tract contains within it a microenvironment of bacteria that influences the host animal in many ways. The microflora can metabolize several nutrients that the host cannot digest and converts these to end products (such as short-chain fatty acids), a process that has a direct impact on digestive physiology. The microbiota directs the assembly of the gut-associated lymphoid tissue, helps educate the immune system, affects the integrity of the intestinal mucosal barrier, modulates proliferation and differentiation of its epithelial lineages, regulates angiogenesis, modifies the activity of the enteric nervous system, and plays a key role in extracting and processing nutrients consumed in the diet. Despite these important effects, the mechanisms by which the gut microbial community influences host biology remain almost entirely unknown. Recent molecular-based investigations have confirmed the species diversity and metabolic complexity of gut microflora, although there is much work to be done to understand how they relate to each other as well as the host animal. It is almost a century ago that Eli Metchnikoff proposed the revolutionary idea to consume viable bacteria to promote health. Since that time, the area known as probiotics has made dramatic progress, particularly during the past 2 decades. The last 20 yr have also seen the emergence of a new, related area of study—prebiotics. Use of these 2 ideas—providing live nonpathogenic bacteria as well as substrates for their growth—have potential to help optimize the health of animals by manipulating the gastrointestinal tract in positive ways.

Key words: digestive physiology, microorganism, probiotic, prebiotic, production

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DESCRIPTION OF PROBLEM

There is a tendency to regard all microorganisms as harmful, to equate bacteria with germs. Nothing could be further from the truth. The number of nonpathogenic species far exceeds the number of pathogenic species, and many of the known bacteria are in fact useful, even essential for the continued existence of life on earth. One example of a beneficial group of microorganisms are those that inhabit the gastrointestinal tract (**GIT**) of animals. The GIT harbors harbors an incredibly complex and abundant ensemble of microbes [1, 2]. The intestine is in contact Nothing could be further from the truth. The with components of this microflora from birth, number of nonpathogenic species far exceeds yet little is known about their influence on nor-the number of pathogenic species, and many mal development and physiology. The GIT is of the known bacteria are in fact useful, even more densely populated with microorganisms essential for the continued existence of life on than any other organ and is an interface where earth. One example of a beneficial group of *mi-the microflora may have a pronounced impact* on animal and human biology [2]. The bacterial population of the human cecum and colon is numerically large with at least 10^{13} cfu/g [3].

Similar values have been reported for other omnivores such as pigs [4]. Bacteria compose about 40 to 55% of solid stool matter [5]. Throughout millions of years of evolution, animals have developed the means for supporting complex and dynamic consortia of microorganisms during their life cycle. A transcendent view of vertebrate biology, therefore, requires an understanding of the contributions of these indigenous microbial communities to host development and adult physiology. The fragile composition of the gut microflora can be affected by various factors such as age, diet, environment, stress, and medication [6]. As with most complex ecosystems, it appears that most species cannot be cultured when removed from their niches [6, 7]. Although a full definition of biodiversity awaits systematic application of molecular enumeration techniques, such as genotyping DNA encoding 16S rRNA [rDNA] genes [8, 9, 10]. More than 50 genera and at least 500 to 1,000 different species are distributed along the length of the GIT [11]. The dominant organisms in terms of numbers are anaerobes including bacteroides, bifidobacteria, eubacteria, streptococci, and lactobacilli, and others, such as enterobacteria, also may be found, usually in fewer numbers [11, 12]. Generally, bacteroides (including those that can utilize a wide range of polysaccharides) are most numerous and can compose more than 30% of the total. Recent evaluation of the microflora ecology of the chicken intestine using 16s rDNA determined that *Lactobacilli* is the predominate organism in young birds, and the population of *Bifidobacterium* dominates in older birds [13].

Colonization begins at birth and is followed by progressive assembly of a complex and dynamic microbial society [14]. Assembly is presumably regulated by elaborate and combinatorial microbial-microbial and host-microbial interactions predicated on principles refined over the course of animal evolution. Comparisons of rodents raised without exposure to any microorganisms to animals that have assembled a microbiota since birth, or those that have been colonized with components of the microbiota during or after completion of postnatal development, have revealed a range of host functions affected by indigenous microbial communities. For example, the microbiota directs the assembly of the gut-associated lymphoid tissue [15], helps educate the immune system [16, 17], affects the integrity of the intestinal mucosal barrier [18, 19, 20], modulates proliferation and differentiation of its epithelial lineages [21, 22], regulates angiogenesis [23], modifies the activity of the enteric nervous system [24], and plays a key role in extracting and processing nutrients consumed in the diet [25]. The microflora can metabolize proteins and protein degradation products, sulfur-containing compounds, and endogenous and exogenous glycoproteins [12]. Some organisms grow on intermediate products of fermentation such as H2, lactate, succinate, formate, and ethanol and convert these to end products including short-chain fatty acids (**SCFA**), a process that has a direct impact on digestive physiology [26]. Although the mechanisms by which bacteria assert these effects on the GIT remain essentially unknown, research in this area is focusing on elucidating these mechanisms as well as manipulating the bacteria and the gastrointestinal environment toward achieving optimal health through probiotics and prebiotics.

THE ROLE OF MICROORGANISMS ON DIGESTIVE PHYSIOLOGY

SCFA Production

The SCFA increase from undetectable levels in the ceca of day-of-hatch chicks to the highest concentration at d 15 of age as the enteric microflora becomes established [27]. The basic fermentative reaction in the human colon or chicken cecum is similar to that in

obligate herbivores: hydrolysis of polysaccharides, oligosaccharides, and disaccharides to their constituent sugars, which are then fermented, resulting in an increased biomass [28]. Carbohydrate hydrolysis is affected by a number of bacterial cell-associated and secreted hydrolases that can digest a range of carbohydrates, which monogastric animals cannot. Fermentation yields metabolizable energy for microbial growth and maintenance and also metabolic end products. Nitrogen for protein synthesis can come from urea (via the urease reaction), undigested dietary protein, or endogenous secretions. The principal products are SCFA together with gases [CO2,CH4, and H2] and some heat [29]. Carbohydrates entering the large intestine can alter gut physiology in 2 ways: physical presence and fermentation. Effects of SCFA can be divided into those occurring in the lumen and those arising from their uptake and metabolism by the cells of the large bowel wall. The SCFA are the principal luminal anions. They are relatively weak acids with p*K*a values of 4.8, and increasing their concentrations through fermentation lowers digesta pH [29]. The SCFA also serve as an important source of energy for the gut wall, providing up to 50% of the daily energy requirements of colonocytes [28, 30]. Fermentable carbohydrates can alter the microbial ecology greatly by acting as substrates or supplying SCFA. Much attention has been directed toward the study of specific beneficial lactic acid bacteria, rather than the flora as a whole [30], however, the SCFA have diverse functions with regard to host and microbial physiology.

Blood Flow and Muscular Activity

Studies in vitro have shown that incubation with SCFA (as the sodium salts) at concentrations as low as 3 m*M* dilate precontracted colonic resistance arterioles in isolated human colonic segments [31]. Greater colonic blood flow has been observed with infusion of acetate, propionate, or butyrate (separately or as a mixture) into the denervated canine large bowel [32]. The mechanism of action of SCFA on blood flow does not involve prostaglandins or α-or β-adrenoreceptor linked pathways [31]. The mechanisms of action may involve local neural networks as well as chemoreceptors together with direct effects on smooth muscle cells [33]. The SCFA produced in the colon and entering the portal circulation seem to influence the upper gut musculature. These actions are important for the maintenance of the function of the whole gastrointestinal system, not just the colon. It is expected that greater blood flow enhances tissue oxygenation and transport of absorbed nutrients.

Enterocyte Proliferation

In rats, SCFA stimulate the growth of colorectal and ileal mucosal cells when they are delivered colorectally or intraperitoneally [34, 35]. In addition to promoting growth, the major SCFA (especially butyrate) appear to lower the risk of malignant transformation in the colon [36]. Secondary bile acids are cytotoxic, and in rats fed deoxycholate plus cholesterol, cell proliferation as measured by incorporation of [3H]thymidine was increased [37]. Some of the effects of SCFA may be due to low intracolorectal pH rather than any specific SCFA. At a pH of 6, bile acids are largely protonated and insoluble and so would not be taken up by colonocytes [38]. Additionally, lower pH inhibits the bacterial conversion of primary to secondary bile acids [39, 40] and, therefore, lowers their carcinogenic potential.

Mucin Production

Evidence has been presented that mucus production and release is stimulated locally by endogenous production of SCFA by gut microflora [41, 42, 43]. Additionally, some studies have been completed evaluating the influence of specific beneficial or probiotic organisms on mucin production. In vitro studies with *Lactobacillus plantarum* 299v suggest that the ability of organisms to inhibit adherence of attaching and effacing organisms to intestinal epithelial cells is mediated through their ability to increase expression of MUC2 and MUC3 intestinal mucins [44, 45]. The benefits of probiotics mediated through intestinal mucin upregulation may have broader applicability than enteropathogen intervention in poultry. Several investigators have shown that the increase in mucin production following probiotic administration inhibited replication, disease symptoms and shedding of rotavirus in humans [46, 47, 48, 49]. In the proximal colon, an increase in the butyrate concentration altered crypt depth and the number of mucus-containing cells; the increase in butyrate was highly correlated with the number of neutral-mucin-containing cells [50, 51].

Probiotic, Prebiotic, and Synbiotic

The use of lactic acid bacteria as feed supplements goes back to pre-Christian times when fermented milks were consumed by humans. It was not until last century that Eli Metchnikoff, working at the Pasteur Institute in Paris, evaluated the subject from a scientific basis. Metchnikoff, documented a direct link between human longevity and the necessity of maintaining a healthy balance of the beneficial and pathological microorganisms residing in the human gut. Metchnikoff's 1908 Nobel Prize in physiology was awarded for his discovery of phagocytes and other immune system components, but his accurate description of vital elements in the body's intestinal flora is equally notable. He developed and prescribed to his patients bacteriotherapy (i.e., the use of lactic acid bacteria in dietary regimens) [52]. In support of this he cited the observation that Bulgarian peasants consumed large quantities of soured milk and also lived long lives. He had no doubt about the causal relationship, and subsequent events have, in part, confirmed his thesis. He isolated what he called the Bulgarian bacillus from soured milk and used this in subsequent trials. This organism was probably what became known as *Lactobacillus bulgaricus* and is now called Lactobacillus del*brueckii* subsp. *bulgaricus* which is one of the organisms used to ferment milk and produce yogurt [52]. After Metchnikoff's death in 1916 the center of activity moved to the United States. Knowledge available at that time suggested the use of *Lactobacillus acidophilus* and many trials were carried out using this organism. Encouraging results were obtained, especially in the relief of chronic constipation [53]. In the late 1940s interest in the gut microflora was stimulated by 2 research developments. The first was the finding that antibiotics included in the feed of farm animals promoted their growth. A desire to discover the mechanism of this effect led to increased study of the composition of the gut microflora and the way in which it might be affecting the host animal. Second, the more readily availability of germ-free animals provided a technique for testing the effect that the newly discovered intestinal inhabitants were having on the host. This increased knowledge also showed that *L. acidophilus* was not the only *Lactobacillus* in the intestine, and a wide range of different organisms came to be studied and later used in probiotic preparations [54].

A probiotic is defined as a live microbial food supplement that benefits the host by improving its intestinal microbial balance [55]. The presence of normal gut microflora may improve the metabolism of the host bird in various ways, including absorptive capacity [56], protein metabolism [57], energy metabolism and fiber digestion [58], energy conversion [59], and gut maturation [60]. Balanced colonic microflora and immunostimulation are major functional effects attributed to the consumption of probiotics [55]. Many probiotic effects are mediated through immune regulation, particularly through balance control of proinflammatory and antiinflammatory cytokines [61, 62]. However, probiotics can only be effective if the requirements for their growth are present in the GIT. The concept of prebiotics is relatively new; it was developed in response to the notion that nondigestible food ingredients (e.g., nondigestible oligosaccharides) are selectively fermented by one or more bacteria known to have positive effects on gut physiology. Bacteria fed by a preferential food substrate have a proliferative advantage over other bacteria [63]. Some prebiotics have shown to selectively stimulate the growth of endogenous lactic acid bacteria and Bifidobact*eria* in the gut to improve the health of the host [63]. Probiotic numbers have been enhanced by prebiotics that selectively stimulate the growth and activity of one or a limited number of bacterial species already resident in the large intestine, and, thus, improve host health [12]. In this way, prebiotics selectively modify the colonic microflora and can potentially influence gut metabolism [63]. However, the bacterial nutrient package will not be advantageous without the presence of the targeted, beneficial bacteria, and likewise the live microbial product will not succeed if the environment into which it is introduced is unfavorable [64]. The concept of synbiotic has been proposed recently to characterize foods with both prebiotic and probiotic properties as health-enhancing functional foods [42].

Role of Microorganisms in Poultry Production

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 [65], and maximum absorption capacity is attained by 10 d of age [66]. 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 posthatch 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 in the intestinal size and morphology [65]. Maturational changes also affect the epithelial cell membranes, a major mechanical interface between the internal environment of the host and the luminal contents [67]. Studies on nutrition and metabolism during the early phase of growth in chicks may help in optimizing nutritional management for maximum growth [68]. By dietary means it is possible to affect the development of the gut and the competitiveness of 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 nature of these additives are well understood, but the manner by which they benefit the animal is not [69].

Probiotics as an Alternative to Antibiotics for Control of Bacterial Pathogens in Poultry
Bacterial antimicrobial resistance in the medical and agricultural fields has become a serious problem worldwide. Antibiotic-resistant strains of bacteria are an increasing threat to animal and human health, with resistance mechanisms having been identified and described for all known antimicrobials currently available for clinical use. There is currently increased public and scientific interest regarding the administration of therapeutic and subtherapeutic antimicrobials to animals, due primarily to the emergence and dissemination of multiple antibiotic-resistant zoonotic bacterial pathogens [70]. Social pressures have lead to creation of regulations to restrict antibiotic use in poultry and livestock production. There is a need to evaluate potential antibiotic alternatives to improve disease resistance in high intensity food animal production. Nutritional approaches to counteract the debilitating effects of stress and infection may provide producers with useful alternatives to antibiotics. Improvement of disease resistance of animals grown without antibiotics will not only benefit the animals' health, welfare, and production efficiency but is also a key strategy in the effort to improve the microbiological safety of poultry products [70].

During the last 4 yr, our laboratory has worked toward the identification of probiotic candidates for poultry that can actually displace *Salmonella* and other enteric pathogens that have colonized the gastrointestinal tract of chicks and poults. Published studies [71] have indicated that after more than 8 million enteric organisms were screened for competition in vitro, 36 organisms were identified that had the ability to exclude *Salmonella* in neonatal poultry. Additional *Lactobacillus*-related isolates [72] were eventually found that were even more efficacious in the treatment of *Salmonella*-infected chicks and poults. In laboratory challenge studies, 80 to 90% reductions in *Salmonella* recovery rates from challenged chicks treated with Floramax probiotic culture were typical. By selecting flocks infected with *Salmonella* preslaughter, we have demonstrated that treating such flocks, approximately 2 wk prior to slaughter, with Floramax can markedly reduce environmental *Salmonella* recovery from commercial turkeys and broilers [73]. Treatment of idiopathic enteritis in commercial poults with Floramax also compares favorably to selected antibiotic therapy in recent studies [74]. Large-scale commercial trials have indicated that appropriate administration of this probiotic mixture to turkeys increased body weight gain at processing by approximately 230 g with over 120 flocks evaluated [75], with similar performance gains observed in more limited commercial trials with broilers. Administration of dietary lactose at a very low concentration (0.1%) greatly enhanced the growth rates of pro-biotic turkeys under commercial conditions and furthered reduced total production costs [75]. These data indicate that selection of therapeutically efficacious probiotic cultures with marked performance benefits in poultry is possible and that defined cultures can sometimes provide an attractive alternative to conventional antimicrobial therapy.

Comparisons Among Genotypic 16S rRNA, MIDI, and Biolog Identifications of FM-B11 Lactic Acid Bacteria
As a blend of facultative and obligate bacteria, the composition of lactic acid bacteria poses a unique problem for microbial identification. The identification techniques of choice for facultative anaerobes are biochemical analyses, but the standard identification system for

lactic acid bacteria is cellular fatty acid profiling. However, these phenotypic methods can yield variable results. Genotypic methods that rely on comparisons of 16S rRNA sequences from unknown bacteria are proving to be valuable for use in a wide range of genera and are not sensitive to variable culture conditions. Genotypic 16S rRNA identification of organisms from probiotic cultures may be more reliable than the current standard microbial techniques applied separately to different microbial groups. Although there are many new experimental molecular identification techniques, such as microarray hybridization and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry, sequence analysis of 16S rRNA is the predominant molecular technology currently available for microbial identification [76]. The 16S rRNA sequences of many bacteria species in a population may be analyzed simultaneously with denaturing gradient gel electrophoresis or random fragment length polymorphism analyses. The detailed information needed to identify each species represented in the complex microbial population of a probiotic product can only be fully obtained from the 16S rRNA at the level of the nucleotide sequence. As an example, we have devised an identification scheme using the MIDI System ID from 2 different private laboratories [77, 78] and the Biolog ID System [79] and the 16S rRNA Sequence Analyses [80] for identification of the individual component bacteria present in the commercial probiotic Floramax (Table 1).The results of that study show that the complex populations of bacteria present in Floramax are not easy to accurately identify, especially with phenotypic techniques. Genotypic identification by 16S rRNA gene sequence analysis has potential to improve the accuracy of bacterial identification, especially as the contents of sequence databases become more comprehensive. Conventional technologies can detect human pathogens, because they are well-established in comparative databases, but emerging and opportunistic pathogens are not. These results support a suggestion by the MIDI company [81] to use 16S rRNA sequence analysis to identify obligate and facultative anaerobes, such as those in FM-B11 (Floramax). Although ambiguity exists between different methods of identification of nonpathogenic probiotic bacteria, identification of known pathogens is much more consistent. Therefore, the use of fully defined cultures for competitive exclusion or probiotic use are still inherently safer than undefined cultures or those where organisms are identified after the culture has been produced.

CONCLUSIONS

The interest in digestive physiology and the role of microorganisms has generated data whereby human and animal well being can be enhanced and the risk of disease reduced. New molecular techniques allow accurate assessment of the flora composition, resulting in improved strategies for elucidating mechanisms. Given the recent international legislation and domestic consumer pressures to withdraw growth-promoting antibiotics and limit antibiotics available for treatment of bacterial infections, probiotics and prebiotics can offer alternative options. New advances in the application of synbiotics (compatible probiotics and prebiotics) are directed toward producing significant changes in gut physiology and provide even higher levels of health as well as increasing performance parameters.

Metchnikoff founded the research field of probiotics, which is aimed at modulating the intestinal microflora. However, other parts of the body containing endogenous microflora or problems relating to the immune system may also be candidates for probiotic therapy. Research has shown that probiotics have potential for addressing human health issues such

as vaginal candidiosis [82], dental caries [83, 84], allergies [85], autoimmune diseases [86], urogenital infections [87], atopic diseases [88], rheumatoid arthritis [89], and respiratory infections [90]. Current research is still heavily biased toward gastrointestinal applications for probiotics, such as chronic constipation [53], chronic diarrhea [91], inflammatory bowel disease [92], irritable bowel syndrome [93], and food allergy [94]; but the possibilities for affecting many areas of health are numerous. Much research has been completed in efforts to understand and apply the natural benefits of nonpathogenic bacteria, but there is much still to do.

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Table 1. Comparisons between MicroSeq, MIDI, and Biolog identifications of FM-B11 (Floramax) lactic acid bacteria (LAB)

LAB ID	16S RNA sequencing (first 500 bp) Microbial ID Inc.	MIDI system ID Micro Test Lab Inc.	MIDI system ID Microbial ID Inc.	Biolog ID Department of Poultry Science, University of Arkansas
18	*Pediococcus parvulus*	*Enterococcus cecorum*	*Lactobacillus gasseri*	Unable to identify
24	*Weissella confusa*	*Lactobacillus casei*	*Lactobacillus casei*	*Clostridium clostridiiforme*
27	*Weissella confusa*	*Lactobacillus casei*	*Lactobacillus casei*	*Weissella confusa*
29	*Pediococcus parvulus*	*Lactobacillus delbrueckii-bulgaricus*	*Lactobacillus delbrueckii-bulgaricus*	*Lactobacillus hamsteri*
36	*Lactobacillus salivarius*	*Lactobacillus cellobiosus*	*Lactobacillus casei*	*Weissella confusa*
37B	*Weissella confusa*	*Pediococcus acidilactici*	*Pediococcus ruminis*	Unable to identify
40	*Weissella confusa*	*Lactobacillus casei*	*Lactobacillus cellobiosus*	*Weissella paramesenteroides*
44	*Weissella paramesenteroides*	*Lactobacillus fermentum*	*Lactobacillus fermentum*	Unable to identify
46	*Lactobacillus salivarius*	*Lactobacillus helveticus*	*Lactobacillus sanfranciscensis*	*Lactobacillus salivarius*
48	*Lactobacillus salivarius*	*Lactobacillus helveticus*	*Lactobacillus gasseri*	*Lactobacillus salivarius*
52	*Pediococcus parvulus*	Unable to identify	*Lactobacillus cellobiosus*	Unable to identify

USO DE LA EXCLUSIÓN COMPETITIVA EN EL CONTROL DE *Salmonella enteritidis*, DESEMPEÑO E INMUNIDAD EN POLLOS DE CORTE

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Introducción

El buscador finlandés Esko Nurmi en 1973 fue el primero en aplicar el concepto de Exclusión Competitiva (CE) en aves1, también conocido como Concepto de Nurmi, que puede ser bien definido como "Establecer, inicialmente, una microflora intestinal adulta, previene la colonización de enteropatógenos"2. A través de la Exclusión Competitiva, el ave queda protegida de la transmisión horizontal de Salmonela transmitida por el medio ambiente e también de la ración y la transmisión vertical, que se da por la reproducción, queda también minimizada3. La CE demostró ser efectiva en la digestibilidad de los nutrientes en las aves4. El objetivo del trabajo fue averiguar la eficacia del concepto de CE, frente a una posible contaminación horizontal de Salmonela, y también evaluar los datos zootécnicos y parámetros de inmunidad de aves tratadas con CE.

Materiales y métodos

Fueron elaborados dos experimentos, uno en UNESP de Jaboticabal (EP1), donde fue evaluada la eficacia de CE frente a un desafío de *Salmonela enteritidis*, las aves fueron sacrificadas para el análisis de la cantidad de células viables de Salmonela en los contenidos cecales; y otro en USP en Pirassununga (EP2), donde las aves fueron monitoreadas sorologicamente en el D1, D21 e D42 para niveles de anticuerpos frente la vacuna de Newcastle y también recolectados los datos zootécnicos.

Resultados

(EP1) Los resultados están descritos en la Tabla. CE redujo la presencia de *S. enteritidis* Nal/Spc en el contenido cecal en el grupo tratado cuando fue comparado con un grupo control ($p<0.01$). La CE fue también comprobada con media dosis sobre el que es recomendado (Group B) y los resultados fueron muy favorables ($p<0.01$).

Tabla. Número de células viables en Log_{10} de *S. enteritidis* Nal/Spc en contenido cecal de 8 aves cinco días después desafío.

	Birds								
Groups	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
A_1	4.47	2.0	5.04	2.0	2.0	4.6	4.69	2.0	
A_2	4.69	4.47	2.0	2.0	5.0	3.78	4.27	5.53	
μ(A)									3.66 A ±1.38
B_1	7.0	2.0	6.47	2.0	2.0	6,47	2.0	2.0	
B_2	2.0	6.0	2.0	6.47	6.0	2.0	2.0	6.47	
μ(B)									3.93 A± 2.27
C_1	8.11	6.47	6.57	6.60	7.38	7.3	6.54	6.84	
C_2	7.34	6.56	7.53	6.78	6.11	6.36	5.84	6.5	
μ(C)									6.80B ±0.58

Grupos: A1 e A2 = grupo de aves tratadas con CE; **Grupos: B1 e B2** =grupo tratado con ½ dosis de CE
Grupos C1 e C2 = grupo control

Prueba de Tukey ($p < 0.05$). **[Nota editor: en el texto se indica p<0.01]**

Conclusión

El resultado mostró que el concepto de Exclusión Competitiva es eficiente en el control de enterobacterias, en especial *Salmonela enteritidis*, lo que es fundamental no solo en la avicultura como también en la salud pública. Independiente la dosis aplicada, CE puede ser una herramienta importante en la sustitución de antibióticos en las raciones consumidas por aves. El restante del estudio EP2 esta siendo concluido para mejorar la explicación de los beneficios del concepto de exclusión competitiva.

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COMPARACIÓN DE LOS NIVELES DE ABSORCIÓN DEL SULFATO DE GENTAMICINA ADMINISTRADO POR DIFERENTES VIAS (ORAL, INTRAVENOSA, INTRAMUSCULAR) EN POLLOS DE ENGORDA CLINICAMENTE SANOS Y CON DIARREA

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RESUMEN

El sulfato de gentamicina (S. G.) es un antibiótico aminoglucósido de amplio espectro utilizado para tratar afecciones bacterianas de etiología gram negativa principalmente, su alta naturaleza catiónica excluye a la mayoría de los aminoglucósidos de el tracto gastrointestinal de individuos clínicamente sanos, su actividad se limita de manera local cuando se utiliza por esta vía y no alcanza valores significativos de absorción.

Material y metodo
El trabajo consistió en administrar sulfato de gentamicina por vía oral a pollos clínicamente sanos y pollos clínicamente con diarrea, a dosis de 15 y 25 mg/kg P.V. tomamos muestras de sangre a diferentes tiempos, dichas muestras se les extrajo el suero sanguíneo y se midió la presencia de antibiótico por medio de la técnica concentración media inhibitoria con sensidiscos.

Resultados
En los animales que se administro S. G. vía oral y que presentaban diarrea, las muestras mostraron halos de inhibición bacteriana similares a la de los animales que se les administro el mismo antibiótico a la misma dosis pero por vía intramuscular (I. M.), en cambio animales que no presentaban diarrea y se les administro S. G. vía oral no presentaron halos de inhibición bacteriana de significancia.
Del mismo modo se comparo con animales clínicamente diarreicos en donde la vía de administración fue intravenosa (I. V.) el halo de inhibición bacteriana de estos animales fue mayor en comparación con las demás vías (oral, I.M.).

Conclusiones
Por todo lo anterior se puede concluir que el S. G. administrado por vía oral en pollos clínicamente sanos no muestra niveles de inhibición bacteriana según el método empleado. El S. G. administrado por vía oral en pollos clínicamente con diarrea muestra niveles de inhibición bacteriana similares a los mostrados cuando la vía de aplicación es I.M.

EFECTO DE LA TEMPERATURA AMBIENTAL DURANTE LA CRIANZA SOBRE LOS INDICADORES PRODUCTIVOS EN EL POLLO DE ENGORDA

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INTRODUCCIÓN

Es un hecho conocido que la temperatura termoneutral para los pollitos en la primera semana de vida se encuentre entre 33° y 35°C. La oscilación de la temperatura, tanto arriba como debajo de la termoneutralidad en la primera semana puede influenciar de manera marcada el desempeño de las aves hasta edad de venta. Se puede definir a la zona de bienestar térmico como un rango de temperatura ambiente en que la tasa metabólica es mínima y la homeotermia se mantiene con un menor gasto energético, de este modo la energía destinada, la utiliza para mantener su temperatura corporal, cuyo rango óptimo es de 40.0 a 40.1 °c. El manejo inicial es fundamental para el desempeño zootécnico del pollo de engorda, y de la misma forma, la preparación de las casetas, la recepción de los pollitos, y el manejo de la primera semana de vida son muy importantes en el ciclo de vida del ave. El desarrollo del pollito en la primera semana de vida es una condición relevante para el desempeño, ya que los procesos fisiológicos de hipertrofia celular, maduración del sistema termorregulador e inmunológico, el crecimiento y la diferenciación de la mucosa gastrointestinal sufren intensos procesos de adaptación en este periodo. En las líneas pesadas, el emplume es más tardío, consecuentemente las aves son más sensibles a las temperaturas durante las tres primeras semanas. Cuando logramos obtener un buen desarrollo inicial y uniformidad en las dos primeras semanas a través de manejo, ambiente y nutrición tendremos aves más resistentes a estrés calórico y con un menor índice de ascitis y otras enfermedades metabólicas; asimismo, las aves serán más resistentes a las infecciones a través de una buena respuesta inmunológica. La integridad del tubo digestivo, proporcionara una mejor absorción y consecuentemente mejor conversión alimenticia y ganancia de peso. Los pollitos

una vez nacidos, se comportan como animales homeotermos, la cantidad de nutrientes disponibles para el crecimiento se reduce en las aves en ambiente frío, teniendo como consecuencia un menor desempeño productivo. Este trabajo tiene como objetivo evaluar los parámetros productivos de pollos de engorda criados bajo dos protocolos de temperatura y manejo diferentes.

MATERIAL Y MÉTODOS

Esta prueba se llevo a cabo en casetas de ambiente natural ubicadas en Aguascalientes. Se utilizaron 230,218 pollos de engorda de un día de edad estirpe Ross x Ross distribuidos en ocho casetas convencionales equipadas con bebederos, comederos automáticos y criadoras infrarrojas.

Grupo A: 115,824 pollos distribuidos en cuatro casetas, dos de machos y dos de hembras y criados a 33 °C ambientales sin cámara de crianza.

Grupo B: 114,374 pollos distribuidos en cuatro casetas, dos de machos y dos de hembras y criados a 34.5°C ambientales con cámara de crianza
La temperatura ambiental fue monitoreada cada ocho horas durante la crianza con termómetros de máximas y mínimas; asimismo, se llevo el control de la temperatura de la cama mediante el uso de termómetros laser.

Se evaluaron el consumo de alimento por ave terminada, conversión alimenticia, kilos de carne producidos, peso promedio del pollo en granja al final de la parvada (en el caso de hembras fueron destinadas a pollo rosticero), ganancia diaria de peso e índice de productividad.

RESULTADOS

En el cuadro 1 se muestran los resultados de conversión alimenticia, kilos de carne producidos, peso promedio del pollo en granja, consumo de alimento por ave terminada, ganancia diaria de peso e índice de productividad. Como se puede observar en el caso de las hembras criadas a 34.5ºC se produjeron 365kg de carne más, consumiendo 28g menos de alimento, ganaron 0.9g más por día y el índice de productividad fue 8.58 puntos mejor que las hembras criadas a 33ºC. En

el caso de los machos criados a 34.5ºC se produjeron 5905kg más de carne, consumieron 288g mas de alimento; sin embargo, la conversión alimenticia fue 0.05 mejor, ganando 1.2g más por día, por último el índice de productividad fue 15.55 puntos mejor que los machos criados a 33ºC.

Cuadro 1. Parámetros productivos en pollos de engorda criados a 33 y 34.5°C				
PARÁMETROS EVALUADOS	***H 33°C***	***H 34.5°C***	***M 33°C***	***M 34.5°C***
CONVERSION ALIMENTICIA	1.855	1.814	2.228	2.178
KILOS DE CARNE PRODUCIDOS	46590	46955	50995	56900
PESO PROMEDIO DEL POLLO EN GRANJA	1.712	1.735	2.388	2.574
CONSUMO DE ALIMENTO POR AVE TERMINADA	3.175	3.147	5.32	5.608
GANANCIA DIARIA (GRAMOS)	42.1	43	50.8	52
INDICE DE PRODUCTIVIDAD	212.92	221.5	185.89	201.44

DISCUSIÓN Y CONCLUSIONES

Como se pudo observar la diferencia de temperaturas durante la crianza fue un factor importante en el rendimiento productivo de los pollos de engorda, por lo que mantener la termoneutralidad en las aves será crucial para el adecuado desarrollo de las aves y la óptima utilización del alimento que proporcionamos a las aves. Es importante mencionar que durante esta prueba las aves que llegaron a las casetas cuya temperatura ambiental era de 34.5 ºC en el caso de las hembras pesaron 38.24g vs 43g de las hembras criadas a 33 ºC. Para los machos los criados a 34.5 ºC el peso de recepción fue de 41.05g vs 44.42g de los criados a 33 ºC. Podemos concluir que crianzas calientes ayudaran a las aves a concluir su desarrollo orgánico permitiéndoles alcanzar y/o superar el rendimiento productivo de aves cuyo peso a la recepción es mayor.

EVALUACIÓN DEL CONSUMO DE AGUA Y GANANCIA DE PESO EN POLLOS DE ENGORDA TRATADOS CON TRES DIFERENTES PROGRAMAS ANTIMICOPLÁSMICOS

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INTRODUCCIÓN

El agua es esencial para cualquier organismo viviente y las aves no son la excepción; sin embargo, el agua es uno de los nutrientes que frecuentemente olvidamos monitorear. La provisión de agua abundante y fresca a las aves será siempre recompensada con salud y buenos resultados. Como parte de las medidas de bioseguridad en las granjas es recomendable realizar de forma rutinaria y periódica, una o dos veces al año pruebas de calidad del agua de las zonas en donde se encuentran las granjas, dichos análisis deben incluir: pruebas microbiológicas y físico-químicas estas últimas deben incluir determinación de la dureza del agua, pH, evaluación de nitratos y nitritos, sodio, cloro, sulfatos, calcio, hierro, magnesio y manganeso. Es importante conocer la calidad del agua que suministramos a las aves ya que el agua es una vía práctica y sencilla para la administración de vacunas, ácidos orgánicos y medicamentos.

En la industria avícola existen diversas situaciones en las que las aves deben o necesitan ser medicadas y podemos hacerlo por diferentes vías tales como parenteral y/o oral en este último caso la medicación puede ser vía alimento o agua de bebida. Sin embargo, bajo ciertas condiciones patológicas el consumo de alimento puede estar disminuido, mientras que el consumo de agua usualmente se mantiene o incrementa. Asimismo, es más fácil y rápido medicar el agua que el alimento, en ocasiones cuando algún tratamiento no esta funcionando como esperamos y este fue aplicado vía alimento tenemos que esperar a que se termine ese alimento en las líneas de comedero o las tolvas para que el pollo comience a consumir el nuevo tratamiento; sin embargo, cuando se medica en el agua en cuestión de horas podemos considerar duplicar la dosis que se esta aplicando o bien cambiar el tratamiento de forma inmediata, esto último nos puede ayudar a minimizar las pérdidas económicas asociadas a una terapia antimicrobiana tardía.

Aunque en la mayoría de las ocasiones el agua es utilizada como una vía terapéutica una vez que se han presentado los signos clínicos de una enfermedad, es importante mencionar que también puede utilizarse como una vía para el tratamiento preventivo de algunas patologías, tal es el caso de la micoplasmosis aviar.

El objetivo de este trabajo es presentar los resultados de consumo de agua y ganancia de peso de tres programas antimicoplasmicos uno suministrado vía alimento y otra vía agua de bebida.

MATERIAL Y MÉTODOS

Esta prueba se llevo a cabo en una caseta experimental ubicada en el municipio de Arenal, Jalisco en pollos de engorda estirpe ross. El diseño de tratamientos fue el siguiente:

1. Alimento no medicado + agua con antimicoplasmico soluble A
2. Alimento medicado + agua sin medicar
3. Alimento no medicado + agua sin medicar
4. Alimento no medicado + agua con antimicoplasmico soluble B

Los tratamientos fueron administrados de los 9-15 días y de los 39-41 días de edad.

Cada tratamiento consto de 5 replicas con 50 pollos cada uno. Diariamente se evaluó el consumo de agua y las aves fueron pesadas semanalmente hasta cumplir los 42 días de edad.

RESULTADOS

El consumo de agua se vio disminuido durante el primer y segundo día de evaluación (9 y 10 días de edad) siendo el grupo mas afectado el medicado con el antimicoplásmico soluble A cuyo consumo fue de 1191ml (día 9 de edad) este

consumo fue menor con respecto a los grupos de alimento medicado y antimicoplásmico soluble B cuyo consumo promedio fue de 1682ml y 2081ml respectivamente.

Para el segundo día de evaluación el consumo de agua en el grupo medicado con antimicoplásmico soluble A se incremento con respecto al día anterior 1877ml vs 1191ml; sin embargo, el consumo de agua con respecto a los otros tres grupos fue menor. Durante los siguientes 5 días de tratamiento no se observo diferencia en el consumo de agua de los pollos (Cuadro 1) y aun durante la segunda fase de medicación no se observo diferencia en el consumo de agua.

Cuadro 1. Media del consumo de agua (ml)±desviación estándar de pollos de engorda medicados con tres programas antimicoplásmicos diferentes

TRATAMIENTO	Edad (días)						
	9	10	11	12	13	14	15
ANTIMICOPLASMICO SOLUBLE A	1191.2 ± 170	1877.4 ± 287	2917.8 ± 67	2993 ± 13	5977 ± 12	5998 ± 4	6000 ± 0
ALIMENTO MEDICADO	1682.4 ± 474	2294.2 ± 264	2945.8 ± 29	2999.6 ± 0.89	5984.8 ± 19	5999 ± 2	6000 ± 0
SIN MEDICAR	1531.6 ± 253	2518.6 ± 299	2937.6 ± 34	2998 ± 4	5990 ± 17	6000 ± 0	6000 ± 0
ANTIMICOPLÁSMICO SOLUBLE B	2081.4 ± 221	2303.4 ± 271	2950.2 ± 15	2995.4 ± 8	5981 ± 21	5999 ± 2	6000 ± 0

En relación a la ganancia de peso semanal durante la primera semana los pesos no exhibieron diferencia; sin embargo, el peso en los pollos tratados con el antimicoplásmico soluble A fue de 14-24g menor con respecto a los otros tres grupos. Los grupos de alimento medicado y antimicoplásmico soluble B fueron los mas altos a la semana 5. El grupo sin medicar a la semana 5 de edad exhibió el peso más bajo. A las 6 semanas de edad los grupos de alimento medicado, sin medicar y antimicoplásmico soluble B obtuvieron los pesos más altos; sin embargo, el peso del grupo tratado con el antimicoplásmico soluble B fue menor con respecto a los grupos de alimento medicado, sin medicar y con antimicoplásmico soluble B (Cuadro 2)

Cuadro 2. Ganancia de peso semanal en pollos de engorda tratados con tres diferentes programas antimicoplásmicos

Columna1	ANTIMICOPLÁSMICO SOLUBLE A	ALIMENTO MEDICADO	SIN MEDICAR	ANTIMICOPLÁSMICO SOLUBLE B
Recepción	44.48	43.616	43.872	43.8
1a. Semana	147.576	147.568	147.16	143.216
2a. Semana	368.864	392.024	392.88	382.56
3a. Semana	763.48	774.15	772.37	771.45
4a. Semana	1330.02	1353.96	1361.64	1368.23
5a. Semana	1898.98	1931.32	1878.33	1920.90
6a. Semana	2433.75	2518.17	2504.78	2498.20

DISCUSIÓN Y CONCLUSIONES

Si bien es cierto que el agua es un medio de fácil y práctico acceso para llevar acabo planes terapéuticos, es importante considerar que algunos antimicrobianos pueden disminuir temporalmente el consumo de agua, dependiendo del tiempo por el cual se prolongue este efecto negativo en el consumo de agua se puede afectar en diferente grado la ganancia de peso, como fue el caso del antimicoplásmico soluble A. Debido a esto es importante contar con registros diarios del consumo de agua en las granjas, con la finalidad de detectar cualquier cambio en el consumo de agua asociado a algún tratamiento terapéutico. Asimismo, es importante mencionar que el éxito o fracaso de los tratamientos vía agua de bebida es influenciado considerablemente por la calidad de la misma ya que algunos minerales, bacterias, materia orgánica, etc., podrían interactuar con los antibióticos provocando que el producto no se encuentre en la cantidad adecuada en el agua y por lo tanto no se absorba la cantidad necesaria de antibiótico para resolver el problema que se este tratando. Por último es importante recordar que debe contarse también con un programa de sanitización del sistema de abastecimiento de agua con la finalidad de eliminar el biofilm y residuos de estabilizadores de vacunas o antibióticos, como parte del programa de bioseguridad de las granjas.

EVALUACION SENSORIAL DE LA CARNE DE POLLO ALIMENTADO CON DIFERENTES CONCENTRACIONES DE INULINA

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Palabras clave: inulina, prebiótico, pollo, sensorial, carne.

Introducción

El medio ambiente interno y externo de animales y humanos proporciona constantes y variados cambios en la salud y el bienestar. Al enfrentarse con estos cambios, los animales han desarrollado una amplia diversidad de mecanismos de defensa. Esto comprende barreras físicas y funcionales para la invasión, la capacidad a responder y eliminar organismos dañinos, la transformación potencial de sustancias dañinas en substancias benéficas para el organismo (Buddington, et al. 2002). Pero no siempre pueden eliminar esas substancias dañinas por lo que se les adicionan antibióticos (Xu, et al. 2003) en los alimentos creando una desestabilización en la flora intestinal y logrando alterar la integridad de las superficies epiteliales impidiendo la absorción de nutrientes por eso es necesario remplazar los antibióticos y buscar otras alternativas como las fibras fermentables encontrándose entre ellas la inulina, la oligofructuosa y otros olisacaridos no digestibles (NDO), que representan orígenes de nutrientes para las bacterias residentes y ayudan a prevenir la invasión de bacterias patógenas. Estos fructanos poseen propiedades funcionales y nutricionales que pueden tener aplicaciones para la nutrición animal. (Propst et al. 2003). La inulina y los oligosacariodos, resisten la digestión en el tracto gastro intestinal superior ,estando disponibles como sustrato para la microflora (Roberfroid et al. 1998) y muestran aumentar el crecimiento de bífido bacterias y lactobacillus, pero inhiben el crecimiento de Escherichia Coli y salmonella en el intestino , estos fructanos han demostrado disminuir el olor de los componentes fecales, reducir el colesterol en suero, aumentar la absorción de nutrientes y estimular el sistema inmune (Jenkins, et al. 1999). La suplementaciòn en las dietas para pollos de algunos oligosacáridos no digestibles identificados como prebióticos cambian las proporciones y las características de las bacterias residentes (Gibson & Roberfroid, 1995).

La estabilidad de la microflora intestinal deberá minimizar los desordenes entericos y estará asociada con la alta productividad. Se debe tener en consideración los efectos que los nutrientes tienen sobre la microflora intestinal, a saber; proteínas y aminoácidos, energía resultante del almidón o la grasa, fibra, niveles minerales en la dieta y balance electrolítico. La reducción de los costos esta siendo de mayor interés para el productor de aves. Sin embargo, la sustitución de componentes en la dieta con una alternativa de bajo costo requieren

de la evaluación no solamente del rendimiento de las aves y las características de la canal, sino también del resultado de la calidad de la carne (Poste. L.M., 1990). La calidad puede ser definida como la suma de demandas del consumidor con respecto a los comestibles (Woodward and Wheelock, 1990). De acuerdo a Wrick (1995), lo que esperan los consumidores de la carne es que sea saludable, rica en proteínas, baja en grasa, tierna y que tenga un sabor y color típico. Las recomendaciones dietéticas favorecen el consumo de menos grasas saturadas (Ruiz. et al., 2001). Por estas razones, un aumento de grasas insaturadas en la carne de pollo puede ser un alimento directamente que beneficia al consumidor. El porciento de crecimiento es el más importante para establecer programas productivos para la crianza de pollos, para ahorrar tareas y alimento permitiendo así la producción anual de pollos, minimizando así los costos de producción (Yusrizal and Chen T.C. 2003). La inclusión de Oligofructuosa de 0.375 % producen aves pesadas de 47 días, mejora el porciento del peso de la pechuga y disminuye la grasa de la misma. El uso de los prebióticos y probióticos en dietas para humanos ha dado esperanzas a los investigadores que los efectos vistos en humanos puedan ser vistos también en aves Brighenti et al, 1999 y Davinson et al. 1998 ha mostrado una reducción de los niveles de colesterol en suero de humanos consumiendo inulina. El efecto de disminuir el colesterol en hámster (Trautwein et al, 1998) y perros han sido mostrados también (Diez et al, 1998). Los cerdos alimentados con probióticos muestran un nivel bajo significativo de colesterol en suero (Gilliland et al 1985). Para medir y cuantificar las características y atributos que contribuyen a la buena calidad de un producto alimentario terminado o ingrediente modelo, las cuales son percibidas por los sentidos humanos, se utiliza la evaluación sensorial que es una técnica de medición y análisis siendo tan importante como los métodos químicos, físicos o microbiológicos, resultando muy útil y decisiva a la hora de lanzar un nuevo producto al mercado de alimentos. (Ibáñez, 2001). Este tipo de análisis tiene la ventaja de que la persona que efectúa las mediciones lleva consigo sus propios instrumentos de análisis, o sea sus cinco sentidos.

Definición del problema

Como influirá la inulina a diferentes concentraciones en la dieta del pollo de engorda en la calidad de carne

Justificación

La necesidad de contar con alimentos de origen animal inocuos para el consumo humano, marca la pauta para la investigación y desarrollo de medios para lograr una mejor calidad alimenticia que requiere un mercado cada más exigente. El énfasis esta ahora en que los gerentes de las granjas adopten un enfoque proactivo hacia la salud intestinal, incluyendo la detección temprana de la enfermedad y su pronto tratamiento junto con un énfasis en el sólido manejo del medio ambiente.

La presión que existe para eliminar el uso de los promotores de crecimiento de la producción avícola debido a un riesgo percibido sobre la eficacia de los antibióticos, implica la urgencia de la necesidad de contar con estrategias alternativas para controlar los problemas infecciosos y mejorar sus parámetros productivos. Se ha logrado un cierto éxito en este ámbito, pero algunas de las estrategias alternativas han tenido efectos negativos en materia económica y de bienestar de los animales.

Los métodos de producción de pollos sin la necesidad de emplear productos químicos tales como promotores de crecimiento y antibióticos, asegurara el acceso de los productos avícolas a la mayor gama posible de mercados.

La solución al problema debe tomar en consideración los aspectos económicos, el bienestar de las aves, la factibilidad de aplicación en la práctica y el deseo que tienen los consumidores de adquirir productos libres de compuestos químicos. Esto puede incluir la aplicación de prácticas novedosas de manejo y nutrición. El abuso y mal manejo de los antibióticos, tiene como consecuencia que se encuentren residuos de estos en los tejidos de las aves y al mismo tiempo estos residuos lleguen al consumidor.
En términos de producción podemos esperar un pollo más pesado y magro, con mejor conversión de alimento , siendo un pollo mas natural esta es una de las estrategias a seguir para la utilización de inulina que es una de las tantas alternativas para suplir la utilización de antibióticos y promotores de crecimiento en pollos de engorda, desafortunadamente este producto no ha sido ampliamente estudiado existiendo grandes espacios vacíos sobre el conocimiento y la aplicación de la inulina en los procesos digestivos del pollo y de la calidad de la carne.

OBJETIVOS GENERAL Y ESPECIFICOS

Objetivo General

La adición de inulina en la dieta del pollo de engorda mejorara la calidad de la carne y disminuirá la grasa de la canal.

Objetivos Específicos

Evaluar el potencial de la inulina sobre la calidad de la carne.
Evaluación sensorial de la carne de pollo

MATERIAL Y METODOS

Diseño de estudio

Se trata de un estudio experimental, prospectivo, longitudinal y comparativo que se realizo en el Centro de Bachillerato Tecnológico Agropecuario y el Departamento de Nutrición Animal del Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán " en el cual se utilizaron un total de100 pollos para

engorda (*Gallus gallus*), de la estirpe Ross, machos. de los cuales se formaron cuatro grupos de la siguiente manera:

Grupo 1 en la dieta incluirá 0.1% de inulina.
Grupo 2 en la dieta incluirá 0.2 % de inulina.
Grupo 3 en la dieta incluirá 0.4% de inulina.
Grupo 4 en la dieta incluirá una dieta estándar para pollo de engorda (grupo testigo).

Los pollos fueron alimentados desde los 0, a 46 días con las dietas experimentales, se les proporciono el alimento y agua a *ad libitum.* El muestreo consistió en sacrificar a los pollos por dislocación cervical y extraer pierna, muslo y pechuga, la toma de muestra se realizo el día 46 de edad del pollo.
Las muestras permanecieron en refrigeración al momento de su obtención, para después llevar a cabo la evaluación sensorial.

Cálculo del tamaño de la muestra.

El tamaño de la muestra se determino utilizando la siguiente formula.

$$n = (z2\ \sigma 2\ )/d2$$

Donde:
n = número de muestra.
z = valor de $\alpha/2$ (0.05) bajo la curva.
σ = desviación estándar esperada.
d = margen de error esperado.

Mecanismos de asignación del tratamiento.

- Las dietas de los pollos se formularon de la siguiente manera:
- 23 % de proteina cruda
- 3.2 mcal de EM para los cuatro grupos con la variante de la inclusión de la inulina.
- Las dietas se formularon de acuerdo al NRC. 1994. Nutrient Requirements of Poultry.
- Criados en piso con cama de viruta de pino. La temperatura de recepción del pollito fue de 34°C , los días 1, 2 y 3, 32°C del día 4 al 7 30°C y de 8 al día 16 28°C. El día 18 al 46 la temperatura fue de 25 ° C. Se utilizara un programa de iluminación de 23 hrs. de luz continua por 1 de oscuridad las primeras dos semanas y para la tercera semana de iluminación natural hasta el final del estudio.

CRITERIOS: a) Inclusión, b) exclusión y c) eliminación.

a) Inclusión.
Pollos sin signos aparentes de enfermedad
Pollos con desarrollo morfológico de acuerdo a su especie
Pollos con comportamiento de acuerdo a los de su especie.

b) Exclusión
Pollos con signos aparentes de enfermedad
Pollos sin un desarrollo morfológico de acuerdo a la especie
Pollos con un comportamiento anormal, no de acuerdo a su especie.

c) Eliminación
Pollos que hayan muerto durante el experimento por cualquier causa.

Variables a medir
Variables principales

- Color, olor, apariencia y sabor
- Cualidades sensoriales de la carne de pollo en :
- Muslo
- Pechuga
- Pierna.

EVALUACION SENSORIAL DE LA CARNE DE POLLO

Se utilizo una prueba afectiva que se realizo con consumidores (personas no entrenadas en técnicas sensoriales) y en condiciones que no fueron ajenas o extrañas para utilizar o consumir el producto en estudio. La evaluación sensorial se realizo en el Laboratorio de Evaluación Sensorial del Departamento de Tecnología de Alimentos de la Dirección de Nutrición, por el método propuesto por Pedrero y Pangborn 1989, se midió de acuerdo a una escala hedónica, mediante cuatro niveles de aceptación a 30 jueces (por cada corte de carne) no entrenados (muy agradable, agradable, indiferente desagradable), se evaluó color, olor, sabor y apariencia de los diferentes cortes (pechuga, muslo y pierna). Se utilizaron 8 pollos de cada tratamiento y de cada pieza, cocida y deshuesada colocándose una porción en platos identificados con los diferentes tratamientos para la degustación de los jueces.

RESULTADOS Y DISCUSIÓN

El nivel de aceptación de apariencia para pierna T3, T5, T2, T4 y T1, (grafico 1), pechuga (grafico 2) en orden descendiente fue: el T1, T4, T2, T3 y T5, en muslo no se encontró diferencia. Con respecto al color en pierna el de mayor aceptación T5, T3, T2, T4 y T1, (grafico 3), en pechuga fue primero T1, T4, T2, T3 y T5, (grafico 4), en muslo no se encontró diferencias. Para olor en ninguna de las piezas se encontró diferencia. En el sabor de la pierna de mayor a menor la

preferencia fue T1, T4, T3, T2 y T5. (grafico 5), Para el muslo la preferencia fue de T3, T4, T1, T2 y T5, (grafico 6), para la pechuga no se encontró diferencia. Se puede concluir que con excepción de de la pierna en cuanto a color, los consumidores prefirieron pollos que no contenían promotores de crecimiento y pigmentos.

Gráfico 1

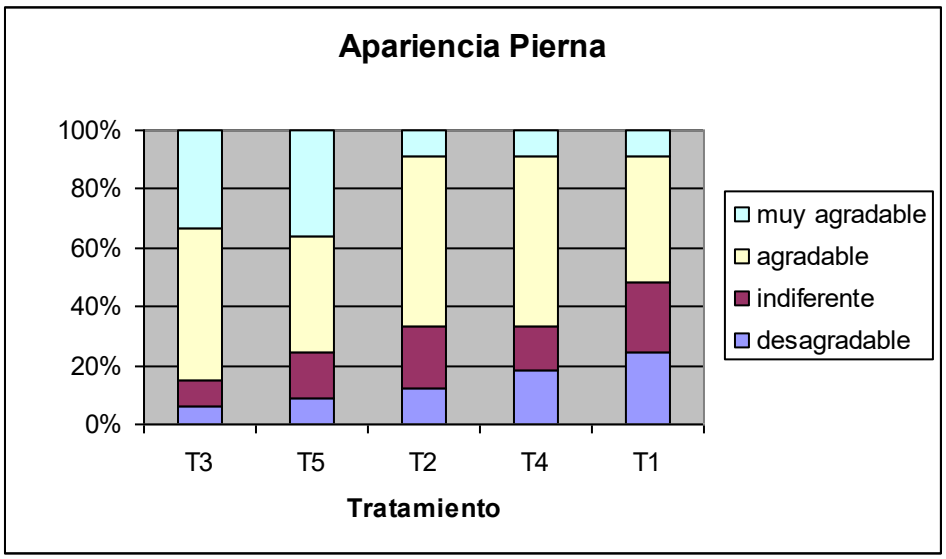


Gráfico 2

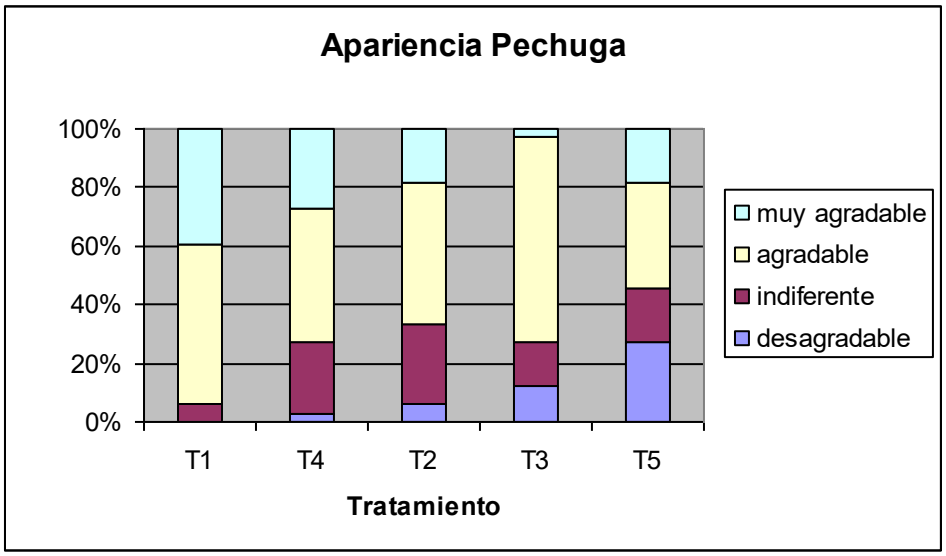


Gráfico 3

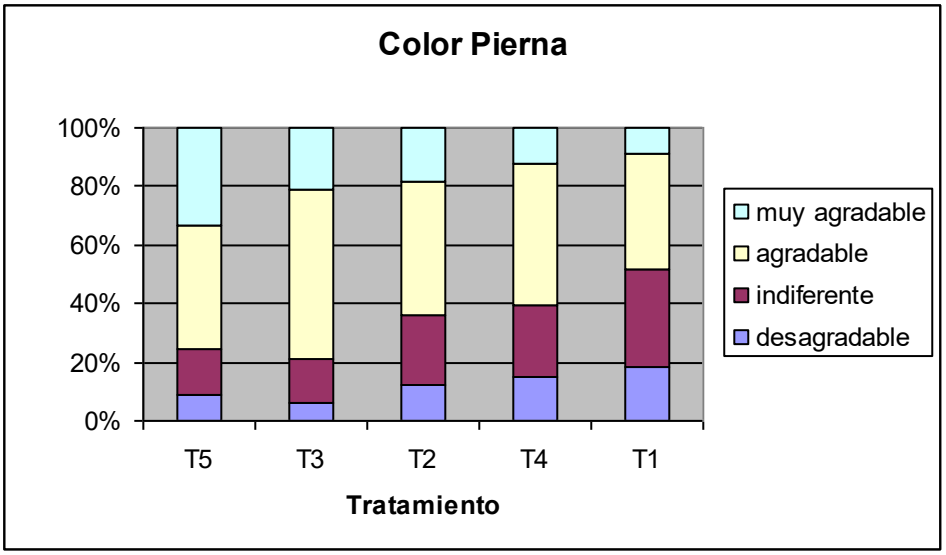


Gráfico 4

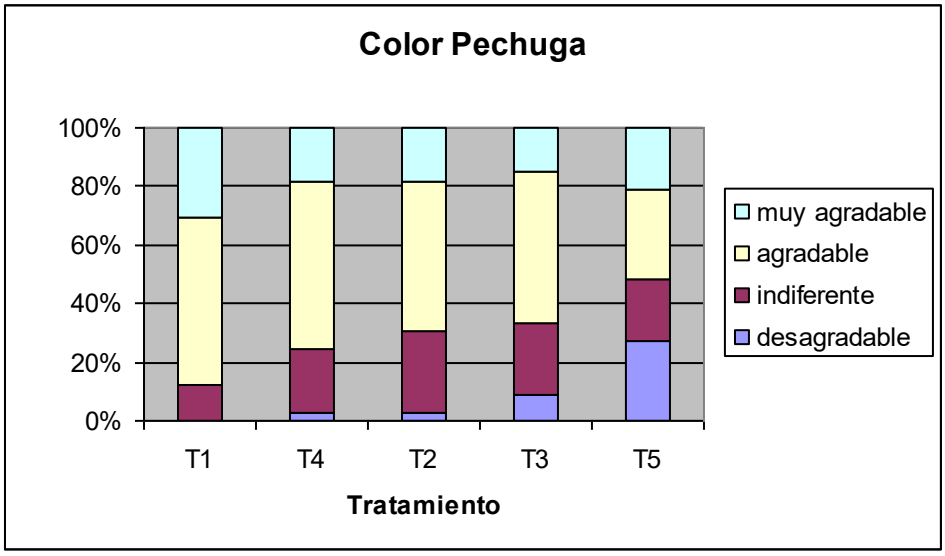


Gráfico 5

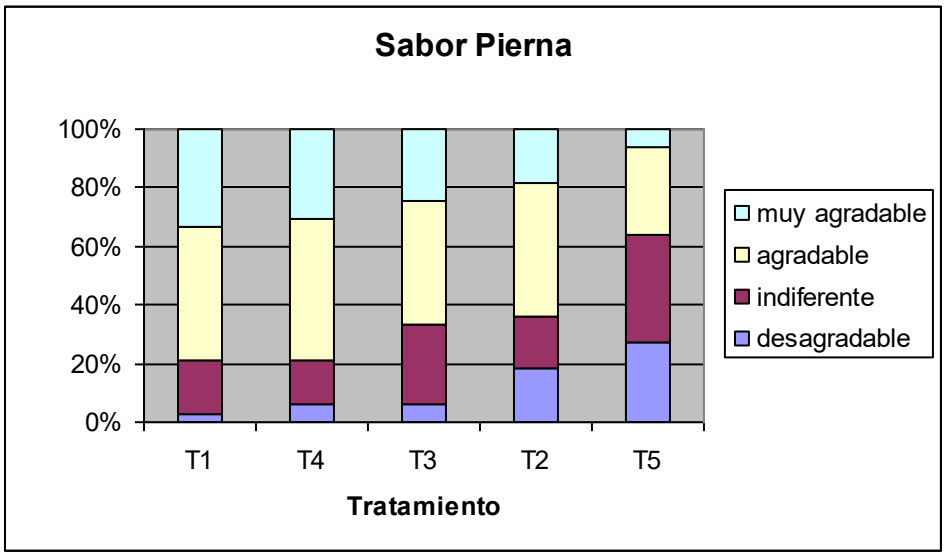
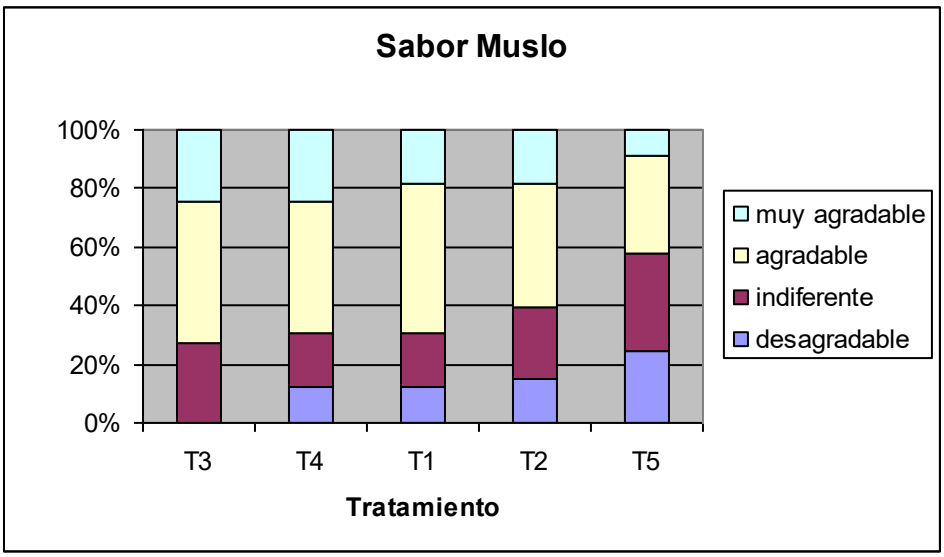


Gráfico 6



ANÁLISIS ESTADÍSTICO

Se analizaron los datos de la evaluación sensorial empleando un diseño completamente aleatorizado, con la prueba no parametrica de Kruskan-Wallis, del programa de SPSS 6.1.

Beneficios esperados

Como ya se menciono anteriormente, la eliminación de promotores de crecimiento en las dietas de pollos y la inclusión de la inulina en las dietas supone una innovación, puesto que al estudiarse los efectos de esta ultima tendrá efectos positivos en la calidad de la carne de pollo, para ofrecer al consumidor un alimento rico y nutritivo para buenas practicas de su alimentación.

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Effect of a *Lactobacillus* spp-Based Probiotic and a Prebiotic on Turkey Poult Performance With or Without *S. enteritidis* Challenge

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Abstract

To evaluate the effect of a *Lactobacillus*-based probiotic culture (LPC) for the first three consecutive days in combination with lactose on feed as prebiotic, four trials were performed. Two treatments were included in these studies: LPC [dietary lactose (0.1%) continuously on feed and probiotic culture (~10^6cfu/mL) in the drinking water] and untreated controls. Due to the high variability in body weight, 100 poults were weighed and only those with body weight between the mean ± 1 STD were included in the experiment. Poults were placed in a Biosecurity level II isolation room that had been disinfected and provided with fresh softwood shavings. Three hundred and twenty selected poults in each trial were tagged, challenged with ~10^4 cfu of *S. enteritidis* (trials 1 and 2), and randomly divided in two treatments with 4 replicates each one (n= 40 poults). Body weight (BW) was evaluated on days 1, 7, and 14 in trials 1, 2, and 3 and at day 1, 8, and 18 for trial 4. In *Salmonella* challenged poults, a significant increment ($P<0.05$) in body weight was observed in trial 1, and trial 2 at 7 and 14 days. Feed conversion ratio (FCR), calculated at the end of the experimental period, was significantly ($P<0.05$) improved in the LPC group compared to the control group in both trials (1 and 2). In contrast, unchallenged turkey poults (trials 3 and 4) showed no difference ($P>0.05$) in either body weight or FCR at the different evaluation times. These data suggest that dietary lactose with appropriate probiotic organisms may enhance performance of poults when they are challenged with a mild dose of *Salmonella.*

Description of the problem

The poultry industry produces a high nutritive food for human consumption in a very short period of time. To maximize the genetic potential of broiler chicks for production, they need to be in a healthy status as well as having diets that meet their requirements for optimal production. The use of probiotic cultures in the poultry industry for pathogen control and chick performance enhancement has gained attention recently due to the increasing restriction for use of antibiotics as growth promoting agents. A probiotic organism like those of the genera *Lactobacillus, Pediococcus,* and *Bifidobacterium,* among others, is a live microorganism that exerts a beneficial effect on the host by enhancing immune response, nutrient absorption, and pathogen control. On the other hand, a prebiotic is defined as food ingredient that selectively stimulates the growth and the activity of a particular bacterial community in the gastrointestinal tract (GTI) but that is also resistant to the digestive process undergoing in the GIT allowing to bypass the upper gastrointestinal section and be used by the beneficial bacteria in the lower sections of the GIT where they colonize.

The objective of this study was to evaluate the effect of a commercially available *Lactobacillus spp*-based probiotic culture in combination with dietary lactose (0.1%) as prebiotic, on turkey poults performance with or without *Salmonella challenge* at day of hatch.

Material and Methods

Salmonella challenge

A poultry isolate of *S. enteritidis* (Phage type 13A) selected for resistance to Novobiocin-nalidixic acid (NO/NA) antibiotics was grown in Tryptic soy broth (TSB) for approximately 24h with passes in fresh media every 8h. Cells of the last pass were washed three times in 0.9% sterile saline by centrifugation (3000x*g)*, and the concentration of viable cells of the inoculum was determined spectrophotometrically. The stock solution was serially diluted and confirmed by colony counts on replicate samples (0.1mL/ replicate) that were spread plated on Xylose Lysine Deoxycholate (XLD) Agar plates containing 25µg/mL NO and 20 µg/mL NA. For *Salmonella* recovery evaluation, a method previously described was used [1].

Turkey Poults

One-day of hatch commercial cross turkey poults were obtained from a commercial hatchery and allocated randomly in floor pens with new pine shavings litter. Due to the high variability in body weight, 100 poults were weighed and three hundred twenty poults with body weight between the mean ± 1 standard deviation were included in the experiment in each trial. Antibiotic-free feed formulated to meet or exceed NRC recommendation for critical nutrients for day-of-hatch poults [2] and water *ad-libitum* were provided on both experiments according to the experimental design.

Experimental design

In each trial, three hundred and twenty turkey poults were placed in a isolation room of the Poultry Health Laboratory of the University of Arkansas-Fayetteville. Poults were randomly grouped into two treatments with 4 replicates each one (n=40 poults). Before placement, poults were challenged with *S. enteritidis* (~10^4 cfu) by oral gavage in trials 1 and 2. Turkey poults in trial 3 and 4 were not *S. enteritidis*-challenged. The treated group (LPC) received a probiotic culture for the first three consecutive days and dietary lactose (0.1%) as prebiotic continuously on the feed whereas the untreated group received plain water and feed. Body weight was registered at days 1, 7, and 14 for trials 1, 2 and 3, and at days 1, 8, and 18 for trial 4. Feed consumption was recorded during the experimental period in order to evaluate feed conversion ratio at the end of each trial.

Probiotic administration

Commercially available *Lactobacillus* spp-based probiotic (IVS/WYNCO) was ten-fold diluted in fresh MRS broth to have 10^8 cfu/mL. Thirty-five milliliter of this dilution were added to a 3,430mL

water along with 35mL of skim milk to stabilize the culture. Fresh treated water with a final concentration of ~10^6 cfu/mL was provided daily for the first three days of the experiment. A sample of the culture was ten-fold diluted for enumeration on MRS agar plates.

Statistic analysis

Differences between groups of body weight and feed conversion rate were determined by one-way analysis of variance using the General Linear Models procedure. Significant differences ($P<0.05$) were further separated using Duncan's multiple range test and commercial statistical analysis software [3].

Results and Discussion

To establish and maintain a successful infection, microbial pathogens have to evolve a variety of strategies to invade the host, avoid or resist the innate immune response, damage the cells, and multiply the specific and normally sterile region [4]. The establishment of a pathogen affects the ability of the host to absorb dietary nutrients that are reflected at the end of the growout period as poor performance of the flock. Recently, our laboratory has been working in the isolation of probiotic organisms mainly from healthy adult chickens and their evaluation as the ability to inhibit the growth of pathogenic organisms under *in vitro* and *in vivo* studies with promising results (Unpublished data from our Laboratory). Several studies have been conducted with these organisms to control *Salmonella* infection but not on turkey poults performance.

The results of these studies (Table 1) indicate that the administration of a selected probiotic culture (~10^6cfu/mL) during the first three consecutive days of life and low concentration (0.1%) of lactose as prebiotic continuously on feed during the experimental period significantly increased ($P<0.05$) the body weight of challenged turkey poults at days 7 (trial 1: LPC=175.5±1.62g vs. Control=163.3 ±3.1.97g; trial 2: LPC= 104.3±1.25g vs. Control=96.1±1.23 g) and 14 (trial 1: LPC= 382.3 ± 3.58g vs. Control= 344.4 ± 3.60g; trial 2: LPC= 226.8 ± 2.69g vs. Control=206.1 ± 2.68g).

Although there was no difference in feed intake, feed conversion ratio was significantly improved ($P<0.05$) in the treated group compared to the untreated group (Trial 1: LPC= 1.772 ± 0.065kg *vs*. Control= 2.022 ± 0.087kg; trial 2: LPC=1.967 ± 0.042kg. *vs.* Control= 2.102 ± 0.033kg.). Further, unchallenged turkey poults showed no difference in any of the variables evaluated, body weight and FCR, at the end of the each experimental period (day 14 in trial 3 and day 18 in trial 4).

Several papers have been published about the beneficial effect of using dietary [1, 5] or soluble lactose in feed [6] at different concentrations on *Salmonella* infection. *Salmonella* reduction was associated with an increase concentration of acetic, propionic, lactic, and butyric acid in the ceca [1,6], which is a primary place of *Salmonella* multiplication. Gulsen *et al.,* (2002) reported that the inclusion of 2.5% lactose and 3.8% of dried whey during a growth out period increased

performance of broiler chick and this was associated with an increase in intestinal villi length during the started period that favored nutrients absorption.

On the other hand, competitive exclusion and probiotic cultures have shown to reduce *Salmonella* colonization in turkey poults [7] and improve turkey poults performance [8]. The combination of a probiotic culture and different prebiotic has been reported to improve broiler chicks performance [9,10]. Higgins et al. (2005)'s results showed a beneficial effect of the probiotic culture when poults were under exposure of a moderate *Salmonella* infection under commercial condition and this is in agreement with our observation. Our results may suggest that the probiotic culture in combination with a prebiotic reduced the impairment of the intestinal wall that favored the nutrient absorption; however more studies need to be conducted to evaluate our hypothesis.

Consclusions and applications

1. The combination of a *Lactobacillus* spp-based probiotic culture and lactose probiotic (0.1%) continuously on feed improved body weight performance and FCR in *Salmonella*-challenged turkey poults.
2. These results suggest that ingestion of lactic acid bacteria may help to reduce colonization of pathogens in the GIT.
3. Lactose as prebiotic may help keep high number of lactic acid bacteria in the gastrointestinal tract to better exert their beneficial effects.

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Table1. Effect of a *Lactobacillus* sp-based probiotic culture combined with Lactose (0.1%) prebiotic on performance of turkey poults challenged with or without *Salmonella enteritidis* (~10^4 cfu) at day of hatch.

			Body weight ± Standard error (g)*			**FCR (Kg)***
Trial Number	**Treatment**	***S. enteritidis* challenge**	**D1**	**D7**	**D14**	**D1-D14**
Trial 1	Control	Yes	56.3 ± 0.18 a	163.3 ± 1.97 b	344.4 ± 3.60 b	2.022 ± 0.087 a
	LPC	Yes	56.3 ± 0.19 a	175.5 ± 1.62 a	382.3 ± 3.58 a	1.772 ± 0.065 b
Trial 2	Control	Yes	50.8 ± 0.23 a	96.1 ± 1.23 b	206.1 ± 2.68 b	2.102 ± 0.033 a
	LPC	Yes	50.7 ± 0.24 a	104.3 ± 1.25 a	226.8 ± 2.69 a	1.967 ± 0.042 b
Trial 3	Control	No	58.9 ± 0.23 a	156.4 ± 1.35 a	363.1 ± 3.28 a	1.500 ± 0.045 a
	LPC	No	58.0 ± 0.23 a	151.7 ± 1.21 b	361.4 ± 3.43 a	1.525 ± 0.024 a
			D1	**D8**	**D18**	**D1-D18**
Trial 4	Control	No	51.3 ± 0.31 a	138.2 ± 1.49 a	356.2 ± 6.29 a	1.614 ± 0.056 a
	LPC	No	51.2 ± 0.28 a	134.9 ± 1.35 a	352.6 ± 3.70 a	1.681 ± 0.018 a

* Data with different superscript in the same column and trial indicates statistic difference ($P<0.05$).

EVALUACION DE DIETAS BAJAS EN PROTEINA PARA POLLOS DE ENGORDA

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Con el objeto de evaluar el comportamiento productivo, el rendimiento en la canal, grasa abdominal y la pigmentación de la piel de pollos de engorda, al ser alimentados con dietas bajas en proteína. Se utilizaron 312 pollitos mixtos, de 1 día de edad. Los pollitos se distribuyeron en 12 lotes de 26 aves cada uno. Los 3 tratamientos fueron con dietas tipo prácticas sorgo + pasta de soya, iniciación, crecimiento y finalización (testigo) y dietas con una disminución de la proteína cruda en 2.5 puntos porcentuales, para cada etapa, pero con contenidos similares de lisina, metionina + cistina y treonina para cada tratamiento; así mismo, otro tratamiento fue similar al anterior menos 60 Kcal/Kg de EM en la dieta. Se empleó un diseño completamente al azar con 3 tratamientos, cada. Se llevaron registros de parámetros productivos. Al final del estudio, se midió el rendimiento en canal, la pigmentación amarilla de la piel en frió y finalmente se pesó la grasa abdominal. Los resultados En ganancia de peso, conversión alimenticia, rendimiento en canal y pigmentación amarilla de la piel no existió diferencia ($P>0.05$) entre tratamientos. Para consumo de alimento, este fue mayor ($P<0.05$) en el tratamiento en donde las dietas además de tener 2.5% menos de proteína, tenían 60 Kcal de EM menos por Kg de alimento. Sin embargo, la grasa abdominal fue ligeramente mayor ($P<0.08$) en las aves alimentadas con las dietas bajas en proteína. Se concluye que la formulación en base aminoácidos, empleando los aminoácidos sintéticos lisina, metionina y treonina permite reducir en 2.5% el nivel de proteína de las dietas para pollos de engorda.

Palabras Clave: Aminoácidos, Pollos, Dietas bajas, proteína

Efecto de la presencia de micotoxinas sobre la vacunación contra la coccidiosis aviar

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La nutrición animal, en gran parte, se basa en el consumo de granos y sus derivados, estos son cosechados todo el año bajo condiciones climáticas diversas lo que afecta la calidad de los productos finales al ser invadidos por hongos tanto en campo como en el almacén (la bodega, el silo). Dentro de las principales especies de hongos productores de micotoxinas se encuentran los géneros *Aspergillus* sp y *Penicillium* sp. El efecto que se puede observar en los animales por la presencia de micotoxinas son: necrosis hepática y cambios degenerativos e inducción de neoplasias. En aves se producen diversas reacciones que van desde una mala absorción de nutrimentos, coagulopatía, retraso en el crecimiento, vulnerabilidad a las infecciones e incapacidad para reaccionar a la vacunación. En el presente estudio se evaluó el efecto de las aflatoxinas y las ocratoxinas en aves que fueron vacunadas contra la coccidiosis aviar. Se utilizaran 64 aves de un día de edad, estirpe Ross para aplicar 8 tratamientos. El trabajo experimental tuvo una duración de 28 días, el consumo de agua y alimento fue *ad libitum*. Las aves fueron vacunadas al día de edad y desafiadas al día 21 de edad con 10,000 ooquistes por ave. Las variables a evaluar fueron peso, consumo de alimento, conversión alimenticia, cantidad de ooquistes por gramo de heces y transaminasas sanguíneas. Las alteraciones intestinales macroscópicas se evaluaron según el método de Johnson y Reid al día 28. Las aves que consumieron alimento con aflatoxina tuvieron un menor desempeño productivo. Sin embargo, las aves que consumieron alimento con Ocratoxina y que fueron vacunadas y desafiadas fueron las que presentaron mayor lesión intestinal y eliminación de ooquistes, al compararlo con las aves de los tratamientos control, vacunado y desafiado sin micotoxinas ($P>0.05$).

Palabras claves: coccidiosis; *Eimeria*; aflatoxina; ocratoxina.

MORTALIDAD SÚBITA EN POLLO DE ENGORDA ASOCIADA A ASPERGILOSIS

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INTRODUCCIÓN

La Aspergilosis es una enfermedad de origen micótico ocasionada por hongos del genero *Aspergillus*. La Aspergilosis aguda se caracteriza por brotes intensos en aves jóvenes con alta morbilidad y mortalidad. El blanco primario de este agente es el aparato respiratorio (neumonía caseosa), aunque también puede afectar otros órganos como el sistema nervioso (necrosis caseosa cerebral y cerebelar) y los órganos de los sentidos particularmente ojos (oftalmitis caseosa). El sistema respiratorio de las aves es muy sensible a factores ambientales e infecciosos y como secuela de daño a este sistema pueden observarse casos de ascitis.

El presente trabajo, tiene como objetivo describir el impacto de un caso de Aspergilosis y ascitis en pollos de engorda estirpe Ross machos.

DESCRIPCIÓN DEL CUADRO CLÍNICO

El presente caso se presentó en pollos de engorda estirpe Ross machos, en un núcleo de 147,344 aves. Dicho núcleo estaba conformado por cinco casetas tres de machos y dos de hembras, la cantidad de aves alojadas por caseta de 29,200-29,500.A los tres días de edad se presentó un incremento en la mortalidad del 0.2 % en las casetas de machos, misma que se elevó alcanzando 1.6 % de mortalidad diaria. Los signos clínicos fueron principalmente de tipo nervioso observándose incoordinación, movimientos de carrera, temblor de cabeza y tortícolis, con el paso de los días otro de los signos fue la presencia de ascitis, la cual presentaban del 20-30% de las aves afectadas, dicho signo se hizo evidente después de la primera semana de edad y acentúo después de la tercera semana de edad. Al final del ciclo en el caso de las hembras la mortalidad acumulada fue de 6.6% promedio y en el caso de los machos del 40.6% promedio.

HALLAZGOS MACROSCÓPICOS

Se realizaron necropsias en aves con signología nerviosa, clínicamente sanas y aves retrasadas. En el caso de las aves con signos nerviosos ocasionalmente se observaba reblandecimiento de los lóbulos cerebrales y formación de granulomas en la corteza cerebelar, además de pequeños y escasos granulomas en el parénquima pulmonar. Otras lesiones observadas de manera frecuente fueron aerosaculitis fibrinocaseosa, pericarditis fibrinosa, perihepatitis fibrinosa, dilatación ventricular, hidropericardio, edema pulmonar, atrofia hepática, ascitis y dilatación de la vena cava. Se colectaron muestras de órganos para aislamiento bacteriano, viral y micótico; así como, para histopatología.

HALLAZGOS MICROSCÓPICOS

Los resultados de histopatología indicaron encefalitis y neumonía granulomatosa asociada a la presencia de hifas septadas compatibles con *Aspergillus* sp.

HALLAZGOS MICROBIOLÓGICOS

Los resultados de laboratorio fueron negativos a aislamiento bacteriano y viral. En el caso del aislamiento micótico el resultado fue positivo para *Aspergillus* sp.

CRITERIO DIAGNÓSTICO

Con base en los hallazgos histológicos descritos en encéfalo y pulmón aunados al aislamiento micótico, el diagnóstico fue infección por *Aspergillus sp.*

DISCUSIÓN Y CONCLUSIONES

En este caso el curso de la enfermedad fue agudo y se afecto solo un módulo de la granja y particularmente a los machos, por lo que es posible que la vía de entrada del agente haya sido hematógena, tal vez por el uso de algún medio de vacunación contaminado. La presencia de ascitis se considera una lesión secundaria, consecuencia de la hipertensión pulmonar provocada por neumonía granulomatosa.

COCCIDIOSIS AVIAR Y SU EFECTO EN EL CONSUMO DE AGUA EN POLLOS DE ENGORDA

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INTRODUCCIÓN

Las enfermedades que afectan el tracto intestinal de las aves tienen un impacto considerablemente negativo sobre la eficiencia productiva de las mismas en las diversas ramas de la avicultura. El daño que provocan los agentes patógenos a nivel intestinal repercute directamente sobre la absorción de nutrientes; por lo tanto, sobre la ganancia de peso y conversión alimenticia.

El conocimiento de la arquitectura y función intestinal es fundamental para comprender los diferentes mecanismos de diarrea. El intestino esta conformado histológicamente por una capa mucosa, una submucosa, una muscular de la mucosa y una muscular del órgano. La mucosa intestinal esta constituida por criptas intestinales y vellosidades, las células epiteliales que recubren la vellosidad intestinal tienen diferentes funciones y diferentes estadios de maduración, las células epiteliales que se encuentran en las criptas se encargaran de reemplazar a las células que se encuentran a lo largo de la vellosidad una vez maduras. Las células epiteliales que se encuentran en la parte lateral y media de la vellosidad se encargan de la secreción de líquidos y electrolitos y las células epiteliales que se encuentran en la parte apical de la vellosidad se encargan de la absorción de líquidos y nutrientes.

Dependiendo del tipo de agente y del lugar en donde lesione a la vellosidad intestinal serán los efectos que podremos observar en las aves. Por ejemplo, en casos de coccidiosis aviar durante las fases de esquizogonia y gametogonia, dichos protozoarios ocasionan daño directo a las células epiteliales desde las criptas hasta la porción apical de la vellosidad, las células dañadas serán reemplazadas por células epiteliales inmaduras que no tendrán capacidad para absorber nutrientes, pero que si llevaran acabo funciones de secreción de líquidos y electrólitos. Este proceso de regeneración a nivel intestinal se reflejara con un

incremento en la humedad de las heces y/o cama y con un incremento en el consumo de agua asociada a la perdida de líquidos y electrolitos a nivel intestinal.

Los problemas de salud intestinal en el campo se pueden detectar de forma muy temprana si contamos con registros de consumo de agua por día, si valoramos de manera adecuada el estado de las heces y la cama, además de observar de forma periódica la mucosa del tracto intestinal durante las necropsias. La valoración del peso, uniformidad y conversión alimenticia de la parvadas son de gran utilidad; sin embargo, el efecto negativo sobre estos parámetros lo observaremos varios días después de que el problema se presento, por lo que es importante contar con algún indicador que nos de información de forma inmediata.
El objetivo del presente trabajo es mostrar el efecto sobre el consumo de agua y la ganancia de peso durante un caso leve de coccidiosis.

DESCRIPCIÓN DEL CUADRO CLÍNICO

El caso se presento en una caseta experimental de 2000 pollos de engorda, estirpe cobb, machos. En dicha caseta las aves se encontraban alojadas y divididas en 20 corrales con 100 aves cada uno. El caso se caracterizo por un incremento marcado en la humedad de la cama y las heces, acompañado de un incremento del 50% en el consumo de agua a partir de los 28 días de edad en todos de los corrales. En el cuadro 1 se puede observar el consumo de agua por día promedio por ave.

Cuadro 1. Consumo de agua en pollos de engorda ml por ave por día sin y con problemas de cama y heces húmedas							
Edad (días)							
Parvada	7	14	21	28	35	42	49
A*	47	145	157	174	300	381	400
B**	34	200	210	400	540	540	400
*Parvada con heces aparentemente normales y cama seca **Parvada con cama y heces húmedas							

En relación al peso en el cuadro 2 se muestran los pesos semanales de las aves de aves sin y con problemas de cama y heces húmedas.

Cuadro 2. Ganancia de peso semanal (g) en pollos de engorda sin y con problemas de cama y heces húmedas.						
Edad (días)						
Parvada	7	14	21	28	35	42
A*	162	384	677	1082	1794	2443
B**	172	405	726	1150	1668	2465
*Parvada con heces aparentemente normales y cama seca **Parvada con cama y heces húmedas						

DIAGNÓSTICO PARASITOLÓGICO

A los 35 días de edad cuando la cama se veía totalmente apelmazada y húmeda se colectaron muestras de heces para diagnóstico parasitológico, el conteo de ooquistes por gramo de heces fue de 1,000-9,100 ooquistes por gramo de heces; siendo 1,000 el conteo mas bajo encontrado y 9,100 el más alto. En el conteo diferencial de ooquistes por especie el 62.08% correspondían a *Eimeria acervulina*, el 37.36% a *Eimeria tenella* y el 0.56% a *Eimeria maxima.* En ese momento se decidió administrar un tratamiento con sulfonamidas durante 3 días y volver a realizar un 2º. muestreo de heces un día después del termino del tratamiento. En las muestras de heces evaluadas posterior al tratamiento los conteos de ooquistes eran de 50-500 por gramo de heces.

EVALUACIÓN MACROSCÓPICA DEL INTESTINO

Se realizo la evaluación macroscópica del tracto intestinal a los 28, 35 y 42 días los hallazgos mas relevantes fueron la presencia de abundante cantidad de líquido en el primer tercio del intestino, además de lesiones +1 y +2 de *Eimeria acervulina*.

CRITERIO DIAGNÓSTICO

La evidencia de camas y heces húmedas, el incremento en el consumo de agua, la presencia de líquido en el tracto intestinal, la presencia de lesiones +1 y +2 de *Eimeria acervulina* aunado a los conteos de ooquistes de *Eimeria* sp en heces, podemos concluir que el cuadro clínico descrito correspondió a un caso de coccidiosis leve.

DISCUSIÓN Y CONCLUSIONES

El agua es un nutriente vital y se encuentra involucrada en diversos procesos metabólicos, su consumo es influenciado por numerosos factores. El contar con registros diarios del consumo de agua es de gran utilidad para el médico, no solo porque el agua sea una ruta común para la aplicación de vacunas y tratamientos; siendo importante conocer el consumo de agua para estimar la cantidad de la misma en la que se aplicará una vacuna o bien para determinar la forma en la que se realizará algún tratamiento, si no también porque un incremento o decremento en el consumo de agua puede ser indicativo de problemas de salud. Como se pudo observar en este caso en la parvada B el incremento en el consumo de agua, aunado a la presencia de cama y heces húmedas fueron un indicador de que existía un problema. En este caso aunque los conteos de ooquistes fueron bajos y las lesiones intestinales leves, esto repercutió sobre el peso a los 35 días de edad. Al comparar los pesos obtenidos entre una parvada y otra, la parvada A (1794g) gano 126g mas que la parvada B (1668g) y aunque al final la parvada B supera a la A por 22g, es importante mencionar que la productividad de las aves se mide en horas y cada minuto que el médico tarde en implementar un plan terapéutico ya sea para un problema digestivo, respiratorio, renal etc., las aves dejan de ganar gramos de peso. Debido a esto es importante contar con métodos prácticos que nos permitan valorar la salud de las aves, con la finalidad de establecer un diagnóstico rápido y oportuno.

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Evaluación de diferentes programas de administración de una vacuna comercial contra coccidiosis en pollo de engorda

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INTRODUCCIÓN

La coccidiosis aviar (CA) es la parasitosis de mayor impacto económico (McDougal y Reid, 2003) en la avicultura mundial. Para su prevención y control se ha contado con una gran diversidad de procedimientos, entre ellos el uso de productos químicos y de fermentación bacteriana (Peek et al 2006, Mathis et al, 2006). Sin embargo, debido al rápido desarrollo de cepas resistentes a las drogas anticoccidiales y a la creciente presión de los consumidores para eliminar el uso de fármacos en la crianza de animales para consumo humano, la vacunación es una herramienta en el control de la coccidiosis aviar y su uso se ha incrementado de manera importante en el sector productor de pollo. Las características de pigmentación cutánea del mercado mexicano han limitado el desarrollo de esta herramienta, básicamente por la asociación del efecto colateral sobre la pigmentación que en algunos casos resulta del empleo y manejo inadecuado de la vacuna. Debido a lo anterior, el objetivo de este estudio fue evaluar diferentes vías y tiempos de administración de una vacuna comercial y su protección al desafió en pollos de engorda, mediante la medición del peso corporal, unidades colorimétricas del pigmento amarillo en piel, el número de ooquistes excretados por gramo de heces y el Índice anticcocidial.

MATERIAL Y MÉTODOS

Animales de experimentación. Se utilizaron 162 pollitos de engorda de la estirpe Ross de un día de edad, provenientes de una incubadora comercial que fueron alojados en corrales en piso dentro de las unidades de aislamiento del Departamento de Producción Animal: Aves (DPA: Aves) de la FMVZ de la UNAM, con una duración de 42 días. Se registró el peso corporal de todos los pollos en cada grupo

semanalmente a partir del día 1 de edad. Mientras que la mortalidad se registró diariamente por réplica y grupo.

Alimento. Se administró agua y alimento *ad libitum*, manejando dos fases de alimentación: del día 1 al 21 de edad se les proporcionó alimento iniciador con 21% de proteína cruda (PC), 3000 kilo calorías (kcal) de energía metabolizable (E.M.) y 40 ppm de xantofilas. El alimento finalizador se les proporcionó del día 22 de edad al final de la prueba, con 18% de PC., 3200 kcal de E.M. y 85 ppm de xantofilas.

Ambas fases fueron elaboradas sin coccidiostato y sin promotor de crecimiento, con excepción del grupo VII que recibió 60 ppm de salinomicina durante toda la prueba.

Vacuna. La vacuna tenía un título de 750, 000 ooquistes / ml, con una esporulación del 78% y contenía las siguientes especies: *E. acervulina* (17%)*, E. maxima* (13%)*, E. tenella* (42%) *y E. praecox* (28%). La dosis por pollo fue de 1,570 ooquistes esporulados y viables.

Inóculo de desafío. Al día 21 de edad todas las aves de los grupos I al VII fueron desafiadas en el alimento con un inoculo mixto que contenía 290,000 ooquistes, de los cuales el 73% eran esporulados. El inóculo estaba compuesto por cepas patógenas de campo de *E. acervulina* (23%)*, E. maxima* (27%)*, E. tenella* (12%) *y E. praecox* (38%).

Evaluación del pigmento cutáneo. El pigmento se evaluó con el colorímetro de reflectancia Minolta CR-300 (Minolta, Co. Osaka, Japón) a los 42 días de edad en la zona apterica lateral derecha en 10 pollos de cada grupo.

Aspecto físico de las heces. El aspecto físico de las heces o Índice de excretas (I.E.) se evaluó semanalmente del día 7 al 28 de edad. Se les asignó los siguientes valores 0= normales (típicas), 1= con estrías mucosas, 2= ligeramente líquidas, 3= mucosas y 4= muy líquidas, de acuerdo a la escala de Johnson y Reid (1970).

Conteo de ooquistes en heces. Para evaluar el número de ooquistes eliminados en heces por grupo, así como el porcentaje por especie, se colectaron muestras de heces del día 3, 7, 14, 21, 28, 35 y 42 de edad. El conteo de ooquistes / g de heces (opgh) se realizó con la técnica de McMaster, al microscopio óptico y con

el objetivo 40x.

Grado de severidad de las lesiones intestinales. Al día 7 posdesafío (28 de edad) las aves sobrevivientes de dos réplicas de cada grupo fueron sacrificadas por dislocación cervical e inmediatamente después se les extrajo el intestino. Las lesiones sugestivas a coccidias fueron evaluadas de acuerdo a la escala de Johnson y Reid (1970).

Índice anticoccidial. Se realizó tomando en cuenta el porcentaje de viabilidad, ganancia relativa de peso corporal entre el día 21 y 28 de edad, índice de lesiones de ooquistes al día 28 de edad, y se clasificó como bueno en los valores de 180 a 200, moderado de 160 a 179 y pobre menor a 160.

Análisis estadístico. Los pesos de los grupos y el número de ooquistes fueron sometidos a una prueba estadística de análisis de varianza y las diferencias entre las medias de los grupos se evaluaron con la prueba de comparación múltiple de medias de Tukey.

La severidad de las lesiones se analizó con la prueba no paramétrica de Kruskall Wallis y con la prueba de U de Man Witney que determinó las diferencias entre las medianas de los grupos. Todas las pruebas estadísticas se analizaron con una significancía de ($P<0.05$) (Shirley 1990, Luginbuke RC and Schlotzhaver 1987).

Diseño de los grupos

Las aves fueron distribuidas en 9 grupos, cada uno con tres replicas de 6 pollos. Posteriormente se asignó **aleatoriamente** un tratamiento por grupo: los **grupos I y II** recibieron la vacuna por aspersión al día 1 de edad y se revacunó al **grupo II** al día 10 de edad. Al **grupo III**, se les administró la vacuna en el alimento durante las tres primeras semanas de vida, mientras que los **grupos IV y V** recibieron la vacuna en el agua de bebida al día 3 de edad. El **grupo V** se revacunó al a los 10 días de edad. El **grupo VI** no recibió la vacuna. Al **grupo VII** se le administró la vacuna al día tres de edad y recibió alimento con 60 partes por millón (ppm) de salinomicina durante todo el ciclo. El **grupo VIII** no fue vacunado ni infectado. Mientras que al **grupo IX** únicamente se le administró la vacuna al día 21 de edad por vía oral.

RESULTADOS Y DISCUSIÓN

La vacunación contra la coccidiosis representa una útil y exitosa herramienta en el control de esta enfermedad utilizada a nivel mundial. Las características de pigmentación del mercado mexicano han limitado el desarrollo de esta herramienta, básicamente por la asociación del efecto colateral sobre la pigmentación que en algunos casos resulta del empleo y manejo de la vacuna. Los resultados obtenidos en años anteriores de pruebas han variado; sin embargo, los resultados han sido mejorados conforme se ha perfeccionando la técnica de la vacunación y seguimiento en campo de cada tipo de vacuna.

Lo anterior se puede observar en los resultados obtenidos sobre la medición del pigmento cutáneo que exponen que los grupos vacunados y desafiados presentaron a los 28 días de edad los menores niveles de pigmento en piel durante el tiempo que duró la prueba y se observó una recuperación al día 35 y mayor aún al día 42.

Este decremento en el pigmento se relaciona a que el desafío con *Eimeria* y al efecto negativo que el parásito causa en el tejido intestinal. En infecciones por *Eimeria* la absorción de nutrientes y aditivos del alimento, particularmente el pigmento, se ve reducida debido al daño directo provocado por el parásito; así como por el cambio de la morfología de la mucosa, ya que se produce una hiperplasia general, disminución del largo y número de las microvellosidades, aumento en la diferenciación celular con incremento en el número de células caliciformes; sin embargo, si la infección es controlada, el tejido intestinal puede regenerarse y recuperar su capacidad de absorción (Peek *et al* 2006).

De acuerdo a los resultados del día 42 de edad se puede precisar que los grupos que presentaron mejor pigmentación en piel son el IV, VII, II, V y VIII en orden descendente; estos resultados están en razón de la vía y método de vacunación utilizado y del efecto protector de la vacuna ante el desafió. Se observó que aunque la vacunación en agua no es la vía preferida para vacunar contra coccidia, debido a que el consumo de ooquistes es poco homogéneo (Schering.P.A 1999). En esta prueba observamos que los grupo IV y V estuvieron dentro de los grupos con mayor pigmento amarillo en piel, esto puede deberse a que la replicación de la vacuna fue adecuada desde edades tempranas como puede observarse en el

cuadro 3 y por lo tanto la inmunidad contra coccidia era sólida para el momento del desafío, mientras que el grupo no vacunado y desafiado obtuvo los niveles más bajos de pigmento amarillo en piel en los dos últimos muestreos de la prueba como era de esperarse.

El grupo vacunado por aspersión al día de edad no mostró un nivel adecuado de pigmento durante toda la prueba, pero el grupo que recibió una segunda vacunación por aspersión al día 10 de edad (grupo II) se encontró dentro de los grupos con mejor pigmentación en piel y peso corporal al final de la prueba, a pesar de que presentó altos niveles de ooquistes al día 28; lo que sugiere que la vacuna confirió protección ante una nueva exposición.

El mismo efecto se observó entre los grupos IV y V en relación al peso corporal final de la prueba, donde el grupo V que recibió una revacunación al día 10 mostró mejor peso corporal, mientras que en relación al pigmento cutáneo no hubo diferencia estadística significativa entre ambos grupos.

Estos resultados concuerdan con estudios donde se menciona que una sola vacunación contra *Eimeria* spp en ocasiones no logra conferir una inmunidad completa debido a que la reinfección depende de factores ambientales y de manejo que favorezcan la esporulación de la cepa vacunal y la reinfección de las aves en la caseta, y esta protección adecuada se alcanzará mejor si se administran vacunaciones subsecuentes. Por otra la administración de cantidades bajas de ooquistes en forma continuas y cantidad desuniforme no garantiza una protección adecuada (Chapman *et al*., 2002)

Esto último también se observo en el grupo III que recibió la vacuna en alimento en cantidades bajas en forma continua durante tres emanas y que se encontró entre los grupos que obtuvieron los niveles más bajos de pigmento, ganancia relativa de peso y fue el penúltimo en peso corporal de los grupos vacunados.

En los resultados obtenidos en el índice anticoccidial al final de la prueba se observó que la vacuna utilizada en el estudio protegió adecuadamente a las aves de los grupos IV, V, y VII, El grupo con mejor índice anticocidial al final de la prueba se observó que la vacuna utilizada en el estudio protegió adecuadamente a las aves de los grupos IV, V, y VII.

El grupo con el mejor índice anticoccidial en relación al grupo VIII fue el grupo VII, en el cual se efectuó la vacunación a los tres días de edad y se adicionó salinomicina en el alimento.

Los resultados muestran que la administración de 60 ppm de salinomicina en el alimento durante toda la prueba controló de mejor manera la replicación de ooquistes. Chapman, 2000 y Shirley and Long, 1990 mencionan como una alternativa para el control de la coccidiosis el uso de vacunas con cepas tolerantes a ionóforos como un mecanismo doble y conjunto para reducir o controlar las poblaciones de eimerias nativas y favorecer la replicación de las cepas vacunales. Además, los grupos IV, V y VII coincidieron con los grupos que registraron mayores niveles de coccidias excretadas en heces a edades más tempranas (día 7) (Chapman, 2000; Shirley and Long, 1990).

Lo anterior muestra que una característica importante para obtener inmunidad sólida a la infección es asegurar adecuadas reinfecciones de la cepa vacunal; ya sea mediante vacunaciones subsecuentes o bien en forma natural con un manejo adecuado del material de cama, mas que una vía de vacunación particular (Crouch, 2003).

El grupo VII también tuvo la mejor ganancia relativa de peso entre el día 21 y 28 esto se atribuye a la presencia del producto ionóforo en el alimento. La salinomicina controló la infección por ooquistes de desafío administrados al día 21 y debido a esto fue el único grupo vacunado que no presentó ninguna lesión intestinal a lo largo de la prueba. Este método donde se combina la vacunación con cepas tolerantes a ionóforos y salinomicina en el alimento asegura que se evite la presencia de lesiones intestinales y afectación en parámetros productivos debidos a una replicación masiva de ooquistes de campo y vacúnales, especialmente si esta vacuna contiene cepas no atenuadas o no seleccionada por su baja virulencia. Sin embargo, tiene la gran desventaja de incrementar los costos de producción al incluir para el control de coccidiosis una vacuna y un

coccidiostato ionóforo. La vacuna utilizada en este estudio contiene cepas de moderada virulencia, ya que al administrarla en pollos de 21 días de edad afectó el peso corporal y moderadamente el pigmento cutáneo, también causó una cantidad mayor de ooquistes en duodeno y se relacionó a las lesiones macroscópicas similares a las del grupo no vacunado y desafiado (grupo IV).

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Promedio del pigmento cutáneo en pollo de engorda vacunado contra *Eimeria* spp y desafiados al día 21 de edad

GRUPO		PIGMENTACIÓN CUTÁNEA			
		DÍA 21	DÍA 28	DÍA 35	DÍA 42
I	VACUNA POR ASPERSION DIA 1	9.73 cd	9.085 bcd	10.03 c	13.68 c
II	VACUNA POR ASPERSION DIAS 1 Y 10	10.67 bcd	9.293 bcd	15.45 b	21.03 ab
III	VACUNA EN ALIMENTO POR TRES SEMANAS	13.02 ab	11.741 b	10.1967 c	14.48 c
IV	VACUNA EN AGUA DIA 3	7.69 D	6.862 d	21.13 a	23.16 a
V	VACUNA EN AGUA DIA 3 Y 10	9.45 cd	8.368 cd	15.24 b	19.82 ab
VI	NO VACUNADO	11.02 abc	15.121 a	13.2 bc	10.91 c
VII	VACUNA EN AGUA AL DIA 3 + SALINOMICINA / 3 SEMANAS	11.4 abc	9.578 bc	18.83 ab	22.43 a
VIII	NO VACUNADO Y NO DESAFIADO	14.17 a	17.02 a	20.9 a	20.25 ab
IX	VACUNA AL DIA 21 DE EDAD	12.64 abc	17.29 a	17.72 ab	15.89 bc

*Literales distintas en una misma edad indican diferencia estadística ($P<0.05$)

Numero de ooquistes promedio por gramo de heces en pollo de engorda vacunado contra *Eimeria* spp y desafiados al día 21 de edad

GRUPO		Número de ooquistes / g de heces						
		DÍA 3	DÍA 7	DÍA 14	DÍA 21	DÍA 28	DÍA 35	DÍA 42
I	VACUNA POR ASPERSION DIA 1	0	0	109885 b	83709 ab	32984 cd	4800 bc	562 a
II	VACUNA POR ASPERSION DIAS 1 Y 10	0	0	49824 bc	6348 c	604464 a	10580 ab	2768 a
III	VACUNA EN ALIMENTO POR TRES SEMANAS	0	0	3120 c	93104 ab	52869 cd	360 cd	353 a
IV	VACUNA EN AGUA DIA 3	0	33137 a	391500 a	277760 a	2766 d	2400 bc	1167 a
V	VACUNA EN AGUA DIA 3 Y 10	0	10500 b	5311 c	68634 b	3045 d	2353 bc	293 a
VI	NO VACUNADO	0	0	0	0	478780 ab	16400 ab	1334 a
VII	VACUNA EN AGUA AL DIA 3 + SALINOMICINA / 3 SEMANAS	0	28141 ab	6600 c	64328 b	2480 d	13369 ab	353 a
VIII	NO VACUNADO Y NO DESAFIADO	0	0	0	0	0	0	0
IX	VACUNA AL DIA 21 DE EDAD	0	0	0	0	187220 b	96000 a	2280 a

*Literales distintas en una misma edad indican diferencia estadística ($P<0.05$)

Promedio de peso semanales en pollos vacunados con *Eimeria* spp y desafiados al día 21 de edad

GRUPO		PESO CORPORAL (g) A DIFERENTES DÍAS DE EDAD													
		1		7		14		21		28		35		42	
I	VACUNA POR ASPERSION DIA1	40.4	a	116.1	abc	297.6	a	497.8	d	747.5	c	1180	d	1520	g
II	VACUNA POR ASPERSION DIAS 1 Y 10	40.8	a	117.7	abc	298.8	a	531.5	bcd	772.2	c	1284	a	1650	b
III	VACUNA EN ALIMENTO POR TRES SEMANAS	39.7	a	122.7	ab	328	a	570.8	ab	801.1	c	1039.5	g	1547	f
IV	VACUNA EN AGUA DIA 3	39.5	a	113.2	abcd	288.3	a	448.7	e	811.6	bc	1136.2	f	1602	d
V	VACUNA EN AGUA DIA 3 Y 10	40.9	a	125.1	a	315	a	519.8	cd	882.9	ab	1240	b	1684	a
VI	NO VACUNADO	39.8	a	101.4	d	301.1	a	540.8	bc	666.3	d	868	h	1173	h
VII	VACUNA EN AGUA AL DIA 3 + SALINOMICINA / 3 SEMANAS	39.6	a	122.4	ab	324.4	a	500.7	cd	878.1	ab	1232	c	1598	d
VIII	NO VACUNADO Y NO DESAFIADO	40.7	a	107.8	cd	298	a	561.7	ab	952.1	a	1220	c	1614	c
IX	VACUNA AL DIA 21 DE EDAD	38.9	a	111.6	bcd	303.2	a	585.2	a	881.5	ab	1160	e	1553	e

**Pesos de un igual día de edad con literales distintas son estadísticamente distintas ($P<0.05$).

Peso corporal promedio entre los días 21 y 28 de edad en pollos vacunado con *Eimeria* spp y desafiados al día 21

GRUPO		PESO CORPORAL (g)			
		DIA 21	DIA 28	GANANCIA DE PESO	GANACIA RELATIVA DE PESO
I	VACUNA POR ASPERSION DIA1	497.8	747.5	249.7	63.96
II	VACUNA POR ASPERSION DIAS 1 Y 10	531.5	772.2	240.6	61.63
III	VACUNA EN ALIMENTO POR TRES SEMANAS	570.8	801.1	230.3	58.99
IV	VACUNA EN AGUA DIA 3	448.7	811.6	362.9	92.95
V	VACUNA EN AGUA DIA 3 Y 10	519.8	882.9	363.1	93.0
VI	NO VACUNADO	540.8	666.3	125.5	32.14
VII	VACUNA EN AGUA AL DIA 3 + SALINOMICINA / 3 SEMANAS	500.7	878.1	377.4	96.67
VIII	NO VACUNADO Y NO DESAFIADO	561.7	952.1	390.4	100
IX	VACUNA AL DIA 21 DE EDAD	585.26	881.5	296.3	75.89

Índice de lesiones intestinales siete días posdesafío en pollos vacunados

GRUPO			INDICE DE LESION INTESTINAL				
			DUODENO	YEYUNO	ILEON	CIEGOS	I. LESION
I	VACUNA POR ASPERSION DIA1	**R*-1** $_{n=5}$	0	0	0	2(2+), 1+	1
		***R-2** $_{n=4}$	0	0	1+	2(2+)	1.25
						TOTAL	**2.25**
II	VACUNA POR ASPERSION DIAS 1 Y 10	***R-1** $_{n=6}$	0	1+	1+	2(1+), 2+, 3+	1.5
		***R-2** $_{n=6}$	0	0	0	3(1+), 3+	1
						TOTAL	**2.5**
III	VACUNA EN ALIMENTO POR TRES SEMANAS	***R-1** $_{n=6}$	0	0	0	5 (2+), 3+	2.16
		***R-2** $_{n=6}$	0	0	0	4 (2+), 2 (3+)	2.33
						TOTAL	**4.49**
IV	VACUNA EN AGUA DIA 3	***R-1** $_{n=6}$	1+	0	0	2+	0.5
		***R-2** $_{n=6}$	0	0	0	0	0
						TOTAL	**0.5**
V	VACUNA EN AGUA DIA 3 Y 10	***R-1** $_{n=5}$	0	0	0	1+	0.2
		***R-2** $_{n=5}$	1+	0	0	0	0.2
						TOTAL	**0.4**
VI	NO VACUNADO	***R-1** $_{n=6}$	3(1+)	0	0	2+, 2(3+),4+	2.5
		***R-2** $_{n=6}$	1(1+)	0	0	2(2+), 3+,2(4+)	2.66
						TOTAL	**5.16**
VII	VACUNA EN AGUA AL DIA 3 + SALINOMICINA / 3 SEMANAS	***R-1** $_{n=6}$	0	0	0	0	0
		***R-2** $_{n=5}$	0	0	0	0	0
						TOTAL	**0**
VIII	NO VACUNADO Y NO DESAFIADO	***R-1** $_{n=3}$	0	0	0	0	0
		***R-2** $_{n=2}$	0	0	0	0	0
						TOTAL	**0**
IX	VACUNA AL DIA 21 DE EDAD	***R-1** $_{n=3}$	2(1+)	0	2+	2(1+)	0.5
		***R-2** $_{n=2}$	1+	0	0	2+	1.5
						TOTAL	**2.5**

***Repeticiones de un grupo.**

Indice anticoccidial al día 28 de edad

GRUPOS		INDICE ANTICOCCIDIAL				
		Viabilidad (%)	Ganancia Relativa de Peso (%)	Índice de. Lesiones.	Ìndice de Ooquistes.	Ìndice Anti Coccidiales.
I	VACUNA POR ASPERSION DIA1	87.5	63.96	2.25	2.8	146.2
II	VACUNA POR ASPERSION DIAS 1 Y 10	94.44	61.63	2.5	50.5	103.5
III	VACUNA EN ALIMENTO POR TRES SEMANAS	100	58.99	4.49	4.4	150.8
IV	VACUNA EN AGUA DIA 3	94.11	92.95	0.5	0.2	186.4
V	VACUNA EN AGUA DIA 3 Y 10	100	93.0	0.4	0	192.6
VI	NO VACUNADO	94.44	32.14	5.16	40	82.3
VII	VACUNA EN AGUA AL DIA 3 + SALINOMICINA / 3 SEMANAS	93.75	96.67	0	0.2	190.2
VIII	NO VACUNADO Y NO DESAFIADO	100	100	0	0	200
IX	VACUNA AL DIA 21 DE EDAD	100	75.89	2.5	15.6	155.3

ÍNDICE ANTICOCCIDIAL:

BUENO 180 a 200.

MODERADO 160 a 180

POBRE < a 160

Efecto del promotor de crecimiento *Calcarea carbonica* sobre la onda R del ECG en patos Pekín

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Con objeto de determinar el efecto de *Calcarea carbonica*, como promotor de crecimiento, sobre el corazón de patos se seleccionaron 15 patos Pekin al azar en la quinta semana de edad, cinco de un lote testigo con 155 animales y 10 de un lote experimental con 154, al lote experimental se les suministró 0.2 mililitros del medicamento homeopático *Calcarea carbonica* 200 C, una vez a la semana. A cada individuó se le efectuó una serie de ECG. Para lo cual los electrodos se insertaron subcutáneamente a nivel de las alas en la articulación humero-radial y el tercero en la articulación feromotibiorotuliana derecha. El equipo utilizado fue el Biopac MP35 fisiógrafo interfaz con PC y electrodos de aguja de plata. A todos los animales se les midió la onda R, por ser la más más representativa del trabajo ventricular. Se utilizó un diseño completamente al azar para estimar el Índice de Constancia (IR), dentro de cada grupo y así ajustar los datos a un valor probable promedio (VMPP) acorde con el número de repeticiones obtenidas en cada ave. Se compararon ambos grupos mediante una prueba de t a un nivel de confianza del 95%. Se obtuvieron los siguientes resultados: para el grupo testigo 0.0248 y el experimental 0.0433 mV, con una varianza de 4.395 E-05 y 9.726 E-05, respectivamente. Se concluye que *Calcarea carbonica* 200 C afecta significativamente las ondas R., lo cual sugiere un mayor trabajo ventricular con el medicamento.

PALABRAS CLAVE: ECG, patos, actividad cardiaca, Homeopatía, promotor de crecimiento.

Evaluación del efecto protector de una vacuna comercial para el control de la coccidiosis aviar en pollos de engorda

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INTRODUCCION

La coccidiosis es una infección parasitaria causada por un protozoario intestinal del género *Eimeria*, responsable de causar enteritis de grado variable, afectando el rendimiento del pollo de engorda provocando una disminución en la ganancia de peso, conversión alimenticia y en casos severos causante de muerte de las aves afectadas. Frente al problema del aumento en la resistencia a programas de drogas preventivas el uso de vacunas es en la actualidad la mejor alternativa para el control de la infección, ya que estimula la inmunidad sin causar el fenómeno de resistencia a los anticoccidiales. Se utilizó una vacuna comercial trivalente (*E. acervulina, E. máxima, E, tenella*), vacunando a las aves por aspersión al día de edad por aspersión. Se utilizaran 180 aves (12 grupos en total) de un día de edad, estirpe Ross para aplicar 6 tratamientos con dos repeticiones cada uno. El trabajo experimental tuvo una duración de 56 días, el consumo de agua y alimento fue *ad libitum*. Las aves consumieron alimento de iniciación del día 0 al 21 de edad, posteriormente algunas de las aves recibieron alimento con salinomicina (40 ppm). Las aves fueron desafiadas al día 28 de edad con 50,000 ooquistes por repetición. Las variables a evaluar fueron peso, consumo de alimento, conversión alimenticia, cantidad de ooquistes y humedades relativas promedio en cama de forma semanal. Las alteraciones intestinales macroscópicas se evaluaron según el método de Johnson y Reid al día 28, 35 y 56. Las aves que recibieron vacunación tuvieron un menor desempeño productivo las 3 primeras semanas de vida. De la semana 4 al final del trabajo experimental y después del desafío no se observó diferencias estadística ($P>0.05$) entre los tratamientos que recibieron la vacunación y desafío con el tratamiento control el cual no recibió vacunación ni desafío, incluso el peso y la conversión alimenticia fueron en promedio mejores que el grupo control.

Palabras claves: coccidiosis; *Eimeria*

EFECTO DE LAS AFLATOXINAS Y LAS FUMONISINAS EN POLLO DE ENGORDA COMO MODELO DE ESTUDIO

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RESUMEN

El estudio del efecto de la combinación de las micotoxinas a tomado un gran significando para la salud humana y animal, ya que es la forma en que se presentan estos compuestos en la naturaleza, por lo tanto, el riesgo está relacionado a la presencia de dos o mas metabolitos tóxicos biológicamente activos y su combinación en el alimento, generando un efecto de interacción enmarcado dentro de los principios de toxicología de sinergismo o de aditividad. Se realizaron una serie de trabajos experimentales con la finalidad de ajustar las concentraciones de micotoxinas a utilizar en el trabajo final, del mismo modo estos sirvieron para preparar la metodología de recopilación de datos, muestreo sanguíneo y de órganos, así como de ajustar las técnicas de laboratorio para la evaluación del hematocrito, proteínas, transaminasas y bilirrubinas séricas. En la investigación final se observó que la presencia de aflatoxinas y fumonisinas (AFBs+FBs) disminuyen el peso de las aves ($p<0.05$). El peso relativo de hígado, riñón y bazo se ven afectados en presencia de algún tipo de micotoxina o su combinación respecto al testigo ($p<0.05$). Del mismo modo el hematocrito, proteínas, transaminasas y bilirrubinas se ven afectadas significativamente en las aves que consumieron AFBs+FBs ($p<0.05$). Este trabajo es el primer estudio en México que pone de manifiesto que concentraciones menores a 200 o 300 mg de FB /kg de alimento en combinación con 1 mg de AFB /kg de alimento inciden negativamente sobre el desempeño y la salud del pollo de engorda bajo las condiciones experimentales empleadas.

Palabras claves: Aflatoxina B_1 | Fumonisina B_1| Pollo de engorda | Interacción |