AN ABSTRACT OF THE THESIS OF

GARY L. BRADLEY for the degree of Doctor of Philosophy in Poultry Science presented on March 8, 1994.

Title: Evaluation of Turkey (*Meleagris gallopavo*) Breeder Hen and Market Male Performances when Fed Diets Supplemented with a Yeast Culture Containing *Saccharomyces cerevisiae*

Abstract approved: _____
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Studies investigating the effects of feeding diets containing the yeast, *Saccharomyces cerevisiae* var. *boulardii* (SCB), and a yeast culture (YC) containing *S. cerevisiae* were conducted in market turkeys and Medium White turkey breeder hens.

Increased utilization of dietary gross energy, N, Ca, P, B, K, Mg, and Mn were observed in poults fed a diet containing 1% YC when compared to the control and 1% inactivated YC diets at 4 weeks of age (WOA). It was concluded that the YC must be "biologically active" in order to affect nutrient retention in poults.

Feeding day-old poults diets containing varying amounts of SCB resulted in increased body weights at 3 WOA. Greater body weights and a decrease in the number of mucous-secreting goblet cells per mm of villus height and a decreased crypt depth were observed in poults receiving .02% SCB from

3 to 5 WOA. No dietary differences were observed for either villus height or width. Results indicated that feeding SCB to poults increased body weight and altered gut morphology.

Experiments conducted to evaluate the effects of .25% YC on market male turkey performance resulted in contradictory responses. Supplemental YC increased body weights of turkeys at 5, 8, 11, and 14 WOA in one trial, while no differences and depressed body weights from 2 through 17 WOA were observed in two subsequent trials, respectively. Similarly, feed to gain ratios were improved from day-old to 5 WOA in one trial, while no differences and a higher feed to gain ratio was observed from 2 to 5 WOA in the second and third trials, respectively. Results indicated that unelucidated factors may influence the response of market male turkeys fed dietary YC.

Consistent results have been observed in two trials on the hatchability of fertile eggs (HFE) from hens of three genetic lines fed .5% dietary YC. Early embryonic mortality (0-10 d of incubation) was reduced when hens were supplemented with YC and the HFE was increased in eggs stored less than 9 days in select hen genotypes. Results indicated that the breeder hen's genotype and pre-incubation storage time are factors to be considered when evaluating a YC in turkey breeder hen diets.

Evaluation of Turkey (*Meleagris gallopavo*) Breeder Hen and Market Male Performances when Fed Diets Supplemented with a Yeast Culture Containing *Saccharomyces cerevisiae*

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Typed by Deyette Bradley for Gary L. Bradley

DEDICATION

This Doctoral Dissertation is dedicated to my lovely wife, Deyette, and to our goodly parents.

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EVALUATION OF TURKEY (*MELEAGRIS GALLOPAVO*) BREEDER HEN AND MARKET MALE PERFORMANCES WHEN FED DIETS SUPPLEMENTED WITH A YEAST CULTURE CONTAINING *SACCHAROMYCES CEREVISIAE*

CHAPTER I

INTRODUCTION

The environment in turkey production is very important because it influences bird health, behavior, productivity, and economics. When referring to the turkey's environment, one envisions the bird's external surroundings. The intestinal lumen of the turkey may also be considered as "an external surface", and therefore, should also be regarded as a component of the turkey's "environment". To provide an optimal environment for turkey production, specialized management and housing facilities are required. Some important factors which are provided and carefully managed include: sanitary production facilities, adequate amounts of clean air and water, high biosecurity standards, and optimal temperature ranges. Dietary factors such as recently prepared and properly-balanced rations are also of utmost importance because they affect the bird's health, productivity, and the microbial ecology of the bird's intestinal environment. Diets which supply recommended amounts of bioavailable nutrients are, therefore, provided; however, they do not guarantee optimal efficiency and bird

performance. Consequently, the addition of feed additives such as antimicrobials and feed supplements like direct-fed microbials (DFM) are used to alter the bird's intestinal environment in order to control disease, improve feed efficiency, and help the bird achieve its genetic potential (Headen, 1989; Lyons, 1989; Risley, 1992; Miles, 1993).

The term DFM encompasses a wide range of commercially available microbial products which are generally regarded as safe (GRAS) by the Food and Drug Administration and the Center for Veterinary Medicine (Miles and Bootwalla, 1991; Risley, 1992). Yeasts and yeast cultures are included within this category of DFM and are defined by the Association of American Feed Control Officials (1989) as sources of highly concentrated (approximately 2 X 10^9 colony CFU/ml) viable yeast cells (yeast), or viable yeast cells (approximately 10^7 CFU/ml) and the media on which they were grown (yeast culture).

Feed antimicrobials have been used extensively in the turkey industry to maximize bird production by subsequently controlling the microbial intestinal environment. The use of antimicrobials is especially important in the control of disease because under current management practices, birds of the same age are housed together as a flock. This practice limits the probability of young birds being exposed to the adult's commensal intestinal microbiota which would

otherwise colonize the young birds' gastrointestinal tract and serve as a natural protection barrier against pathogenic bacteria. Although antimicrobials have mainly been used to reduce intestinal pathogenic bacteria (thus counteracting the consequence of raising young birds without adults), most are nonspecific (broadspectrum) medications which also eliminate many of the otherwise resident bacteria such as certain strains of *Lactobacilli* sp., *Enterococci* sp., and *Streptococcus* sp. (Watkins and Miller, 1983; Chesson, 1991).

Consumers of animal meat products are now demanding that antimicrobial usage in the feed of animals reared for human consumption be reduced or eliminated. A viable alternative to the use of feed antimicrobials may be the use of yeast and bacterial DFM (Savage, 1991; Dawson, 1993). Another aspect which may make the use of DFM products more efficacious is the practice of increased nutrient management. The current industrial management of turkey production is affecting the larger environment of the earth and an increasing number of people are becoming concerned with the impact of industrialized poultry production (and subsequent waste production) on the earth's environment. The use of yeast DFM such as yeast cultures may reduce this environmental impact on the earth's water and soil through improved nutrient utilization and subsequent reduced

nutrient excretion (Cromwell and Coffey, 1991; Jensen, 1993; Bradley and Savage, 1994b).

Yeast DFM are thought to assist in balancing microbial populations and provide the bird with additional enzymes, vitamins, and chelated trace minerals (Chad Risley, 1993, Chr. Hansen's Laboratory, Inc., 9015 West Maple Street, Milwaukee, WI 53214, personal communication). In addition, yeast cells have also been reported to possess aflatoxin binding properties which can reduce the toxicity of feeding moldy feedstuffs (Devegowda and Aravind, 1993; Stanley *et al.*, 1993). Although the addition of yeast and yeast cultures to turkey diets have provided improved performance, the lack of consistent results between reports has prevented their general acceptance for use by the turkey industry. The variability observed between reports involving yeast DFM products has not been fully elucidated. Some researchers have hypothesized that certain feed ingredients such as alfalfa may have an influence on the outcome of studies using yeast culture (John Brake, 1994, North Carolina State University, Raleigh, NC 27695, personal communication). Recent research has revealed genetic line differences in response to the feeding of a dietary yeast culture (Bradley and Savage, 1993; Hayat *et al.*, 1993, Savage *et al.*, 1993; Bradley and Savage, 1994a). Other factors such as different types of yeast DFM products, nutrient densities of the

experimental diets, differing managerial practices and environmental or pathological stresses on the bird may also influence the bird's response when fed diets containing yeast DFM products.

The purposes of the research presented in this thesis were to:

1. evaluate the effects of diets containing the yeast, *Saccharomyces cerevisiae* var. *boulardii*, and a yeast culture of *Saccharomyces cerevisiae*^{XP} on the performance of commercial market male turkeys,
2. evaluate the effects of diets containing a yeast culture of *Saccharomyces cerevisiae*^{XP} on Wrolstad Medium White turkey breeder hens, and subsequent progeny performance,
3. evaluate the effects of diets containing *Saccharomyces cerevisiae* var. *boulardii* and a yeast culture of *Saccharomyces cerevisiae*^{XP} on the microscopic morphology of the ileum in commercial market male turkeys, and
4. to attempt to elucidate the modes of action associated with the feeding of yeast DFM products.

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CHAPTER II

REVIEW OF LITERATURE

TURKEY INDUSTRY

The poultry industry has evolved at an exponential rate over the past 50 years. The turkey industry in particular, has evolved from numerous small backyard breeder flocks to larger flocks of fifty turkeys (then considered a large flock) and finally, to vertically integrated companies with flock sizes of 100,000 producing millions of birds per year (Miller, 1986b). The industry has also changed its production and breeding programs from a seasonal operation which mainly focused on providing holiday turkeys for Thanksgiving and Christmas to a system which supplies fresh whole turkeys and value-added products such as turkey roasts, whole breast, deboned gourmet breast, legs, hot dogs, bacon, bologna and turkey ham throughout the year (Miller, 1986b; Cook, 1990).

The nutritional outlook of the turkey industry has also changed rapidly. Prior to the 1920's, little information was available on the nutrition of the turkey, and backyard flocks were fed nutritionally unbalanced diets. The diets these backyard flocks received consisted of table scraps, various grains such as wheat and corn, and limited foraging

on range grasses. Years later, the early turkey producer (raising flocks of fifty) would feed a starter ration to poults up to six weeks of age consisting of cottage cheese, green onion tops, and plenty of pepper (to encourage the poult to drink water). The grower ration (from 6-8 weeks of age) which was fed when birds were on the range, consisted of a combination of mash feed mixed with a small amount of grain (mostly corn and oats which was referred to as scratch). Larger proportions of these grains were added to the diet as birds matured and grew larger (Miller, 1986b). Surprisingly, these early flocks were fairly successful in producing turkey meat in spite of their nutritionally unbalanced feeding practices. Most toms were marketed at 25 to 26 weeks of age and weighed on the average of 10.9 kg while hen body weights averaged approximately 6.3 kg at the same age (Miller, 1986b). Today (1994), toms are generally marketed between 17 and 20 weeks of age and weigh 10.5 to 16.3 kg while hens marketed at younger ages (15 to 18 weeks of age) weigh 6.3 to 8.1 kg. Feed to gain ratios of both sexes have also improved dramatically with the improved feeding, genetics, and management techniques currently practiced (National Research Council, 1984; Moreng and Avens, 1991; Sell, 1991; Nicholas Turkey News, 1993a,b). These dramatic improvements in turkey performance and the rapid expansion of the turkey industry are due to

progressive and innovative changes by an industry not reluctant to change ideas and technologies associated with producing quality least-cost turkeys. These changes have been augmented by other scientific advances in nutrition, genetics, physiology, pathology, and by the industry's ability to respond quickly to consumer demands (Cook, 1990).

Future Trends

According to May (1990), turkey meat production will increase from 2.27 billion kg in 1990 to 4.45 billion kg by the year 2000. This is a 96 percent increase in only ten years. Even if May's predictions are not completely realized, it is evident that the poultry industry must expand to meet the increased consumer demand for poultry meat and its value-added products. Factors cited which will boost consumer demand for poultry meat products include:

- versatility in the development of further processed meat products such as poultry hot dogs, bologna, bacon, ham and sausage,
- continued growth in the fast food industries, and
- exportations to other countries.

Goals of the Industry

Maximizing profitability is the goal of poultry producers and integrated companies. As the poultry industry expands to satisfy demands, it must also increase its production efficiency in order to maximize the economic advantages. According to Wentworth (1993), "the poultry industry has relied on a high return on past investments in the development and adoption of new techniques". This willingness to adapt has been a key to the continued success of the industry.

Three significant ways in which we may attempt to improve the efficiency of the poultry industry are through nutrition, genetics, and management. Improvements in nutritional efficiency are economically essential. Approximately 70% of production costs are associated with the purchase and manufacture of feeds (Moreng and Avens, 1991; Harry Nakaue, 1991, Oregon State University, Withycombe Hall, Corvallis, OR 97331, personal communication; Firman, 1993). As a result, greater profits can be achieved by small nutritional improvements through increased knowledge and new technologies in the feeding industry. One concern of poultry nutritionists is the rapidly changing genetics of the turkey and the continual struggle to keep abreast of the nutritional needs of each of the improved lines (Thomas Savage, 1992, Oregon State

University, Withycombe Hall, Corvallis, OR 97331, personal communication). According to Jensen (1993), practical poultry diets are generally in excess of the National Research Council (1984) suggested nutrient requirements. This excess is referred to as the "safety margin". This "safety margin" is believed to be crucial because adequate amounts of nutrients are necessary to maximize each bird's genetic growth potential, thereby maximizing efficiency and profitability. This "safety margin" if excessive, however, will be to the detriment of the producer's already modest profit margin. If this margin could be prudently reduced then the profitability of poultry production would increase. The practice of overformulating feed rations, in addition to being costly, leads to an excess of nutrients in the excreta. Currently, the excreted nutrients of greatest concern are nitrogen and phosphorus (Barton, 1992; Malone, 1992; Swick and Ivey, 1992). The nutrient-rich excreta is an additional challenge for the poultry producer because of the potential for environmental contamination of soil and water caused by improper land application of this poultry waste product (Shih, 1993). Nutrient management legislation is currently (1993) pending in Pennsylvania, the consequences of which could result in future legislative activities in other states which would also regulate the disposal of poultry waste products (Patterson and Lorenz,

1993). Besides the environmental problem of excess nutrients in the feed, there is new evidence that this excess may also be detrimental to the bird's performance (Jensen, 1993). Clearly, minimizing the practice of overformulation, or eliminating it through nutritional advances, would benefit the turkey industry in many ways.

FEED SUPPLEMENTS AND CONSUMER CONCERNS

Unidentified Growth Factors

Feed manufacturers and nutritionists are attempting to maintain a "nutritional insurance policy" to ensure the optimization of feed efficiency and growth of poultry by selecting ingredients, which for reasons unidentified, improve animal performance or efficiency. The ingredients which are associated with this phenomenon are referred to as having unidentified growth factors (UGF). Today, ingredients with acknowledged UGF are still used in some complex poultry rations such as pre-starter and breeder diets for improved performance. In the past, researchers identified feed ingredients having UGF by replacing the known UGF, such as dried whey and fishmeal, with the ingredient in question. When this procedure was applied to a yeast culture (YC), researchers found that the YC also contained an UGF (Scott *et al.*, 1982). After identifying

ingredients that contained an UGF, researchers would then analyze the ingredients for their common nutrient composition thereby determining which nutrient(s) were responsible for the UGF. Although most of the growth factors in fermentative products such as YC and Brewer's dried yeast have been identified, some explanations of increased efficiencies remain to be elucidated (Waibel *et al.*, 1988; Gardner *et al.*, 1992; Stanley *et al.*, 1993).

Responding to Consumer Demands

1) Concerns About Antimicrobials

There is a keen concern about the use of antimicrobials in poultry feed and their potential effect on meat quality (Lyons, 1990; Dawson, 1993a). There have been television and newspaper reports about antibiotic-resistant pathogenic bacteria entering the human food chain from animal food sources. This news coverage has elevated and spread concern to an increased number of consumers (Spika *et al.*, 1987; Lucio-Martinez, 1993). In addition, the recovery of antimicrobial residues from polyphosphates in chickens, and the political debate in the beef industry over the use of implanted hormones, spreads doubt and confusion on the morals of the entire animal production industry (Raine, 1988).

2) Food Safety

Bacterial contamination occurs in many animal food products. Consequently, concerns about the potential contamination of meat products with pathogenic bacteria are legitimate, and the transmission of pathogenic bacteria from the gastrointestinal tract of poultry to the consumer is a health concern (Hinton, 1988). Due to the high publicity given to the contamination of poultry meat and egg products, however, most consumers incorrectly ascribe a higher risk of bacterial contamination to poultry meat as compared to other animal food products. The industry is attempting to change this perception by reducing the incidence of bacterial contamination through consumer handling education, changes in processing management, and through the use of competitive microbial exclusion which minimizes the presence of intestinal pathogenic bacteria (Schlleifer, 1985; Corrier *et al.*, 1993; Cox *et al.*, 1993; Hollister *et al.*, 1993; Kopek *et al.*, 1993; Musgrove *et al.*, 1993; Stern *et al.*, 1993). The technology of competitive exclusion and the modification of meat processing procedures have experimentally reduced pathogenic bacteria on whole carcasses (Blankenship *et al.*, 1993; Kaniawati *et al.*, 1993; Kim and Doores, 1993; Kopek *et al.*, 1993; Musgrove *et al.*, 1993; Shackelford *et al.*, 1993; Stern *et al.*, 1993; Wallner-Pendleton *et al.*, 1993).

3) Biotechnology in the Food Industry

Biotechnology is the manipulation and use of the biological sciences for economic or other perceived benefits in order to improve a given process (Lyons, 1986a,b). Biotechnology has been in use for centuries [e.g., the Egyptians used yeast as a leavening agent some 6,000 years ago (John Gauwitz, 1993, Western Yeast Company, P.O. Box 257, Chillicothe, IL 61523, personal communication). Through the use of biotechnology and a greater understanding of biochemistry, the pharmaceutical industry is now able to generate antimicrobials on an industrial level using either genetically engineered microorganisms or chemical processes using special catalysts. If, however, consumers continue to question the use of antimicrobials in animal feeds, their usage may be discontinued by the poultry industry (Lyons, 1989). A promising alternative to antimicrobials in poultry feeds is the use of beneficial microorganisms such as yeast or bacteria to decrease the number of pathogenic organisms (Headen, 1989; Miles, 1993).

4) Environmental Concerns and Animal Welfare

The extended environment of the earth is a growing consideration and the industry must institute tighter controls on waste disposal, bird management, and other

practices in which the poultry industry impacts society and the environment (May, 1990; Appleby *et al.*, 1992). In addition, with the increase in concern for the welfare and treatment of animals, some groups of "well educated" people have gone to the extreme of suggesting that all animals have divinely given rights which are being violated by the industry's use of these animals for monetary profit. An increasing number of people view the industry as "inhumane" because young birds are raised without their natural parents and in a living space which is perceived as too small. This public perception and the change in laws which govern the use of farm animals will affect the way the poultry industry operates (Carlson, 1993). Probable changes will include:

- providing more space per bird,
- allowing free range animals, and
- providing birds free from feed additives and other "chemicals" (Appleby *et al.*, 1992).

The use of beneficial microorganisms as animal feed supplements may be implemented to a greater extent in the future because of regulations on the use of feed additives and public demands for more "chemical free" products.

5) Acceptance of Food Microbials

Consumers are concerned about the use of antimicrobials in animal feed and believe that fermentation by-products

such as yeast cultures and other beneficial bacterial supplements are safe and natural. Furthermore, they believe that when live microbial supplements are added to poultry feed, they do not detract from the meat product's wholesomeness (Dawson, 1993a). These beliefs may be due to the currently accepted use of many varieties of beneficial bacteria and yeasts in further processing of dairy products such as yogurt, cheese, new products such as *acidophilus* milk, and the common use of yeast in the baking and brewing industries (Klaenhammer, 1982; Kim and Gilliland, 1983; Sandine, 1990). The acceptance of animal food microbials may also be due to the fact that humans have evolved using microbes to process their food and have eaten these foods containing microorganism for centuries (Savage, 1991).

Direct-fed Microbials (DFM)

According to Miles and Bootwalla (1991), Metchnikoff in 1907 was the first to suggest that the health of an animal may depend on a properly balanced gut microflora. Further work by Metchnikoff in 1908, as reported by Hutcheson (1991), revealed that people drinking fermented milk had a reduced incidence of digestive disturbances. This concept of using specific microbes to enhance the intestinal microbial population is termed "probiotic" (Hamilton and Proudfoot, 1991; Miles and Bootwalla, 1991).

The term "probiotic" has been used in general conversations to mean a microbial product used as a feed supplement. The literal meaning is "for life" as opposed to antibiotic "against life". Examples of microorganisms which are used as probiotics include: single bacterial preparations, a mixture of different bacterial species, lyophilized dry yeast, yeast cultures, bacterial and yeast mixtures (dry and liquid forms), and other microbes such as fungi (Muirhead, 1992). Miles and Bootwalla (1991) stated that Parker in 1974 defined the word "probiotic" to mean "organisms and substances which contribute to the intestinal microbial balance". This definition is vague and could include antibiotic and microbial extracts. Fuller (1989) defined a "probiotic" as: "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". Fuller (1988, 1989) believed it was essential for the microorganisms in the product to be "live" in order to affect the microbial balance. Hutcheson (1991) outlined seven criteria which aid in defining a probiotic:

1. a culture of specific microorganisms;
2. it implants in the animal to which it is fed;
3. must be viable for the effective altering of intestinal populations of both beneficial and pathogenic organisms;

4. must be maintained in a dry form for stability purposes;
5. is temperature dependent;
6. is dose dependent; and
7. is host-specific.

It is interesting to note that many probiotics marketed today do not satisfy these criteria (Muirhead, 1992).

Designation of DFM

In 1989, the United States Food and Drug Administration (FDA) mandated the use of the term direct-fed microbials (DFM) instead of "probiotics". Therefore, for the balance of this review the designation DFM will be used. In addition, the term "microbiota" rather than "microflora" will be used to describe the microbials in the gastrointestinal tract because most biologists consider microorganisms to belong to the kingdom "protista" and not to the plant kingdom, or "flora" (Savage, 1991). The FDA further defined DFM as "a source of live (viable) naturally-occurring microorganisms" (Miles and Bootwalla, 1991; Pendleton, 1992). Risley (1992a) suggested an alternative definition for DFM as being "live, naturally-occurring bacterial supplements given to animals at times of stress or on a continual basis". Although Risley (1992a) refers only to "bacterial supplements" as being DFM, fungi and yeast are

also included as DFM products. Consequently, the definition established by the FDA is more general and encompassing. The terms "live" or "viable" require further definition as research has revealed that although a yeast cell may be reproductively "non-viable," the enzymatic processes may remain (Mathews and van Holde, 1990a; Headen, 1992). DFM products have been evaluated by the FDA and the Center for Veterinary Medicine and are generally recognized as safe (GRAS) by these regulatory agencies (Miles and Bootwalla, 1991; Risley, 1992a). The general categories (genus) of microbes used in DFM are:

Lactobacillus	*Streptococcus*
Bacillus	*Bacteroides*
Bifidobacterium	*Leuconostoc*
Pediococcus	*Propionibacterium*
Aspergillus	*Candida*
Saccharomyces	*Torulopsis*

(Gilliland, 1988; Hutcheson, 1991; Miles and Bootwalla, 1991; Muirhead, 1992; Risley, 1992a). When DFM are fed to animals as feed supplements, these microbes are thought to manipulate the type and numbers of microorganisms in the intestinal tract.

Need for DFM

1) Poultry Waste Management

As turkey production becomes geographically more concentrated, environmental factors such as waste disposal may become a limiting factor. For instance, land and ground water contamination due to excessive amounts of nitrogen and phosphorus is a concern in the United States especially in the production-dense poultry areas (Cole, 1993). Problems such as overformulation of feed by the producer and low availability of nitrogen and phosphorus to the bird result in unnecessarily high levels of these products in the poultry waste (Sweerczek, 1986; Cromwell and Coffey, 1991; Jensen, 1993). Thayer *et al.* (1978) discovered that feeding *Saccharomyces cerevisiae* as a DFM to breeder turkey hens increased the availability of phytate phosphorus from the feed through the action of the yeast's enzymes. Consequently, the addition of a yeast culture to poultry diets could reduce the requirement for total dietary phosphorus thus decreasing the concentration of phosphorus in the excreta and reducing the feed cost. Yeast products are also being used in preliminary tests to convert poultry by-products such as feather waste to a high quality and highly bioavailable protein supplement (Ted Sefton, 1993,

Altech Inc., 3031 Catnip Hill Pike, Nicholasville, KY 40356, personal communication).

2) Growth and Adaptation in Poultry Industry

The turkey industry needs to adapt in order to take advantage of the increased technological advantages currently available. The use of biotechnology in order to incorporate microbial digestive enzymes and fermentative products or microbes themselves into poultry rations may provide environmental and economic advantages. Although fungi, yeast, yeast extracts, and yeast cultures have been used in the feed industry since the early 1950's, their use has been sporadic because of inconsistent positive effects. The use of DFM may allow the industry to effectively increase the efficiency of feed utilization , lower dietary nutrient constraints, and maximize profitability. An efficient use of poultry feedstuffs would reduce nutrient losses in the feces and aid in waste disposal challenges currently facing the poultry industry (Cole, 1991; Wenk and Messikommer, 1991; Wyatt, 1992; Cole, 1993). Furthermore, the reduced use of antimicrobial products as growth promotants creates a need which could be met with DFM.

3) Disease Prevention

It has been reported in the New England Journal of Medicine that "Food animals are a major source of antimicrobial-resistant salmonella infections in humans and that these infections are associated with antimicrobial use on farms" (Spika *et al.*, 1987) The number of reported food-associated outbreaks of salmonellosis throughout the world are growing as the consumption of poultry meat products increases. Consequently, the consumer has perceived poultry meat as the main source of Salmonellosis outbreaks (Stavric, 1987). To reduce consumer concerns, more wholesome poultry meat products must be produced. According to Hamilton and Proudfoot, (1991) and Gilliland (1988), DFM may replace antibiotics which are used to control pathogenic bacteria (Visek, 1978). In fact, this is believed (by some) to be the most important benefit of DFM as an animal feed supplement (Parker, 1974; Dawson, 1993a; Miles, 1993; Sainsbury, 1993).

4) Stressful Environments

The environment for turkey production (poultry houses) has been shown to harbor a variety of pathogenic microorganisms (Pinello *et al.*, 1977). In an effort to reduce the number of pathogenic microorganisms, producers

clean and disinfect the poultry facilities after each flock and observe strict biosecurity practices. However, in the event that these measures are insufficient and the birds are exposed to an increased number or a strain of pathogens for which they have a low immunity, the birds are placed under a stressful condition. This stress may express itself as an increased incidence of disease, increased requirements for certain nutrients, or increased flock morbidity. If this stress is not ameliorated promptly, profitability will decrease. The practice of feeding DFM supplements is thought to aid in times of stress by enhancing the immune system (Gedek, 1987; Kung, 1992; Miles, 1993; Sainsbury, 1993).

YEAST

General Description

The term "yeast" is a generic name used to identify certain microscopic organisms. In botanical terms, a yeast is classified as a fungus. The biological definition of a yeast is a single-celled fungus having cell walls (Rose, 1988). The first person to microscopically observe the yeast cell was the Dutch scientist, Anton van Leeuwenhoek, and since then, we have learned a great deal about yeast (Krutzman, 1990).

Types of Yeasts Used in Poultry Feed

There are numerous yeast DFM products available as animal feed supplements. These DFM products contain some of the following microorganisms: strains of *S. cerevisiae* (*S. cerevisiae boulardii* and *S. cerevisiae*[1026]), *Candida utilus, Torulopsis,* (torula yeast), *Aspergillus oryzae,* and *A. niger* (Scott *et al.*, 1982; Cheeke, 1991; Martin, 1992; Muirhead, 1992). Yeast DFM products are available in dry, liquid, or paste forms and consist of yeast cultures, yeast and other fermentation products, or concentrated active-dry yeast cells (Muirhead, 1992). Non-fermentative yeast cells are not considered DFM and are generally by-products from the brewing or distilling industry. Some of these by-products include: brewer's dried yeast, torula dried yeast, grain distillers dried yeast, and molasses distillers dried solubles (Scott *et al.*, 1982).

Saccharomyces cerevisiae

The name "saccharomyces" means "sugar fungus". This name reflects the need of this yeast for sugar as a substrate while "cerevisiae" refers to its association with the production of a variety of beers. *S. cerevisiae* exists as single egg-shaped cells which measure 5 to 10 microns in width and 5 to 12 microns in length (Rose, 1988). The

common manner for *S. cerevisiae* to replicate is by budding. The buds, when mature, break off from the mother cell. Yeast also have the capability of utilizing one of two metabolic pathways which either do not require oxygen (anaerobic) or require oxygen (facultative anaerobes) as a terminal electron receptor (Matthews and Webb, 1991; Risley, 1992b). According to Dawson, (1993a) The American Type Culture Collection maintains in excess of 1000 distinct strains of *S. cerevisiae* which can be differentiated by biochemical and genetic testing. A given strain of *S. cerevisiae* may have a unique enzymatic profile which will aid in the digestion of certain nutrients such as proteins, lipids, and carbohydrates. Companies choosing these microbes have selected the strain which they feel suits their purposes.

YEAST PRODUCTS

Yeast and Yeast By-Products

1) Yeast as a Major Feed Ingredient

Most yeast products available to the animal feed market today are present in the form of feed supplements and not as a major feed ingredient (Miles, 1993). An exception is yeast grown on gas-oil and n-paraffin. Yeast grown in this

manner is not yet economical to produce as an animal feed ingredient where soybean meal and fishmeal are readily available (Waldroup and Flynn, 1975; Waldroup and Hazen, 1975; Yoshida, 1975). Although not readily used for poultry, this single-cell protein source is an excellent source of protein, energy, and phosphorus. This "cultivated" yeast contains between 55 and 65% crude protein on a dry weight basis and has a high nucleic acid content of 6 to 18%. Because of the high acid content, animal growth is depressed due to the inadequate metabolism of nucleic acids. The inadequate metabolism causes insufficient nitrogen excretion which results in uric acid deposits in the body. Because most non-DFM yeast products are deficient in portions of their amino acid profile (low cystine and methionine), they are best used as protein supplements (van Weerden *et al.*, 1970; Waldroup and Flynn, 1975; Waldroup and Hazen, 1975; Litchfield, 1983; Miles, 1993).

2) Beneficial Nutrients from Yeast Products

Yeast and yeast by-products are nutrient rich sources of highly digestible proteins, simple carbohydrates, and vitamins (Maurice and Jensen, 1978; Verachtert *et al.*, 1990). This high bioavailability of amino acids from yeast is due in part to the presence of the B-complex vitamins

which aid in the amino acid utilization. The term "B-complex" vitamins was originally used to describe the vitamins found in yeast. (Gontard, 1950a,b; Lyons, 1986c; Hutcheson, 1991; Tadtiyanant *et al.*, 1993). Dried brewer's grains (10%) was successfully used as a protein supplement in diets fed to laying hens (Eldred *et al.*, 1975; Tadtiyanant *et al.*, 1993). Brewer's yeast and torula yeast have been popular in reducing leg disorders and improving growth and feed efficiency in poultry (Plavnik and Scott, 1980; Bolden and Jensen, 1985; Charles *et al.*, 1985). According to Scott (1987), niacin and biotin present in yeast culture and yeast by-products have been the key to the reduction of "hock and bowed leg disorders" in turkey poults and ducks.

3) Yeast Metabolites

Yeasts are unique in their ability to synthesize complex proteins from nitrogenous sources such as ammonia and ammonium salts. Bhattacharjee (1985) has identified the pathway where yeast are able to synthesize lysine. He concluded that this occurred if the gut environment permits enzymatic yeast activity and the ammonia nitrogen (produced by other intestinal microorganisms) is reclaimed by active yeast cells within the gut. Consequently, enhanced nitrogen retention may result from the direct synthesis of amino

acids (such as lysine) and whole proteins by the yeast cells within the digestive tract (van Weerden *et al.*, 1970).

Yeast Cultures as DFM

The official definition of a yeast culture provided by the Association of American Feed Control Officials (1989) is:

> "A dry product composed of yeast and the media on which it was grown, dried in such a manner as to preserve the fermenting capacity of the yeast"

This definition encompasses a wide range of products, thus, animal responses may differ when fed yeast cultures from different commercial sources. Yeast cultures from various companies may be unique in the strain of yeast used, cell viability, colony forming units per gram, growing conditions, and culture contents (Dawson, 1993a). These factors play important roles in the final product, nutrient profile, and metabolic activities of the yeast cells (Williams, 1989; Bui and Galzy, 1990). Currently, only a few companies which produce and market their own products reveal the strain or strains of yeast being used in their culturing process. Also companies do not identify on their product labels their fermentation process. This information is maintained as proprietary to enable the companies to compete more favorably and maintain their unique products. Since the genetic regulation in yeast cells is dependent on

the concentration of oxygen, nutrients, and temperature; the metabolites produced depend greatly on the environment of fermentation and processing (Zitomer and Lowry, 1992; Hargrove and Berdanier, 1993). For example, according to Williams (1989), an unidentified company uses high concentrations of zinc to control yeast cell division in the latter stages of fermentation. This procedure results in yeast cells which contain high concentrations of chelated zinc. Although some information can be kept proprietary, the media on which the yeast is grown must be stated on the label (Rose, 1988).

YEAST CULTURES IN ANIMAL FEEDS

Yeast Culture Supplementation

There are two basic types of yeast cultures available for animal feed supplementation: 1) those which contain a high level of viable yeast cells, and 2) those which contain a low number of viable cells and metabolites or "nutrilites" which the yeast cells have secreted during fermentation and have been preserved in the drying process (Chr. Hansen's Biosystems, 1991). The literature supports the use of yeast cultures for improved animal performance in many species including: dairy and beef cattle, sheep, goats, horses, swine, rabbits, dogs, chickens, and turkeys

(Thayer and Jackson, 1975; Phillips and von Tungeln, 1985; Glade and Biesik, 1986; Glade and Sist, 1987; Harrison *et al.*, 1988; Sefton, 1989; Hollister, *et al.*, 1990; Hughes, 1990; Lowe, 1991; McDaniel, 1991a,b; McDaniel and Sefton, 1991; Dawson, 1993a).

Not all animals may benefit from a DFM in the diet (Miles, 1993). In any population of animals there are those individuals which may derive only slight benefits from the use of DFM. Conversely, there may be individual animals who need the extra protection and advantage of a product designed to control the microbial balance within its digestive tract. However, more studies are required on individual animals in order to establish the validity of these statements. The immunocompetence of an individual animal and its response to YC products may well be factors to consider.

Management of Yeast Cultures

A large number and a wide variety of yeast and yeast culture products exist in today's feed supplement market (Muirhead, 1992). Each yeast DFM manufacturer has outlined how and when their product should be used. It has also been shown that certain strains of yeast are required in combination with specific feed ingredients and animal species in order to obtain the desired response. This is

evidenced by the number of nutritionists advocating selected yeast cultures and their subsequent incorporation in specific animal feeds (Boyett, 1990).

Identifying DFM in Feed

To ensure the proper incorporation of DFM products into animal feeds, the identification of yeast products may become important. Since specific strains are used, strain identification techniques must be specific. One such identification technique currently available is "DNA fingerprinting". This technique verifies that the given yeast is present in the manufactured feed. This verification is accomplished by comparing genomes of known yeast strains with the genome of the yeast strain(s) recovered from the feed (Moore and Headen, 1992; Moore, 1993).

Yeast Cultures (YC) in Poultry Rations

The supplementation of turkey rations with yeast products began in the 1950's when fermentative by-products and yeast cultures were used in the poultry industry. The scientific community has been puzzled for many years because of increased performance when certain feed ingredients such as fermentation products were used. As mentioned earlier,

these are referred to as UGF (Scott *et al.*, 1982; Brewer, 1983). Although most of these UGF have been elucidated, subtle improvements in feed efficiency and reproductive performance when using UGF remain unclear. Because of conflicting results among research reports on the incorporation of YC and other DFM into animal rations, scientists have not come to a general conclusion regarding DFM (Jernigan *et al.*, 1985). Savage and Mirosh (1990a,b) reported that selected lines of turkeys may benefit from the inclusion of YC in the diet. Supplementation of broiler breeder diets with a YC resulted in earlier semen production in the males and increased hatchability of eggs from caged females (McDaniel and Sefton, 1991). Hayat *et al.* (1992, 1993) observed an improved hatchability of fertile eggs in cross-bred hens fed a diet containing a YC. In subsequent studies using cross-bred turkey females, Bradley and Savage (1993, 1994) further demonstrated an improved hatchability of fertile eggs with the addition of a YC to the breeder hen diet within selected lines. Thayer *et al.*, (1978) observed that hens better utilized phytate phosphorus in phosphorus deficient diets when the deficient diets contained a YC. It has also been revealed that the feeding of a YC reduced the incidence of Colibacillosis in turkey poults (Barnes, 1987; Kumar, 1991). To the contrary, Brake (1991) reported no effect of feeding YC to broilers, broiler breeder hens, nor

on subsequent progeny performance. Similar results have been reported by Edmonds and Teeter (1983) using both broiler and layer type chickens and by Day *et al.* (1987) using caged laying hens. Variability in the response to DFM is not confined to yeast DFM. Bacterial DFM have also demonstrated great variability in improving the performance as measured by body weights and feed conversions of turkeys. Some authors have reported positive effects of supplemental bacterial DFM (Francis *et al.*, 1978; Brown, 1991, Nahashon, *et al.*, 1993) while others have shown no response (Anonymous, 1977; Potter *et al.*, 1979; Damron *et al.*, 1981).

FACTORS INFLUENCING YEAST CULTURE PERFORMANCE IN ANIMAL PRODUCTION

Genotype

1) Nutritional Differences

According to Scott *et al.* (1982) an animal's body is regulated by the genotype received from its parents. This not only is apparent phenotypically but metabolically as well. The ability of an animal to synthesize enzymes for the production or absorption of required nutrients differs with the animal's genotype. Variations among breeds and strains of chickens in their nutrient requirements have been

elucidated. Newkirk *et al.* (1993) demonstrated a strain interaction affecting egg production when xylanase was added to the diet. It is important to note that differences in dietary requirements encompass not only nutrient quantities, but the requirements for a nutrient class may differ as well (Scott *et al.*, 1982).

2) Other Biological Differences

The immunocompetence of individual animals may be a factor when assessing the influence of a DFM (Miles, 1993). Genetically dissimilar animals may respond differently to an immunologic challenge. Sharaf *et al.* (1989) demonstrated that genotype is a factor in determining the immune response to vaccination in different strains of turkeys. A genotype by dietary YC interaction has also been reported by Bradley and Savage (1993, 1994) when evaluating the hatchability of fertile eggs from three lines of Medium White turkey breeder hens. Furthermore, Savage *et al.* (1993) have described a genotype and dietary YC interaction for parthenogenesis in turkey eggs. Interactions may also exist between a dietary YC and diet composition (Williams *et al.*, 1991). Consequently, it would appear that some of the unexplained variations observed when DFM are studied may be illuminated as the interactions of genotype and environment are better understood (Dawson, 1993b; Thomas Savage, 1993, Oregon State

University, Withycombe Hall, Corvallis, OR 97331, personal communication). Differing geographical locations have also produced different responses when bacterial DFM were fed (Miles *et al.*, 1981). As more studies are conducted to evaluate the interaction of DFM, genetics, nutrition and management, the poultry industry will be able to utilize DFM to more consistently improve the efficiency of the industry.

Heat Stress

Stress, in its various forms, alters the gut microbiota and disrupts the normal balance of commensal microorganisms (Miles *et al.*, 1981). Consequently, the addition of DFM to the feed would benefit the bird in providing a constant inoculation of commensal microorganisms and additional enzymes. There are published reports in the poultry industry of contained economic loss due to stress in birds fed diets containing DFM (Kung, 1992; Dawson, 1993a; Miles, 1993). Jones *et al.* (1993) demonstrated an improved performance in broilers fed a DFM which were heat stressed (41 C for 6 h) post-hatching and then inoculated with *Salmonella typhimurium*. Research by Hughes and Jones (1987) demonstrated that heat stressed laying hens maintained egg production at a higher rate when fed diets containing 1.25% yeast culture.

Feed Manufacturing Practices

Pelleting poultry rations at temperatures of 70 to 80 C is common in the industry (Headen, 1992). Because the feed pelleting process may inactivate the reproductive ability of yeast cells (and has been proven to kill all bacterial DFM), the practice of pelleting feeds may not be compatible with DFM products (Risley, 1992b). Although yeasts are more able than bacteria to survive adverse conditions, the pelleting process does inactivate the reproductive capability of yeast cells (Rose, 1988). The degree of inactivation is dependent upon the yeast strain; its heat resistance; and the temperature, humidity, and pressure used in the pelleting process (Andrews, 1991; Lewis, 1991; Headen, 1992; Brown, 1993). It remains unclear as to whether the reproductive viability of the yeast should be considered when evaluating a yeast culture or whether the metabolic activity is the only factor of real importance (Headen, 1992). Studies have demonstrated that non-viable or "thoroughly dead" yeast cells are still capable of enzymatic processes such as the process of fermenting sugar into ethanol (Mathews and van Holde, 1990b; Headen, 1992). In addition, studies with the irradiation of yeast cells has also proven that enzymatic activity is still intact even after the yeast has been "killed" (Headen, 1992). For instance, the enzymes within a yeast cell and the culture media (such as phytase) have

been proven to retain as much as 83% of their activity when pelleted at 83.8 C even though the yeast cell has been rendered reproductively inactivated. Phytase activity declined substantially when the pelleting temperature exceeded 83.8 C (Ward, 1993). To avoid this loss in enzymatic activity, liquid products have been developed which allow the effective application of the yeast culture to the feed after the pelleting process (Ward, 1993). In order to verify that the yeast DFM has been properly applied, researchers have developed a DNA fingerprinting procedure which detects the yeast DFM in finished feeds (Moore and Headen, 1992; Moore, 1993).

Because the viability of the yeast DFM does not appear to be as important as those of bacterial DFM (Gedek, 1987; Headen, 1992), the pelleting of feeds containing yeast DFM appears to be more efficacious. However, the yeast culture's resistance to compression may affect the pelleting quality of the feed (Bui and Galzy, 1990).

Feed Handling

1) Feed Storage

The manufacturer's recommendations should be closely followed when using and storing DFM to ensure maximum viability of the microorganisms. Viability of the

microorganisms in the feed could be seriously impaired due to prolonged or inappropriate storage conditions such as exposure to high temperatures and humidity (Sainsbury, 1993).

2) Feed Hydration

Yeast cultures should be stored in a cool, dry place to prevent heat and moisture from activating the yeast. If the yeast is allowed to activate, it may grow in the feed and consequently utilize the feed's nutrients before the feed can be fed to the animal (Muirhead, 1992; Jack Garrett, 1993, Diamond V Mills, Inc., P.O. Box 74408, Cedar Rapids, IA 52407, personal communication).

Antimicrobial and DFM Interactions

The modes of action for both growth promoting antibiotics and DFM are not clearly understood, however, researcher have determined that trace elements such as selenium inhibit yeast growth in concentrations above 100 μM. Consequently, it is difficult to predict the response of animals when these feed additives and mineral supplements are simultaneously included in the diet (Hinton, 1988; Matthews and Webb, 1991). Many DFM are not compatible with currently available antibiotics and anticoccidial drugs

(Tortuero, 1973; Francis *et al.*, 1978; Castaldo, 1991). Poultry nutritionists must be aware of the information available on possible interactions as well as encourage new research in this area in order to better understand the synergistic or antagonistic effects (Dawson, 1993b).

CHANGES IN INTESTINAL MORPHOLOGY DUE TO GUT MICROBIOTA

The ability of poultry to adapt through alterations in the anatomical structure, metabolism, and overall functioning of the intestine is affected by nutrients, intestinal microbiota and other environmental changes (March, 1979; Fethiere and Miles, 1987; Parker, 1991). The intestinal environment may vary in the concentration of non-digestible matter, available nutrients, and the number and type of microorganisms present. For example, chickens with normal gut microorganisms have heavier and longer digestive tracts than germ-free birds. This difference is due in part to a thickened intestinal wall caused by a larger lamina propria and an increase in lymphatic tissues. Furthermore, the pH of the duodenal contents and the enterocyte turnover rate in chickens are higher when normal microbiota are present (Scott *et al.*, 1982; Fuller and Coats, 1983). Since the intestinal tract is the single most demanding organ in the body in terms of energy and protein requirements, a small change in its demands or increase in its efficiency

would greatly impact the overall performance of the bird (Neutra, 1988; Chesson, 1991). The epithelial cells along the entire length of the intestinal tract are constantly adapting to the microorganisms in the lumen through altering the morphology of the epithelial cells (Boedeker, 1984; Chesson, 1991). Features and activities such as a smaller brush border, increased microvillus area, increased crypt depth, and greater mitotic activity have been attributed to the presence of microorganisms in the gastrointestinal tract (Fuller and Coats, 1983; Boedeker, 1984; Chesson, 1991). Ultimately, this intimate contact may be either detrimental or beneficial to the ability of the enterocyte to absorb the required nutrients from the digesta (Fuller and Coats, 1983).

Dietary Factors Influencing Gut Morphology

Ritz *et al.* (1993) have reported that the mucosa of the intestine responds to amylase supplementation by increasing the mean villus length in the jejunal and ileal regions (thus increasing the absorptive surface area) in poults. Earlier results by Raudati *et al.*, (1991) also noted that some enzymes can enlarge segments of the chicken's intestine. In contrast, dietary factors such as fiber have not been shown to affect intestinal morphology in poults (Vilaseca *et al.*, 1993).

Enhanced Immunocompetence

With the exposure of environmental microorganisms to the epithelial surface of the intestines, the ability of the turkey to respond defensively with a strong immune system is critical. Microorganisms can gain entrance to the young poult's intestine through various routes: oral, nasal, and naval passages (Cox *et al.*, 1993). An important component of the defense response is the ability of the intestinal wall to adapt through stimulation of the gut-associated lymphoid tissue (GALT). The GALT is composed of lymphocytes surrounding the epithelial cells which can migrate and defend against foreign substances (Neutra, 1988; Lillehoj, 1993). An increase in the GALT has been observed in a preliminary study by Samuel Nahashon (1992, Oregon State University, Withycombe Hall, Corvallis, OR 97331, personal communication) following the incorporation of a bacterial DFM in the diet of SCWL laying chickens. According to several researchers (Gedek, 1987; Kung, 1992; Sainsbury, 1993), a benefit of adding DFM to the diet is to stimulate the immune system.

When DFM are added to poultry diets, a mild inflammation occurs in the intestinal wall resulting in the stimulation of the immune system and a consequential decrease in bird performance as the bird diverts more of its nutrient resources to the immune system. As long as the DFM

does not cause a significant pathological burden to the host (through the production of toxins or by causing a lowering of the enzymatic activity and absorbability of the enterocyte), the state of eubiosis is beneficial to both host and parasite (Gedek, 1987). To the contrary, antibiotics have been shown to reduce the immune response instead of enhancing it as would a DFM (Derieux, 1980).

POSSIBLE MODES OF ACTION

Increased Bioavailability of Nutrients

Before an animal's performance can be enhanced, the animal's diet must be deficient in a required nutrient or the bird must be in a pathologic state whereby its genetic potential cannot be fully expressed. In these sub-optimal conditions, a DFM or antibiotic can enhance the animal's growth by increasing the bioavailability of feed ingredients or decreasing morbidity due to pathogenic organisms. This increased performance could also be due to a net increase in absorbed nutrients, a sparing of nutrients by certain related substances, or by nutrient repartitioning (Chesson, 1991). In addition, due to the absorptive properties of the yeast cell wall, the yeast cells could absorb or chelate nutrients such as zinc from the media during the fermentation process. These concentrated micro-nutrients

would consequently be available for absorption by the animal or other intestinal microorganisms (Phillips and von Tungeln, 1985; Rose, 1988; Hughes, 1990). Consequently, increased availability of nutrients would perhaps lead to an improved feed to gain ratio and greater body weights. Evidence supporting this theory was reported by Madrigal *et al.* (1993) who observed an increase in the efficiency of feed utilization with the addition of a yeast DFM in the diet of broilers.

Enhanced Reproductive Performance in Poultry

McDaniel and Sefton (1991) observed an increased hatchability of fertile eggs when a YC was included in a broiler breeder hen's diet. Although no mode of action was discussed, it was thought to be associated with zinc absorption. Studies conducted with Medium White turkey breeder hens have demonstrated improved performance when YC was added to the diet of select hen genotypes as previously noted (Savage and Mirosh, 1990a,b; Hayat *et al.*, 1993, Bradley and Savage, 1994). To the contrary, Brake (1991) observed no improvement in reproductive performance with broiler breeders fed a dietary YC.

Metabolic Activity of Yeast

1) Oxygen Scavenger

Yeast have a high affinity for molecular oxygen. This oxygen-scavenging ability is much greater than any oxygen-absorbing chemical catalysts (Rose, 1988). Consequently, as yeast cells consume the oxygen present in the intestines, the oxygen concentration in the gut is further reduced and the proliferation of anaerobic bacteria is favored (Leeson and Major, 1990). Anaerobic growth stimulation has been shown experimentally by Harrison *et al.* (1988) where the numbers of anaerobic and cellulolytic bacteria concentrations were increased in cows fed diets containing a YC.

2) Phytase Activity

Phytate is a well known natural anti-nutritional factor present in the most common cereal grains used in poultry rations. Phytate is the principal form of storage for phosphate and inositol in most plant seeds. Some authors refer to phytic acid and/or its salt (phytate) as an "anti-nutritional factor" because of its strong chelating ability. This chelating action tightly binds nutrients such as calcium, zinc, and copper in the gastrointestinal tract

thereby reducing the availability of these nutrients to the animal (Savage *et al.*, 1964; Waldroup *et al.*, 1964; Erdman, 1989; Cheeke, 1991; Power, 1993; Ward, 1993). In most feed grains, only 30% of the total phosphorus present is available unless the feed is pre-soaked. Phytase functions in the gastrointestinal tract and can remain active at high temperatures (40 C) and in a wide pH range (Sooncharernying and Edwards, 1993). The lack of intestinal phytase activity is significant to the poultry industry because approximately 50-80% of the total phosphorus in a ration is bound as phytic acid (Kung, 1992; Power, 1993; Ward, 1993). Calcium and phosphorus content as well as the ratio of calcium to phosphorus is important to the turkey industry because imbalances or inadequate levels of these minerals have been implicated as contributing to leg weaknesses such as tibial dyschondroplasia (Brown, 1992; Stadelman, 1993).

The concept of nutrient management in relation to poultry waste management is becoming acute since pollution of soil and ground water is becoming a public and industry concern (Barton, 1992; Malone, 1992). Nutrients of importance in waste management are nitrogen and phosphorus. Fortunately, enzymes (such as phytase) have the potential to increase the bioavailability of minerals and other dietary nutrients which decrease nutrient loss and potential subsequent environmental pollution. Some of the enzymes

that increase nitrogen retention include the phytases, hemicellulases, and beta-glucanases (Patterson, 1993; Ward, 1993). In rations where the phytase enzymes have been used (1,000 enzyme units per kg of diet), phytate phosphorus utilization has been increased as much as 10% (Patterson, 1993; Ravindran *et al.*, 1993). Thayer and Jackson (1975) cited a study conducted *in vitro* by Kirby where he added a 10% yeast culture to finely ground corn and measured phytin bound phosphorus. The yeast culture contained phytase activity and as a consequence released 70% of the bound phosphorus in 4 hours. Furthermore, 100% of the bound phosphorus was released after 24 hours of fermentation at 37.7 C. Using this data, Mason (1974) estimated that the phytase activity of 1.25% added yeast culture could replace up to .15% of the available dietary phosphorus. He stressed, however, that the yeast culture had to be capable of active fermentation in the bird.

3) Increased Mineral Retention

Thayer and Jackson (1975) noted an improved performance of breeder turkey hens fed phosphorus deficient diets that contained a yeast culture. They concluded in a subsequent study (Thayer *et al.*, 1978) that the increased performance was due to an enhanced utilization of dietary phytate phosphorus due to the microbial phytase activity. The

results obtained by Thayer *et al.* (1978) are especially significant because breeder hen diets high in calcium, for proper egg shell formation, inhibit phytase activity. Microbial phytase from a dietary supplementation of a yeast culture may be most beneficial to poultry where phytase activity may be limited such as young poultry, breeder hens, and developing embryos or birds being fed diets containing high amounts of fiber or calcium (O'Dell *et al.*, 1964; Ward, 1993). Guevara *et al.* (1977) demonstrated increased tibia ash content when broiler chickens were fed diets low in phosphorus and supplemented with a yeast culture (3%). The increase in tibia ash content of birds supplemented with yeast culture is significant since the normal microbiota of the bird has little or no role in the utilization of phytate phosphorus (Reddy *et al.*, 1989). Benefits from phytate destruction have been reported: calcium, zinc, copper, iron, and manganese become more bioavailable through the action of yeast phytase (O'Dell *et al.*, 1964; Savage *et al.*, 1964; Scott, 1987; Erdman, 1989; Ward, 1993). Furthermore, Erdman (1989) discovered that otherwise insoluble metal-phytate complexes formed in the gut are hydrolyzed by the gut microbiota of rats at neutral pH and subsequently, the absorption of calcium was increased. Erdman (1989) has also discovered that baker's yeast is a rich source of phytase and that bread leavened with yeast has a greater mineral

digestibility in humans than do chemically leavened and unleavened breads.

4) Increased Nitrogen Retention

Decreasing the amount of phytic acid in the digesta reduces the protein-phytate complexes that are much more resistant to proteolytic enzymes. Ward (1993) cites studies conducted by Van der Klis and Versteegh in 1991, who reported an increase of 2% nitrogen absorption in laying hens when fed a microbial phytase supplement. Although 2% nitrogen retention is numerically small, the economic savings to the poultry industry would be very large because of increased feed efficiency and decreased nitrogen content in poultry waste.

The quality of available protein to the bird is also an important consideration since birds cannot produce sufficient quantities of the indispensable amino acids required for optimal growth. Phytate has an affinity for the basic amino acids such as lysine, arginine, and histidine which are among the ten indispensable amino acids for poultry (Scott *et al.*, 1982). Consequently, the destruction of phytate may increase the absorption of these important amino acids which are indispensable for poultry (Ward, 1993). Skadhauge (1983) believes that the recycling of uric acid nitrogen by microbes in the ceca could result

in increased absorption of the synthesized amino acids and free ions bound in the uric acid for possible resorption. Further evidence by Nahashon *et al.* (1993) has demonstrated increased nitrogen and calcium retention in Single Comb White Leghorn laying hens fed diets containing a bacterial DFM product. Increased nitrogen retention has also been observed in horses supplemented with a YC (Glade and Biesik, 1986). Therefore, microbial phytase is a means of increasing nutrient retention through the destruction of phytic acid.

5) Increased Energy Utilization

The efficient utilization of dietary energy is an aspect of poultry nutrition which needs to be improved. Savage *et al.* (1985) reported decreased fat deposition in market turkey hens fed a yeast culture may be attributed to enhanced energy utilization. Charles *et al.* (1985) reported that YC significantly improved growth rate and feed conversion in broiler studies using various feed grade fats. Tonkinson *et al.* (1965) also demonstrated an increased utilization of dietary fat in laying hens when fed diets containing 3% YC.

Improved Intestinal Microbial Balance

1) Importance of Intestinal Microbial Balance

Soon after the poult hatches, a unique microbial population becomes established on the poult's body parts which are exposed to the external environment. These body locations include the skin, feathers, the scales on the feet, and the intestinal tract which has a large surface area (Neutra, 1988). The microbial populations which are quickly established on these areas depend entirely on the organisms and their ability to propagate (Lev and Briggs, 1956; Phillips and von Tungeln, 1985). Under ideal conditions where birds are reared by the parents, non-pathogenic microorganisms called "commensal microbiota" become established first and keep transient pathogenic microorganisms from establishing themselves. However, under current (1993) management practices, birds are raised in groups where all the birds are the same age. When stressed by a sudden change in the environment (such as a different feed or fluctuation in temperature), the animal and its resident microbiota may be unable to adapt as quickly as the transient or lower numbered pathogenic populations of microbes (Wagner and Thomas, 1978; Phillips and von Tungeln, 1985). If these transient pathogenic bacteria are able to reproduce, the bird may be subjected to disease. If this

stress continues, the pathogenic bacteria will eventually invade the host's internal environment gaining access to the rest of the body through the blood or other body fluids (Miles, 1993). After pathogens have gained access to the bird's tissues, the danger exists for this contamination to be spread to the human population via the eventual consumption of the meat product (Dawson, 1993a; Miles, 1993). According to Stavric (1987), the establishment of adult commensal microorganisms in the small intestine of birds can occur in two weeks and up to four weeks in the ceca. Preventing pathogenic microorganisms from proliferating during this crucial time is important.

2) Increased Commensal Microorganisms

One aspect of improving the intestinal microbial balance is not simply reducing the number of deleterious bacteria, but increasing existing resident populations of beneficial gut microorganisms (Gedek, 1987; Miles, 1993; Sainsbury, 1993). A balanced microbial population is essential to optimize the efficient utilization of the nutrients in a feed (Lev and Briggs, 1956). According to Dawson (1993a), one important aspect of feeding high concentrate diets to ruminants is the prevention of a lactic acid overload. Similar effects might also occur in the bird's crop which is commonly fed high concentrate diets.

For example, Fuller (1973, 1975, and 1977) observed a large number of commensal bacteria in the crop such as *Lactobacillus acidophilus* which adhere to the crop epithelium and produce high levels of lactic acid. The avoidance of excess levels of lactic acid in the crop may be accomplished through the addition of a yeast DFM to the feed. This DFM subsequently stimulates populations of bacteria which can metabolize lactic acid with the consequential stabilization of the pH in the crop. In addition, the yeast's cell wall may also serve as a pH buffer resulting in greater feed efficiency (Cartwright *et al.*, 1986).

3) Decreased Pathogenic Microorganisms

The importance of a well balanced microbiota within the gastrointestinal tract of turkeys has been appreciated for many years (Lev and Briggs, 1956; Miller, 1986a; Sweerczek, 1986). Thirty years ago, the poultry industry began to rely on prophylactic and therapeutic uses of antimicrobial feed additives in order to suppress disease and achieve an increased efficiency of feed utilization. The major benefit of these antimicrobial products is the control of pathogenic microorganisms and the maintenance of certain beneficial gut microorganisms (Roura and Klasing, 1993). It is possible, however, that DFM could replace antibiotics as an

alternative for growth promotion through control of pathogenic bacteria (Hamilton and Proudfoot, 1991).

The control of pathogenic bacteria is especially important during times of stress or when the birds are young. To prevent the establishment of pathogenic microorganisms during these critical times and in order to maintain desired microbial populations, Miles (1993) believes that a constant influx of non-invasive microbes, such as those supplied by DFM, would prevent the transient and low level pathogenic microbes from colonizing. In support of this claim, Harrison *et al.* (1988) have demonstrated that the addition of YC to the diets of cattle decreases the variation in microbial concentrations within the rumen. Piva (1984) has concluded that yeast cultures indirectly reduce the numbers of *Coliform* and *Enterococci* bacteria in the chicken's intestinal tract through the stimulation of antagonistic bacteria towards those pathogenic bacteria. This antagonism is brought on by stimulating the numbers of *Lactobacillus* and *Bacteroid* bacteria which in turn produce metabolic products such as organic acids, short chain fatty acids, and bacteriocin. Thus, the addition of the yeast culture indirectly affects the containment of these *Coliform* and *Enterococci* pathogenic groups. Studies conducted using turkey poults have also shown an improved resistance to Colibacillosis with the use

of a yeast DFM in the feed (Kumar, 1991). In addition, Dawson (1993a) has observed that the yeast cell wall, whether or not metabolically active, can bind to microbial produced toxins within the lumen.

a) Competitive Exclusion

The skin and mucous membranes of the intestines and air passages are among the most readily recognized forms of self defense against pathogenic microbial invasion. Also of great importance are the commensal microorganisms which inhabit most exterior portions of the body (Weinack *et al.*, 1982; Snoeyenbos, 1989). Researchers have studied the impact of oral administrations to chicks and poults at hatch of microorganisms harvested from the excreta of adult chickens and turkeys. This procedure has protected chicks and poults from certain species of *Salmonellae* (Rantala and Nurmi, 1973; Snoeyenbos *et al.*, 1978; Bailey *et al.*, 1988; Stern *et al.*, 1993).

The competitive exclusionary role of certain commensal bacteria have prompted research on feeding supplemental DFM products to compete with pathogenic microorganisms and reduce their numbers (Kung, 1992). Commensal microorganisms in the intestinal tract are in a delicate balance and DFM are thought to maintain those populations (Risley, 1993). Several studies have been conducted in an attempt to

inoculate the young poult with *L. acidophilus* or *L. acidophilus-Streptococcus faecium* combinations in order to establish a quick colonization of the intestine with known beneficial bacteria (Watkins and Miller, 1983; Chesson, 1991). Since *S. faecium* has been shown to be a significant contributor to the commensal microbiota in poults and other young poultry, a DFM of *S. faecium* (M-74) has been proven to be effective in colonizing chick intestines, thereby competing with the transient microbiota for adhesion cites (Mican, 1976; Soerjadi *et al.*, 1982; Owings *et al.*, 1990). In addition, Stern *et al.* (1993) reported a reduction in the colonization of *Campylobacter jejuni* in broilers by inoculating them at hatch with an antagonistic culture of microorganisms. Therefore, the reduction of undesired microorganisms through competitive exclusion is a viable alternative to antibiotics for the poultry industry.

b) Bio-film Formation

Data in support of DFM's ability to reduce the colonization of deleterious bacteria as salmonella continue to be gathered (Izat and Waldroup, 1990), however, further research is required to establish which DFM have this ability and their functions. One theory on a possible mode of action suggests that the DFM become an integral part of a barrier system known as a bio-film. Gedek (1987)

discovered that a bio-film is developed which forms a barrier between the lumen of the intestine and the enterocyte. This bio-film is composed of a mucosal secretion layer (also called the glycocalix) and physically trapped bacteria which fill in the mesh of the supporting structure. The glycocalix is composed mostly of mucin which is secreted by intestinal epithelial cells known as goblet cells. The secreted mucin consists of polypeptides with oligosaccharide side-chains which are cross-linked by disulfide bonds. This mucin may serve as a habitat and nutrient source for the gut microbiota (Carlstedt-Duke, 1989; Savage, 1991; Miles, 1993). Although not well understood, goblet cell numbers in the mucosa of germ-free rats have been shown to be higher than in conventional rats (Larson, 1989). It is very probable that as the microbiota of the intestinal tract is altered through the use of DFM, the gut ultrastructure will change to accommodate this new microbial environment (Scott *et al.*, 1982; Fuller and Coates, 1983; Chesson, 1991).

c) Flocculation of Yeast Cells

Flocculation is the ability in some strains of yeast to aggregate or bind to neighboring yeast cells when growth conditions are unfavorable. In the intestinal tract of poultry, ideal conditions for yeast growth do not occur and

this may lead to yeast flocculation. Miles (1993) stated that aggregation of microorganisms in the intestine may prevent pathogens from securing access to adhesion receptor sites by creating a physical barrier. If in the gut, yeast cells were able to flocculate, this might aid in protecting the microvilli by allowing the yeast to become an integral part of the glycocalx. Consequently, yeast flocculation may be an important factor in the mode of action of yeast DFM fed to breeder hens receiving diets high in calcium. If in fact flocculation is a factor in the mode of action of a yeast DFM, the process associated with pelleting would not inhibit this phenomenon and breeder diets with high concentrations of calcium may enhance the flocculation of yeast cells (Marshall, 1984).

d) Killer Systems of Yeast Cells

Many microorganisms have the capacity to not only compete with other microorganisms, but also to interfere with their growth and productivity through the production of antibiotics (Savage, 1969). Yeast cells have killer systems which destroy other strains of yeast and bacteria. These killer systems can reduce the number of detrimental yeast and bacterial organisms in the gastrointestinal tract of birds supplemented with yeast DFM. The genes associated with the production of the proteins involved with the killer

system are replicated 10 fold when fermentation temperatures exceed 36 C (Piper and Kirk, 1991; Wickner, 1991). Consequently, these proteins would be produced in the gastrointestinal tract because of the high (41.2 C) environmental temperature present, thereby making it possible for yeast DFM to reduce undesirable yeast strains and bacteria through the production of these antibiotics (Whittow, 1986; Slapack *et al.*, 1987; De Wilde, 1990).

e) Decreased Adhesion by Pathogens

Animals are affected by the adhesion of microorganisms to the epithelial surfaces of the body (Savage, 1985). Factors which inhibit the adhesion of certain pathogenic bacteria to the luminal wall also affect the hosts performance. One of these factors is the presence of lipopolysaccharides which has been shown *in vitro* to reduce bacterial cell surface hydrophobicity, thereby decreasing the adhesion of *S. typhimurium* to inanimate objects (Marshall, 1984). *In vivo* work by Oyofo *et al.* (1989a,b) has demonstrated that *S. typhimurium* was prevented from colonizing the intestines of broilers fed d-mannose. This observation is significant because there are large amounts of mannans and glucans in the walls of yeast cells. These carbohydrates are largely unavailable to the turkey and most other livestock, however, they may be utilized by some

bacteria and may also prevent the adhesion of certain pathogenic bacteria (Bui and Galzy, 1990). Although the precise mode of antibacterial action is unclear, a combination of microbiota which include yeast and fungi have indeed demonstrated antagonistic abilities toward such potentially pathogenic bacteria as *E. coli*, *S. enteritidis*, and *S. typimurium*.

3) Yeast Enzymes

The yeast, *S. cerevisiae*, is known for its enzymatic ability to synthesize various products--collectively referred to as biotranformations (De Mot, 1990; Nikolova and Ward, 1992). One such biotransformation is the manufacturing of certain enzymes in the laboratory which are produced under strict conditions, harvested separately from the yeast, and sold as enzyme supplements. The enzymes and inactive dried yeast cells are then sold as health supplements for humans and supplemental feed ingredients for animals, respectively (Johnson, 1977; Tsiomenko *et al.*, 1987; Bentley, 1989). However, the enzyme-producing industry is changing because of the long and costly processes currently required to gain FDA approval of enzyme health supplements. Many manufacturers market their enzyme supplements as DFM and provide a guarantee on the enzymatic activity of their product in order to avoid the costly and

time consuming FDA approval process (Classen, 1992; Gonzalez, 1993).

The addition of enzymes to the feed or water of poultry is becoming a refined practice since birds may produce insufficient quantities of endogenous enzymes, or lack needed enzymes to efficiently utilize certain dietary cereal grains. Under ideal conditions of minimal stress, the turkey could produce sufficient quantities of endogenous enzymes to absorb the required nutrients from high quality diets. Under current poultry management practices, however, stress is unavoidable. As a consequence, the birds are unlikely to produce sufficient endogenous enzymes for optimal efficiency. Stanley *et al.* (1993), for example, have shown that the suppression of body weight and improper organ development in chicks fed diets containing 5 ppm aflatoxin was eliminated by including .1% of a yeast DFM (*S. cerevisiae*) in the diet. This improved performance was also accompanied by increased serum enzymatic activities (alanine transaminase, creatine phosphokinase, and aspartate aminotransferase) in birds fed the yeast DFM. Mycotoxin concentrations in feedstuffs are variable and may account for variations in performance as well as response to yeast DFM products (Thompson, 1993). Microbial challenges from the environment are also capable of creating stress that can affect the commensal microorganisms which aid in the

digestive and absorptive processes. Consequently, it is possible to counteract these problems and optimize bird health and feed efficiency by including both the microbial enzymes and DFM products (combined) in the feed. The inclusion of DFM enzymes may be exploited where alternative, low-quality energy sources such as wheat, sorghum, and barley are used in poultry rations to counteract the antinutritional factors associated with these grains (Classen, 1992).

Gonzalez (1993) has shown an improved utilization of poor quality forages when yeasts (*S. cerevisiae* and *A. oryzae*) were added to the diets of cattle and sheep. In addition, Potter *et al.* (1991) recently fed α-amylase, xylanase, pectinase, and a protease to Large White male turkey poults receiving diets containing 24% crude protein and noted an increased weight gain from day-old to 5 weeks of age. These results indicate that exogenous sources of enzymes from direct or indirect sources (DFM) may provide increased performance when limiting nutrients are released through enzymatic activity and absorbed by the bird. One of the proposed modes of action of a dietary YC is thought to be the production of enzymes such as amylases, proteases, and lipases by the yeast cells in the intestinal tract or the enzymes may already be present in the YC product. Some

manufacturers provide guaranteed enzyme activities with their DFM products (Muirhead, 1992).

The yeast, *S. cerevisiae*, produces: amylase, invertase, maltase isomaltase, enolase, phytase, aldehyde dehydrogenase, α-glucosidases, and glucoamylases. In addition, yeast cells also demonstrate lipolytic activity (Tsiomenko *et al.*, 1987; De Mot, 1990; Antranikian, 1992). The production of these extracellular and intracellular enzymes is dependent upon the yeast's genetics and the environment in which the yeast was cultured (Schaaff *et al.*, 1989). Specific enzymes can be produced industrially from carefully selected or genetically engineered yeast (Hutter and Niederberger, 1986; Finkelman, 1990; Brearley and Kelly, 1991; Wiseman, 1991). The environment may also be altered in order to produce the preferred enzymes or other products. For example, yeast produces α-glucosidase optimally at a temperature of 42 C and a pH of 6.3 while glucoamylases are produced more abundantly at 25 C and the optimal pH is unknown. Furthermore, yeast enzymes are very specific with respect to their substrate as opposed to bacterial or fungal enzymes (Tsiomenko *et al.*, 1987; Slapack *et al.*, 1987; Antranikian, 1992).

Cartwright *et al.* (1986) demonstrated that yeast cell walls exhibit a buffering capacity which could aid in pH maintenance within the intestine. This is significant

because the pH of the yeast cell's environment is critical to understanding the cell's activities (Lyons, 1990). To maintain optimal growth of yeast cells, the pH is maintained at 4.5 even though the intracellular pH of the yeast cell is approximately 6.5. The intracellular pH would indicate that endogenous metabolic enzymes function best at a pH of 6.5. The proteins and enzymes associated with the transport of nutrients into the yeast cell, as well as cell wall synthesis, however, function optimally around pH 4.5. When yeast cells are grown at a pH other than the optimum, cell replication is slowed. At a suboptimal pH, the cells produce: nucleotides, amino acids, vitamins, and lytic enzymes (Peppler, 1982; Williams, 1989). Normally, these compounds would be incorporated into new cells, but when normal growth is impaired, they are excreted to the external environment. When yeast cells are grown in a high pH environment, the cells synthesize enzymes which catalyze the hydrolysis of the yeast's cell wall. The resulting hydrolysis causes destruction of the cells and their subsequent population decrease within the gut. The consequences of cell lysis within the alimentary canal include the release of proteins, polysaccharides, and lipids into the lumen. Under these conditions, important nutrients would then be available to both the host as well as other microorganisms in the intestinal tract (Rose, 1988). This

may explain the increased cellulytic bacterial populations observed when YC is added to the diets of ruminants (Wiedmeier *et al.*, 1987).

4) Increased Palatability

Yeast cultures in the ration of animals are thought to enhance feed palatability. This concept is not surprising because extracts and hydrolysates of yeast have been used as flavor-enhancing agents and condiments in human foods (Oura *et al.*, 1982). The main flavor-enhancers are nucleic acids, nucleotides, and glutamic acid which are produced by the yeast are therefore present in the YC medium (Rose, 1988). The concept of yeast as flavor-enhancers is receiving interest within the poultry industry as the awareness of taste perception by the fowl is being recognized. According to Appleby *et al.* (1992), the fowl has a well developed sense of taste having between 350 and 500 taste buds. In addition, birds do not like acidic or bitter flavors and unlike mammals, will reject saline solutions. Sweet flavors are also unappealing to fowl. However, the enzymes and "nutrilites" contained and produced by the yeast which are maintained in the yeast culture may stimulate appetite through increasing saliva production (Martin, 1992; Risely, 1992a; Jack Garrett, 1993, Diamond V. Mills, Inc., P.O. Box 74408, Cedar Rapids, IA 52407, personal communication).

Studies by Cantor *et al.* (1983) have revealed that poults possess the ability to distinguish between corn-soy diets with or without 2.5% yeast culture. In these diets, poults preferred the diet containing the yeast culture over the diet without the YC after having consumed the diets for one week. More research is needed to provide evidence of the effect of YC on ration palatability for poultry.

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CHAPTER III

THE EFFECT OF AUTOCLAVING A YEAST CULTURE OF *SACCHAROMYCES CEREVISIAE*[XP] ON POULT PERFORMANCE AND THE UTILIZATION OF GROSS ENERGY, NITROGEN, AND SELECTED MINERALS[1]

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ABSTRACT

A study was conducted to determine the effects on nutrient utilization in turkey poults fed a diet containing an autoclaved yeast culture (YC). Isonitrogenous and isocaloric diets consisting of corn-soy (CS, control), CS + YC (1% XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills, Inc., Cedar Rapids, IA) or CS + 1% autoclaved YC (CS + AYC; autoclaved at 121 C and 12 psi for 35 min) were fed to poults from 1 to 28 days of age (DOA). One hundred and twenty Wrolstad Medium White poults were randomly assigned to 24 battery cages (5 poults per cage, 8 cages per diet) at day old. Poults were fed diets containing .3% chromic oxide from 21 to 28 DOA. Feed and fecal samples were collected between days 26 and 28 and analyzed for gross energy (GE), nitrogen (N), calcium (Ca), phosphorus (P), boron (B), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), and zinc (Zn).

Increased ($P<.05$) utilization of GE, dietary Ca, N, P, B, K, Mg, and Mn were observed only in poults fed the CS + YC diet when compared to the CS and CS + AYC diets. These results indicate that autoclaving impairs the ability of the YC to increase the utilization of selected nutrients in poults at 28 DOA.

INTRODUCTION

The results of incorporating a yeast culture (YC) into poultry diets have produced differing responses. Feeding a YC to market turkeys was reported by Brewer (1983) to have no effect. Similarly, Brake (1991) reported no effects in broiler breeder hens fed diets supplemented with a YC or differences in subsequent progeny performance. To the contrary, other investigators have reported beneficial effects of feeding a YC to select lines of turkey breeder hens (Savage and Mirosh 1990a,b; Hayat *et al.*,1993; Bradley and Savage, 1993).

Thayer *et al.* (1978) noted an improved P utilization in breeder turkey hens fed diets supplemented with a YC and attributed this effect to the activity of a YC containing phytase. Approximately 70% of the P contained in plant seeds is in the form of phytate which is nutritionally unavailable to poultry. This lack of P availability has been attributed to the absence of phytase within the gastrointestinal tract of poultry (NRC, 1984; Perney *et al.*, 1993). The strong chelating ability of the phytate molecule also reduces the bioavailability of Ca, Fe, Mn, Cu, Zn, protein, and energy which results in higher nutrient requirements (O'Dell *et al.*, 1964; Savage *et al.*, 1964; Scott *et al.*, 1982; Scott, 1987; Erdman, 1989; Reddy *et al.*, 1989; Power, 1993; Ward, 1993). Consequently, these higher

amounts of dietary nutrients, concomitantly increase the nutrients excreted which exacerbates fecal waste management (Scott *et al.*, 1982; Perney *et al.*, 1993; Ward, 1993). The degradation of phytate by the production of microbial phytase in the intestinal tract is a key factor in the capacity of poultry to utilize the chelated phytate nutrients (Nelson *et al.*, 1968; Thayer and Jackson, 1975; Scott *et al.*, 1982; Muirhead, 1992; Stanley *et al.*, 1993).

A concern in supplementing poultry feeds with YC is the decreased activity of the yeast which occurs when feeds are pelleted. Headen (1992) reported that following heat treatment of a YC (70 C for 15 min), the yeast's metabolic activity (measured as gas production) was unaffected. When higher temperatures were used (90 C) for the same amount of time, the metabolic activity was reduced 40 percent. It remains unclear whether the metabolic activity can be completely inactivated when the YC is subjected to adverse conditions (Mathews and van Holde, 1990). Consequently, the objective of the present study was to determine the effect of feeding a turkey starter diet supplemented with autoclaved YC on body weight, feed to gain ratios, and the nutrient retention of gross energy (GE), nitrogen (N), calcium (Ca), phosphorus (P), boron (B), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), and zinc (Zn) in poults between 1 and 28 days of age (DOA).

MATERIALS AND METHODS

One hundred and twenty straight run day-old Wrolstad Medium White poults (L-line; Hales *et al.*, 1989) were randomly assigned (5 poults per cage) to 24 battery cages with raised wire floors and wing banded for identification. All birds were reared in a common room with continuous light (24L:0D). Feed and water were provided *ad libitum*, and the poults were weighed individually at 1 and 28 DOA. Feed consumptions were measured by cage for the 28 d period and the feed:gain ratios calculated. A mash corn-soy turkey starter diet (Table III.1) formulated to meet the NRC's nutrient requirements (NRC, 1984) was divided into three equal portions. The first portion served as the control diet (CS). Either 1% YC (XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills Inc., Cedar Rapids, IA), or 1% autoclaved yeast culture (AYC) was added to the two remaining portions of the starter diet (CS + YC and CS + AYC), respectively. The YC was autoclaved at 121 C with 12 psi for 35 min. There were 8 cages of poults fed each diet.

Nutrient retentions were determined by the addition of chromic oxide (.3%) as an inert feed marker to each of the three experimental diets and fed to the poults from 21 to 28 DOA. Chromic oxide passes through the digestive system unabsorbed and chemically unchanged which allows the relative concentrations of chromic oxide in the feed and

feces to be used in calculating the amount of feces derived from a given amount of feed (Scott *et al.*, 1982). Fecal samples from each cage were collected for three consecutive days (26 to 28 DOA), homogenized, and dried in a forced air oven at 26.6 C for 24 h. Dried samples from the 3 daily collections were pooled by cage and ground in a Wiley mill (Arthur H. Thomas Co., Scientific Apparatus, Philadelphia, PA) equipped with a 60 mm mesh screen. The ground samples were then stored at -2.2 C until analyses.

Chemical analyses

Three samples of feed and feces were randomly selected from each of the 8 cages per treatment and the samples were analyzed for GE, N, Ca, P, B, Cu, Fe, K, Mg, Mn, and Zn. The gross energies of the 3 feed and fecal samples were determined using an adiabatic oxygen bomb calorimeter (Parr Inst. Co., Moline, IL) according to procedures outlined by the Association of Official Analytical Chemists (AOAC, 1980) and the GE of each diet calculated (Scott *et al.*, 1982). The N content was measured using a Kjeldahl procedure (AOAC, 1980).

Each of the feed and fecal samples was also analyzed for chromium using acid digestion followed by a spectrophotometric analysis described by Czarnocki *et al.* (1961). The concentrations of Ca, P, B, Cu, Fe, K, Mg, Mn,

and Zn were quantitated using an Inductively Coupled Argon Plasma Analyzer (Thermo Jarrell Ash Model 9000, 8 East Forage Parkway, Franklin, MA) in accordance with the instrument's operations protocol.

The percent retentions of each nutrient from the three diets were calculated according to Edwards and Gillis (1959).

Statistical analysis

Twenty-eight day body weights, weight gains, feed to gain ratios, and calculated percentage retentions of GE, N, Ca, P, B, Cu, Fe, K, Mg, Mn, and Zn were analyzed by one-way ANOVA using the General Linear Models procedure of SAS® (SAS Institute, 1988). Data were summarized on a straight-run sex basis and the cages of birds were used as the experimental units. Values determined to be significant were separated using the Protected Least Significant Difference test (SAS, 1988).

RESULTS AND DISCUSSION

Body weights, periodic weight gains, and feed to gain ratios of poults (1 to 28 DOA) fed diets without and with the 1% YC or 1% AYC are summarized in Table III.2. The sex of each poult was determined at the conclusion of the study and there were no differences ($P>.05$) in sex ratios between dietary treatments. Furthermore, no differences in body weights, or weight gains were observed between sexes. There were also no differences in body weights, weight gains, or feed to gain ratios of straight-run poults among the three dietary treatments at 28 DOA.

The percent retentions of GE, N, Ca, P, B, Cu, Fe, K, Mg, Mn, and Zn of poult diets without (CS) or with either YC or AYC are summarized in Table III.3. The addition of 1% YC to the CS diet (CS + YC) increased the percent retentions of GE ($P<.03$), N ($P<.005$), Ca ($P<.05$), P ($P<.05$), B ($P<.01$), K ($P<.004$), Mg ($P<.004$), and Mn ($P<.05$) when compared to poults fed either the CS or CS + AYC diets. The addition of YC or AYC to the control diet did not influence the retention of Cu, Fe, or Zn. Autoclaving the YC prior to its incorporation in the feed impaired its ability to affect nutrient retentions.

Increased efficiency of energy utilization in diets containing a YC have been reported by other investigators (Tonkinson *et al.*, 1965; Charles *et al.*, 1985; Savage *et*

al., 1985) and were confirmed in the present study. The increased N retention in poults supplemented with YC in this experiment also support similar results by Glade and Biesik (1986) who noted an increased N retention when a YC was added to the diet of yearling horses. Ward (1993) cited studies conducted by Van der Klis and Versteegh in 1991, who reported an increased N absorption of 2% in laying hens fed a microbial phytase supplement. Although the percent retention of N was small, the economic savings to the poultry industry would be substantial due to increased nutrient utilization and the subsequent decrease of N in the excreta. The increased retention of N may also be a result of uric acid recycling in the ceca of poultry by microorganisms. Skadhauge (1983) has hypothesized that the amino acids synthesized by the microbes in the ceca of poultry, as well as valuable ions, may be absorbed from the digested uric acid.

Nahashon *et al.* (1993) demonstrated an increased N and Ca retention in SCWL laying hens fed diets containing a direct-fed microbial (DFM) product of bacterial origin. Increased mineral retention were also demonstrated by Guevara *et al.* (1977) as an increased tibial ash content in broilers fed diets deficient in P and supplemented with 3% YC. The results of this experiment confirmed those findings. The increased tibial ash content of birds

supplemented with yeast culture is significant since the normal microbiota of the poultry have little or no role in the degradation of phytate (Reddy *et al.*, 1989). Thayer *et al.* (1978) attributed the increase of P utilization in turkey breeder hens to the presence of YC which facilitated a more efficient utilization of phytate P. Consequently, the results of this and other studies suggest that phytase activity from the YC is active in the degradation of phytate in the gastrointestinal tract of poultry (Scott, 1987).

The increased retention of K observed in this study may be related to the improved performance of animals supplemented with YC in times of stress (Miles and Bootwalla, 1991; Miles, 1993). According to Scott (1987), K is an important nutrient in turkey production and its requirement is increased in times of stress. Although the retention of Mn is not as crucial in turkey production, the increased retention of Mn observed in this study is significant because the absorption of Mn is important for the prevention of "hock disorders" in turkeys (Scott, 1987).

Although the use of yeast, yeast extracts, and yeast cultures in the feed industry has been sporadic over the last forty years, the incorporation of yeast into poultry diets may provide environmental and economic advantages. In the present study, increased ($P<.05$) retentions of percent GE, N, Ca, P, B, K, Mg, and Mn were only observed in the

diet supplemented with 1% YC while the addition of 1% AYC did not affect nutrient retention. These findings suggest that improved nutrient retentions may be due to the additional phytase activity which can be adversely affected by subjecting YC to high temperatures (Ward, 1993) and pressure (autoclaving). Consequently, the practice of pelleting feeds may reduce the phytase activity associated with YC products and subsequently reduce the YC's ability to improve nutrient retention. These findings also suggest that the application of yeast or yeast cultures with phytase activity to poultry diets can also reduce the requirement for total dietary GE, N, Ca, P, B, K, Mg, and Mn and decrease waste disposal challenges currently facing the poultry industry by subsequently reducing nutrients in the excreta (Wenk and Messikommer, 1991; Wyatt, 1992; Cole, 1993).

Table III.1. Composition of mash turkey starter diets without (CS, control) and with 1% yeast culture (CS + YC) and 1% autoclaved yeast culture (CS + AYC)

Ingredients and analysis	CS	CS + YC	CS + AYC
	---------- (%) ----------		
Corn, yellow (8.9% CP)	41.63	41.21	41.21
Yeast culture[1] (12% CP)	...	1.00	...
Yeast culture, autoclaved[2]	...	...	1.00
Soybean meal (46% CP)	47.18	46.71	46.71
Meat and bone meal (50% CP)	6.00	5.94	5.94
Limestone flour	.75	.74	.74
Fat, poultry blend	1.70	1.68	1.68
Monocalcium phosphate (16% Ca, 21% P)	1.90	1.88	1.88
Salt, iodized	.10	.10	.10
Vitamin premix[3]	.30	.30	.30
Trace mineral premix[4]	.05	.05	.05
L-lysine (78.4%)	.15	.15	.15
DL-methionine (98%)	.19	.19	.19
Coban-60® premix[5] (132 g/kg)	.05	.05	.05
Calculated analysis			
CP	27.90	27.94	27.94
ME, kcal/kg	2,837	2,835	2,835
Ca	1.53	1.53	1.53
Available P	.71	.72	.72
Analyzed[6]			
CP	27.05	27.40	27.32

[1] XP yeast culture®, Diamond V. Mills Inc., Cedar Rapids, IA.
[2] XP yeast culture®, autoclaved at 121 C under 12 psi for 35 min.
[3] Supplied per kilogram of diet: vitamin A, 17,220 IU; cholecalciferol, 6,660 ICU; vitamin E, 30 IU; vitamin B_{12}, 0.006 mg; riboflavin, 12 mg; niacin, 115 mg; d-pantothenic acid, 32 mg; menadione bisulfite, 2 mg; folic acid, 0.9 mg; pyridoxine, 3 mg; thiamine, 1.5 mg; biotin, 0.11 mg; choline, 2,500 mg.
[4] Supplied per kilogram of diet: Mn, 160 mg; Zn, 150 mg; Fe, 120 mg; Cu, 135 mg; I, 1.5 mg; Se, 0.3 mg.
[5] Gratuitously provided by Elanco Inc., Indianapolis, IN.
[6] Kjeldahl analysis.

Table III.2. Twenty-eight day body weights, weight gains and feed to gain ratios of poults[1] fed corn-soy diets without (CS) and with 1% yeast culture[2] (CS + YC) or 1% autoclaved[3] yeast culture (CS + AYC) from 1 to 28 days of age

Diet	Body weight	Weight gain	Feed:gain
	---------- (g) ----------		(g:g)
CS	591	539	1.65
CS + YC	575	526	1.70
CS + AYC	565	515	1.71
SEM	11	11	.02
Source of variation	---------- Probabilities ----------		
Diet	NS	NS	NS

[1] Straight-run poults.
[2] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.
[3] XP yeast culture®, autoclaved at 121 C under 12 psi for 35 min.

Table III.3. Retention of GE, N, Ca, P, B, Cu, Fe, K, Mg, Mn, and Zn in poults[1] fed corn-soy (CS) diets without (CS) and with 1% yeast culture[2] (CS + YC) or 1% autoclaved[3] yeast culture (CS + AYC) at 28 days of age

Diet	GE	N	Ca	P	B	Cu	Fe	K	Mg	Mn	Zn
	(%)										
CS	66.7[b]	72.8[b]	63.7[b]	62.3[b]	40.5[b]	55.4	40.3	51.4[b]	50.5[b]	39.6[b]	42.2
CS + YC	73.9[a]	79.3[a]	72.4[a]	69.7[a]	53.1[a]	65.2	52.4	60.4[a]	60.0[a]	54.0[a]	46.8
CS + AYC	65.3[b]	72.0[b]	63.4[b]	61.4[b]	38.6[b]	55.8	36.0	50.7[b]	49.3[b]	42.3[b]	36.9
SEM	1.9	1.0	2.4	2.0	2.2	3.2	4.6	1.3	1.4	3.4	6.8
Source of variation	Probabilities										
Diet	.03	.005	.05	.05	.01	NS	NS	.004	.004	.05	NS

[1] Straight-run poults.
[2] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.
[3] XP yeast culture®, autoclaved at 121 C under 12 psi for 35 min.
[a,b] Means within a column with no common superscripts differ significantly ($P<.05$).

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CHAPTER IV

THE INFLUENCES OF PRE-INCUBATION STORAGE DURATION AND GENOTYPE ON THE HATCHABILITY OF MEDIUM WHITE TURKEY EGGS FROM HENS FED A DIET CONTAINING A YEAST CULTURE OF *SACCHAROMYCES CEREVISIAE*[KP,1]

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ABSTRACT

Two experiments were conducted to determine the effects of feeding turkey breeder hens of different genotypes a diet containing .5% yeast culture (YC) and its effect on pre-incubation egg storage duration (Experiment 1, eggs stored 0-7 and 8-14 days; Experiment 2, eggs stored 0-4, 5-9, and 10-14 days), hen reproductive performance, and the hatchability of fertile eggs.

Wrolstad Medium White turkey hens, representing three distinct genetic lines, Low (L), High (H), and Cross (C), were fed pelleted 15.4% CP corn-soy breeder diets without or with .5% YC (XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills Inc., Cedar Rapids, IA).

Results of Experiment 1 indicated that early embryonic mortality (Days 0-10) of eggs stored from Days 0-7 was reduced ($P<.01$) in the three lines when supplemental YC was provided in the breeder hen's diet. Hatch of fertile eggs was increased ($P<.02$) in the line C eggs stored from Days 0-7 prior to incubation and there was a significant ($P<.04$) genotype-dietary YC interaction.

Results of Experiment 2 were similar to those in Experiment 1, early embryonic mortality was reduced in eggs stored from Days 5-9 in lines H and C. Significant genotype-dietary YC interactions were noted for egg storage times of Days 5-9 ($P<.05$) and 10-14 ($P<.04$). A genotype-

dietary YC interaction ($P<.01$) was also observed when the hatch of fertile eggs was increased in eggs stored from Days 5-9 in lines H and C but not in line L. These results indicate that the breeder hen's genotype and duration of pre-incubation egg storage time are factors to be considered when feeding diets containing a YC to turkey breeder hens.

INTRODUCTION

One of the current challenges facing the poultry industry centers around improving reproductive efficiency. To meet this challenge and maintain efficient feed utilization, the practice of incorporating antimicrobials or other natural products known as "direct-fed microbials" (DFM) into animal feeds has been exercised (Kung, 1992). Currently, DFM products contain bacteria, yeast, or a combination thereof in dry or liquid forms (Muirhead, 1992). Yeast cultures (YC) are a unique form of DFM which contain viable yeast cells, metabolites, and the media on which the yeast cells were grown (Miles and Bootwalla, 1991).

Research findings regarding the effects of incorporating a YC into poultry diets have been inconsistent. Thayer *et al.* (1978), Brewer (1983), and Brake (1991) reported no effect of dietary YC in balanced turkey and chicken diets. In contrast, other studies have described improved fatty acid digestibility (Tonkinson *et al.*, 1965), reduced abdominal fat content (Savage *et al.*, 1985), enhanced feed efficiency (Day, 1977), improved internal egg quality (Miles and Bootwalla, 1991), and increased organic phosphorus utilization in chicken and turkey hens (Thayer and Jackson 1975; Thayer *et al.*, 1978). More recently, specific studies have shown that YC can influence turkey breeder hen and egg hatchability traits

(parthenogenetic development, fertility, and hatchability) in specific lines (Savage and Mirosh 1990 a,b; Hayat *et al.*, 1992; Bradley and Savage, 1993; Savage *et al.*, 1993). Hayat *et al.* (1993) reported that the beneficial effect of feeding a dietary YC to turkey hens may be influenced by the bird's genome and recommended further studies.

Hatchability of fertile eggs declines as the length of egg storage time prior to incubation is extended (Landauer, 1967). That decline in the hatchability is also influenced by the hen's genotype (Yoo and Wientjes, 1991). Improved management practices including the hen's diet may increase the hatchability of eggs which otherwise could not withstand extended pre-incubation egg storage. In a preliminary report, Bradley and Savage (1993) demonstrated that supplementation of a turkey breeder hen's diet with a YC influenced the hatchability of eggs stored for an extended period of time.

This report describes two experiments conducted to verify the findings of Hayat *et al.* (1993) that the reproductive effects resulting from supplementing a turkey breeder hen's diet with .5% YC are mediated by the hen's genotype. Further, that feeding of a YC can influence the effect of pre-incubation storage on subsequent hatchability.

MATERIALS AND METHODS

Management Procedure

Three lines of Wrolstad Medium White turkey hens from consecutive generations of a divergent selection program for semen ejaculate volumes, Low (L) and High (H) (Hales *et al.*, 1989) and a reciprocal line cross (C; Hayat *et al.*, 1993) were used in two experiments (1 and 2) conducted in consecutive years, 1992 and 1993, respectively. The bird management and feeding programs from day of age to 30 weeks of age (WOA) and facilities used have been previously described (Hayat *et al.*, 1993). In experiment 1, the line C poults were hatched 2 weeks later than lines L and H. The same formulated breeder hen diets were fed in both experiments and were calculated to be isonitrogenous and isocaloric without and with .5% yeast culture (XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills Inc., Cedar Rapids, IA), Table IV.1. The feeds were mixed every four weeks to insure quality.

In Experiment 1, hens were randomly assigned to 20 pens (n=9 hens per pen) with 3 or 4 pens per line per diet. The hens were fed breeder diets without and with .5% YC starting at 38 WOA in L and H lines and 36 WOA in line C. Hen photo-stimulation commenced at 34 WOA (lines L and H) and at 32 WOA (line C), respectively. Eggs were set bi-weekly from

hens between 42 and 52 WOA in the lines L and H and between 40 and 50 WOA in line C (5 hatches). The egg storage interval prior to incubation (14 Days) was separated into two 7-day periods and the eggs identified by the length of egg storage: Days 0-7 and 8-14, respectively.

In Experiment 2, all hens were randomly assigned to 18 pens (n=10 hens per pen, with 3 pens per line per diet), fed the breeder diets with and without .5% YC, and photo-stimulated at 31 WOA. Eggs were collected daily and set bi-weekly from all hens between 37 and 51 WOA (7 hatches). The pre-incubation egg storage interval (14 Days) was partitioned into three periods, Days 0-4, 5-9, and 10-14, and the eggs were marked for identification according to the respective egg storage period.

Different males were used in both experiments were fed a 14% crude protein diet which did not contain any YC. The artificial inseminations of the hens were begun between Days 10 and 14 after oviposition and repeated on a weekly basis as previously described (Hayat *et al.*, 1993).

Variables

Individual hen body weights were measured bi-weekly from 36 to 52 WOA in Experiment 1, and at 4 week intervals (periods) between 30 and 51 WOA in Experiment 2. Feed consumptions were recorded by pens when body weights were

determined. Egg production for each pen was recorded daily and summarized by feeding period and feed (kg) per dozen eggs was calculated based upon an adjusted mean weight of 75 g per egg.

Following the onset of oviposition, the hens were observed throughout the experiments for the occurrence of prolapsed oviducts and broodiness as described by Hayat *et al.* (1993). Egg collection, sanitation procedures, and incubation of eggs for both experiments were also in accordance with the description provided by Hayat *et al.* (1993). All eggs from both experiments were stored in the same storage room maintained between 10 and 13 C and 80 and 85% relative humidity until incubation. Immediately prior to setting, eggs from each pen were enumerated and weights were determined according to pre-incubation egg storage period.

Statistical Analysis

Data from each experiment were analyzed separately by analysis of variance using the General Linear Models procedure from Statistical Analysis Systems Institute (SAS, 1988). All variables were analyzed by blocking across time with the exception of body weight change. Arc sine transformation of percentage data prior to analysis had no affect on the results obtained, therefore, the actual data

percentages were used for all statistical analyses. Means determined to be different were separated using the least significant difference test (SAS, 1988).

RESULTS AND DISCUSSION

Breeder Hen Performance

The change in mean hen body weight and reproductive performance traits from hens receiving corn-soy bean diets without or with .5% YC for Experiments 1 and 2 are summarized in Table IV.2. The inclusion of a dietary YC did not influence the change in mean body weight, hen-day egg production, or mean egg weight within lines during either experiment when compared to those hens not receiving the YC diet. There were no genetic line differences for rate of egg production for either experiment, but line differences were evident in mean egg weight. The mean egg weights of line L were heavier ($P<.05$) than those of lines H and C, Experiment 1. In Experiment 2, mean egg weights were lower ($P<.02$) in line H than those of lines L and C. Genetic line differences in feed efficiency were apparent, the line C hens were most efficient ($P<.01$) in Experiment 1 and least efficient ($P<.04$) in Experiment 2. These observations are similar to those reported by Hayat *et al.* (1993).

There were no differences in the incidence of prolapsed oviducts, broodiness, or hen mortality as a result of dietary YC supplementation or hen genotype in either experiment.

Experiment 1

Incubation Performance

Fertility and embryonic mortality. True fertility, determined at Day 10 of incubation (summarized in Table IV.3) for eggs stored from Days 0-7 and 8-14 prior to incubation were not different with the addition of .5% dietary YC supplementation or between genetic lines.

The early embryonic mortality measured at Day 10 of incubation (Table IV.4) indicated a genetic line difference, the H line hens experienced a higher ($P<.01$) early embryonic mortality than lines L and C. Diets also influenced early mortality. Eggs from hens supplemented with the YC had a reduced ($P<.01$) early embryonic mortality for the pre-incubation egg storage period, Days 0-7. The addition of the YC reduced early embryonic mortality within lines from eggs stored from Days 0-7 in line H ($P<.07$) and in line C ($P<.06$).

Late embryonic mortality (Days 21-28) is summarized in Table IV.5. Line differences ($P<.05$) were apparent for both pre-incubation egg storage time periods while the YC diet had no effect.

The incidences of pipped embryos in the three genetic lines fed diets without and with the supplemental YC are summarized in Table IV.6. The percent of pipped embryos in

the lines L and H were higher (P<.01) than the C eggs stored from Days 0-7. The incidence of pipped embryos in eggs stored from Days 8-14 increased in line L hens fed the YC diet, but was unaffected or reduced in lines H and C, respectively. Therefore, a significant (P<.04) genotype-dietary YC interaction was noted for eggs stored from Days 8-14.

Hatch of Fertile Eggs. Hatchability differences among lines were present for both egg storage periods, Table IV.7. The line H sustained the lowest (P<.01) hatchability in both pre-incubation egg storage periods. Feeding the YC diet increased (P<.02) the line C hatchability of fertile eggs stored between Days 0-7, only. The dietary supplementation of YC reduced hatchability in line L and increased it in line C resulting in a significant (P<.04) genotype-dietary YC interaction of eggs stored from Days 0-7.

Experiment 2

Incubation Performance

Fertility and embryonic mortality. The effects of feeding .5% YC to the three lines of hens on hen fertility are also summarized in Table IV.3. Fertility in line H was significantly lower than lines L and C for eggs stored from Days 0-4 (P<.01), Days 5-9 (P<.09), and Days 10-14 (P<.06).

The addition of YC to the diet of line H hens increased ($P<.04$) the fertility in eggs stored Days 5-9. Fertility in line L, however, was reduced ($P<.07$) in hens receiving diets supplemented with YC in eggs stored Days 0-4. These lines responded differently to the same dietary YC resulting in a significant genotype-dietary YC interaction for eggs stored Days 0-4 ($P<.06$), Days 5-9 ($P<.04$), and Days 10-14 ($P<.06$) prior to incubation.

Early embryonic mortality, summarized in Table IV.4, shows that line H embryos experienced higher ($P<.01$) early embryonic mortality for the pre-incubation egg storage periods of Days 0-4 and 5-9. Feeding the dietary YC reduced early embryonic mortality of eggs stored from Days 5-9 ($P<.04$) and Days 10-14 ($P<.06$) in line H and from Days 5-9 ($P<.01$) in line C. There were significant genetic line-dietary YC interactions during pre-incubation egg storage periods of Days 5-9 ($P<.05$) and Days 10-14 ($P<.04$).

There were line differences for late embryonic mortality in eggs stored from Days 0-4 ($P<.04$) and 5-9 ($P<.06$), Table IV.5. This mortality was reduced ($P<.06$) in line C hens fed the dietary YC for the pre-incubation egg storage time of Days 10-14.

The percent pipped embryos was influenced ($P<.03$) by genetic lines in eggs stored from Days 5-9 as well as the feeding of the YC, Table IV.6. The YC reduced the incidence

of pipped embryos in line L for eggs stored from Days 0-4 ($P<.05$) and in line C for eggs stored Days 5-9 ($P<.07$).

Hatch of Fertile Eggs. Line differences ($P<.01$) in hatchability for eggs stored from Days 0-4 were observed, Table IV.7. The dietary YC increased the hatch of fertile eggs stored from Days 5-9 in lines H ($P<.01$) and C ($P<.05$) only. There was also a genotype-dietary YC interaction ($P<.01$) for hatchability of eggs stored from Days 5-9. These results indicate that the genetic characteristics of the bird influence the effect of the YC on the subsequent hatchability of fertile eggs.

Poult Quality and Livability

The effect of hen genotype and dietary YC on the incidence of poults exhibiting unhealed navels at hatching when eggs were stored Days 0-14 prior to incubation are summarized in Table IV.8. Unhealed navels is a condition observed in newly hatched chicks and poults usually associated with incubation and results in birds that cannot be utilized. The occurrence of unhealed navels was higher ($P<.01$) in line H as compared to lines L and C in Experiment 1 and higher ($P<.05$) than the L line in Experiment 2. The inclusion of .5% dietary YC increased ($P<.08$) the incidence of unhealed navels in line H and numerically reduced the occurrence of unhealed navels in lines L and C,

Experiment 2. This resulted in a significant ($P<.05$) line-dietary YC interaction for the incidence of unhealed navels. There were no differences in post hatching livability (Days 1-10 of age) among poults from hens fed diets without or with .5% YC. The absence of an effect of a dietary YC in maternal diets on progeny performance was also noted by Brake (1991).

The results of these experiments are in agreement with the those of Hayat *et al.* (1993) and confirm that the hatchability of eggs from hens fed a dietary YC can be mediated by the hen's genotype. These results indicate that the animal's genotype should be considered when feeding diets supplemented with a YC. Further, the addition of a YC in the turkey breeder hen's diet appears to have beneficial effects for eggs subjected to pre-incubation storage periods from Days 5-9. This finding suggests that further studies are needed to evaluate the relationship of the breeder hen's diet and pre-incubation egg storage periods in order to provide optimal hatchability. These studies, which evaluated the effect of a dietary YC, provide further incentives which warrant additional investigative studies of YC for enhancing efficient animal production. It may also prove important to identify the YC or other DFM product used if comparisons of experimental findings are conducted.

Table IV.1. Composition of pelleted turkey breeder hen corn-soy bean meal diets[1] without (CS) and with .5% yeast culture (CS + YC)

Ingredients and analysis	CS	CS + YC
	---------- (%) ----------	
Corn, yellow (8.9% CP)	76.25	75.87
Yeast culture[2] (12% CP)	. . .	.50
Soy-bean meal (47% CP)	9.33	9.29
Meat and bone meal (50% CP)	5.08	5.05
Limestone flour	4.14	4.12
Fish meal (65% CP)	2.54	2.53
Fat (blended, animal)	1.01	1.01
Vitamin premix[3]	.53	.53
Monocalcium phosphate (16% Ca, 21% P)	.49	.48
Salt, iodized	.41	.40
Trace mineral premix[4]	.10	.10
DL-methionine (98%)	.02	.02
Selenium premix (200.2 mg/kg)	.05	.05
Biotin premix (220.4 mg/kg)	.05	.05
Calculated analysis		
CP	15.40	15.40
ME, kcal/kg	3,076	3,060
Linoleic acid	1.45	1.45
Ca	2.42	2.41
Available P	.54	.54
Analyzed[5]		
CP, Experiment 1	14.05	13.40
CP, Experiment 2	14.23	14.28

[1] Manufactured by Kropf Feed and Seed, Inc., Harrisburg, OR.
[2] XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills Inc., Cedar Rapids, IA.
[3] Supplied per kg of diet: vitamin A, 8 745 IU; vitamin D_3, 2 915 ICU; riboflavin, 9 mg; d-pantothenic acid, 15 mg; niacin, 58 mg; choline, .58 g; vitamin B_{12}, .015 mg; vitamin E, 2.92 IU; vitamin K, 1 mg; folacin, .58 mg; ethoxyquin, 165 mg.
[4] Supplied per kg of diet: Ca, 1.03 g; Mg, .64 g; Fe, .21 g; I, 13 mg; Zn, .29 g; Co, 2 mg; Cu, 21 mg.
[5] Kjeldahl analysis.

Table IV.2. Influence of corn-soy diets without (CS) and with .5% yeast culture[1] (CS + YC) on mean body weight change, hen day egg production, egg weight, and feed efficiency in three lines of Wrolstad Medium White breeder hens

Line	Diet	Body weight change[2] (kg)	Hen day egg production[3] (%)	Egg weight[3] (g)	Feed per dozen eggs[4] (kg)	Body weight change[5] (kg)	Hen day egg production[6] (%)	Egg weight[6] (g)	Feed per dozen eggs[4] (kg)
		Experiment 1				Experiment 2			
Low	CS	-.78	59.62	77.86	4.08	-.37	60.87	76.18	3.82
	CS + YC	-.80	59.13	77.49	4.07	-.20	60.60	76.10	4.21
	SEM	.06	3.90	.43	.53	.11	2.11	.49	.22
High	CS	-.81	58.00	75.52	3.99	-.29	58.73	75.54	4.13
	CS + YC	-.69	57.12	75.38	4.23	-.26	57.87	75.16	4.14
	SEM	.08	3.50	.38	.34	.15	1.52	.35	.18
Cross	CS	-.82	60.98	75.33	3.78	-.03	57.73	76.05	4.48
	CS + YC	-.73	59.31	74.90	3.96	-.58	58.13	75.68	4.67
	SEM	.06	3.18	.28	.44	.29	2.73	.41	.25
Source of variation		Probabilities							
Line		NS	NS	<.05	<.01	NS	NS	<.02	<.04
Diet		NS	NS	NS	NS	NS	NS	NS	NS
Interaction		NS	NS	NS	NS	NS	NS	NS	NS

[1] XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills Inc., Cedar Rapids, IA.
[2] Measured at 38 and 52 WOA in L and H lines and at 36 and 50 WOA in line C.
[3] Recorded daily in lines L and H from 36 to 52 weeks of age (WOA) and in the C line from 34 to 50 WOA.
[4] Calculated as the feed per dozen eggs having a mean weight of 75 g per egg.
[5] Measured at 32 and 51 WOA.
[6] Measured bi-weekly from 37 to 51 WOA.

Table IV.3. Fertility (%) in three lines of Wrolstad Medium White hens fed diets without (CS) or with .5% yeast culture[1] (CS + YC)

Line	Diet	Days of Pre-incubation Egg Storage Time				
		0-7	8-14	0-4	5-9	10-14
		-- Experiment 1 ---		---------- Experiment 2 ----------		
Low	CS	95.25[a]	94.79[a]	95.70[a]	94.50[a]	97.10[a]
	CS + YC	95.41[a]	93.86[a]	92.20[a]	90.70[a]	94.50[a]
	SEM	1.25	.85	1.08	1.45	1.50
High	CS	96.30[a]	95.58[a]	85.60[a]	83.60[b]	86.10[a]
	CS + YC	94.12[a]	94.94[a]	90.40[a]	91.70[a]	94.50[a]
	SEM	1.41	1.33	2.63	2.35	2.59
Cross	CS	96.18[a]	94.93[a]	94.50[a]	91.50[a]	92.60[a]
	CS + YC	94.89[a]	94.24[a]	93.20[a]	91.60[a]	94.50[a]
	SEM	1.30	1.48	1.28	1.94	1.34
Source of variation		---------- Probabilities ----------				
Line		NS	NS	<.01	<.09	<.06
Diet		NS	NS	NS	NS	NS
Interaction		NS	NS	<.06	<.04	<.06

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.
[a,b] Means in columns within lines with no common superscripts differ significantly (P<.05).

Table IV.4. Early (0-10 d) embryonic mortality (%) in three lines of Wrolstad Medium White hens fed diets without (CS) or with .5% yeast culture[1] (CS + YC)

Line	Diet	Days of Pre-incubation Egg Storage Time				
		0-7	8-14	0-4	5-9	10-14
		--- Experiment 1 ---		---------- Experiment 2 ----------		
Low	CS	4.67[a]	6.03[a]	6.14[a]	7.09[a]	6.47[a]
	CS + YC	3.03[a]	3.21[a]	6.78[a]	9.10[a]	10.20[a]
	SEM	.45	1.15	.52	1.82	1.12
High	CS	7.77[a]	9.83[a]	12.56[a]	16.55[a]	12.79[a]
	CS + YC	6.88[a]	10.56[a]	8.90[a]	10.24[b]	9.21[a]
	SEM	.43	1.05	1.82	1.66	.94
Cross	CS	6.55[a]	4.21[a]	8.92[a]	10.82[a]	10.65[a]
	CS + YC	3.75[a]	5.32[a]	6.73[a]	4.91[b]	11.37[a]
	SEM	.79	.66	1.10	1.02	1.34
Source of variation		---------- Probabilities ----------				
Line		<.01	<.01	<.01	<.01	<.10
Diet		<.01	NS	<.10	<.03	NS
Interaction		NS	NS	NS	<.05	<.04

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.
[a,b] Means in columns within lines with no common superscripts differ significantly (P<.05).

Table IV.5. Late (21-28 d) embryonic mortality (%) in three lines of Wrolstad Medium White hens fed diets without (CS) or with .5% yeast culture[1] (CS + YC)

Line	Diet	Days of Pre-incubation Egg Storage Time				
		0-7	8-14	0-4	5-9	10-14
		----- Experiment 1 -----		---------- Experiment 2		---------
Low	CS	8.57[a]	13.52[a]	7.66[a]	8.56[a]	12.04[a]
	CS + YC	9.84[a]	15.79[a]	8.42[a]	10.36[a]	12.02[a]
	SEM	1.72	1.79	1.66	.93	1.76
High	CS	13.90[a]	18.71[a]	12.90[a]	14.10[a]	11.64[a]
	CS + YC	16.57[a]	18.16[a]	14.11[a]	10.27[a]	14.26[a]
	SEM	.82	1.99	2.64	2.14	2.15
Cross	CS	16.89[a]	14.49[a]	11.40[a]	15.15[a]	17.64[a]
	CS + YC	13.72[a]	13.97[a]	8.09[a]	11.97[a]	13.24[a]
	SEM	1.40	1.50	1.70	1.85	1.16
Source of variation		---------- Probabilities ----------				
Line		<.01	<.05	<.04	<.06	NS
Diet		NS	NS	NS	NS	NS
Interaction		NS	NS	NS	NS	NS

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.
[a,b] Means in columns within lines with no common superscripts differ significantly (P<.05).

Table IV.6. Incidence of pipped embryos (%) in three lines of Wrolstad Medium White hens fed diets without (CS) or with .5% yeast culture[1] (CS + YC)

Line	Diet	Days of Pre-incubation Egg Storage Time				
		0-7	8-14	0-4	5-9	10-14
		----- Experiment 1 -----		---------- Experiment 2 ----------		
Low	CS	13.09[a]	13.32[a]	14.12[a]	15.18[a]	15.40[a]
	CS + YC	17.20[a]	20.35[a]	10.44[b]	14.78[a]	14.88[a]
	SEM	2.24	2.32	1.04	1.63	2.23
High	CS	14.49[a]	12.88[a]	11.46[a]	12.44[a]	15.84[a]
	CS + YC	14.27[a]	11.72[a]	15.19[a]	11.18[a]	17.26[a]
	SEM	1.75	2.25	2.41	1.14	4.60
Cross	CS	10.33[a]	14.97[a]	10.30[a]	13.83[a]	14.71[a]
	CS + YC	9.57[a]	12.75[a]	8.98[a]	9.31[a]	10.83[a]
	SEM	1.30	.93	1.45	1.48	2.28
Source of variation		---------- Probabilities ----------				
Line		<.01	<.06	NS	<.03	NS
Diet		NS	NS	NS	<.08	NS
Interaction		NS	<.04	NS	NS	NS

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.
[a,b] Means in columns within lines with no common superscripts differ significantly (P<.05).

Table IV.7. Hatchability of fertile eggs (%) in three lines of Wrolstad Medium White hens fed diets without (CS) or with .5% yeast culture[1] (CS + YC)

Line	Diet	Days of Pre-incubation Egg Storage Time				
		0-7	8-14	0-4	5-9	10-14
		------ Experiment 1 -----		---------- Experiment 2 ----------		
Low	CS	74.20[a]	68.26[a]	70.90[a]	68.69[a]	65.20[a]
	CS + YC	71.00[a]	62.42[a]	73.70[a]	63.51[a]	61.00[a]
	SEM	2.34	2.52	2.02	2.53	3.06
High	CS	64.21[a]	59.25[a]	59.83[a]	55.40[b]	57.20[a]
	CS + YC	63.61[a]	59.78[a]	59.68[a]	67.27[a]	57.56[a]
	SEM	1.85	.76	2.95	1.94	3.96
Cross	CS	66.52[b]	67.36[a]	67.95[a]	59.19[b]	54.92[a]
	CS + YC	74.17[a]	68.45[a]	73.02[a]	71.71[a]	61.58[a]
	SEM	1.44	2.32	2.77	3.57	3.01
Source of variation		---------- Probabilities ----------				
Line		<.01	<.01	<.01	NS	NS
Diet		NS	NS	NS	<.01	NS
Interaction		<.04	NS	NS	<.01	NS

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.
[a,b] Means in columns within lines with no common superscripts differ significantly (P<.05).

Table IV.8. Incidence of poults hatched with unhealed navels in three lines of Wrolstad Medium White hens fed diets without (CS) and with .5% yeast culture[1] (CS + YC)

Line	Diet	Number of Poults Examined[2]	Unhealed Navels[3] (%)	Number of Poults Examined[4]	Unhealed Navels[3] (%)
		------- Experiment 1 -------		------- Experiment 2 -------	
Low	CS	1236	7.27	847	13.27
	CS + YC	900	6.24	835	9.64
	SEM		.90		1.89
High	CS	811	18.38	652	16.35
	CS + YC	748	15.02	745	22.09
	SEM		1.86		2.25
Cross	CS	779	7.91	662	17.50
	CS + YC	1189	7.74	712	12.71
	SEM		.80		2.94
Source of variation		---------- Probabilities ----------			
Line			<.01		<.01
Diet			NS		NS
Interaction			NS		<.05

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.
[2] n = 5 hatches.
[3] Unhealed navels were defined as open navels exhibiting incompletely retracted yolk sacs.
[4] n = 7 hatches.

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CHAPTER V

THE EFFECTS OF SUPPLEMENTING DIETS WITH *SACCHAROMYCES CEREVISIAE* VAR. *BOULARDII* ON MALE POULT PERFORMANCE AND ILEAL MORPHOLOGY[1]

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ABSTRACT

A study was conducted to determine the effects of three levels of supplemental yeast of *Saccharomyces cerevisiae* var. *boulardii* (SCB) on commercial male poult performance and morphology of the ileum. One hundred and sixty Nicholas (line cross 50-0602) poults were randomly assigned to 16 battery cages (10 poults per cage, 4 cages per diet) from 1 to 21 days of age (DOA). Poults were fed diets (26% CP) consisting of corn-soy (CS, control), CS + .01% SCB, CS + .02% SCB, and CS + .06% SCB. At 21 DOA, a total of thirty poults fed the CS and CS + .02% SCB diets (the optimal level of SCB from 1 to 21 DOA) were randomly selected within each diet, placed in one of 6 cages (5 poults per cage, 3 cages per diet) and fed their respective diet to 35 DOA. Body weights, and feed consumptions were measured weekly between 21 and 35 DOA and morphologic comparisons of ileal tissues were conducted at 35 DOA.

Increased body weights ($P<.004$) at 7, 14 and 21 DOA were observed for poults fed diets containing SCB at .01 .02 and .06% of the diet. No dietary differences ($P>.05$) were observed in feed consumption or feed to gain ratios from 1 to 21 DOA. Increased ($P<.03$) body weights were maintained from 21 to 35 DOA for poults fed .02% SCB, while no dietary differences ($P>.05$) in feed consumption or feed to gain ratios were observed. Histological examination of ileal

sections from poults (35 DOA) fed the CS and CS + .02% SCB diets revealed a decrease ($P<.04$) in the number of goblet cells per mm of villus and a decreased ($P<.02$) crypt depth in poults receiving .02% SCB. No dietary differences ($P>.05$) were observed for either villus height or width. The results of this study indicate that the addition of SCB to the diet of poults increased body weights from 1 to 35 DOA and altered ileal morphology.

INTRODUCTION

Saccharomyces cerevisiae var. *boulardii* (SCB) is a nonpathogenic yeast capable of preventing diarrhea associated with therapeutic antibiotic administration in hospital patients (Surawicz *et al.*, 1989). According to investigators, the administration of SCB to patients undergoing oral antibiotic administration (in which the normal gut microbiota are being disrupted) prevents the proliferation of pathogenic bacteria. In addition, SCB has been reported to reduce mortality resulting from toxicogenic strains of *Clostridium difficile* in hamsters (Toothaker and Elmer, 1984).

There are limited reports of improved bird performance and decreased proliferation of pathogenic bacteria, resulting from the addition of yeast and yeast products (yeast cultures) containing different strains of *Saccharomyces cerevisiae* in poultry feeds (Hamilton and Proudfoot, 1991; Miles and Bootwalla, 1991; Miles, 1993). Stanley *et al.* (1993) reported increased body weights and a decrease in aflatoxicosis of broiler chicks fed diets containing .1% *S. cerevisiae*. However, because SCB is a newly developed yeast strain for use in monogastric feeds, reports on the effects of incorporating this variety (*boulardii*) of *S. cerevisiae* in poultry feeds are limited. Madrigal *et al.* (1993) reported an improved feed utilization

when broiler chicks were fed mash diets containing 50, 100 and 200 g per ton of SCB from 1 to 49 d of age. To date, there have not been any published research on the use of SCB in poult diets.

It is very probable that as the microbiota of the intestinal tract is altered through the use of direct-fed microbials (DFM) such as SCB, gut microstructure may also change in order to adapt to this new microbial environment (Scott *et al.*, 1982; Fuller and Coates, 1983; Chesson, 1991). One such change, although not well understood, has been observed in studies involving germ-free rats in which higher goblet cell numbers of the intestinal villi were observed as compared to those in conventional rats (Larson, 1989). The mucin produced by goblet cells may be important to the gut microbiota because it serves as both a habitat and a nutrient source (Carlstedt-Duke, 1989; Savage, 1991; Miles, 1993). There is no literature, however, describing the effects, if any, of SCB on intestinal morphology of the fowl.

Because of the lack of information on the effects of SCB in turkeys, the following study was conducted to determine the effects of feeding 26% CP diets supplemented with three levels of SCB (.01, .02, and.06%) on weight gain, feed to gain ratios from 1 to 21 days of age (DOA) and to observe the effects of continued feeding of .02% SCB on body

weight, periodic feed to gain ratios (21 to 35 DOA), and ileal microscopic morphology at 35 DOA.

MATERIALS AND METHODS

One hundred and sixty Nicholas line cross (50-0602) male poults were wing banded for identification and randomly assigned to 16 battery cages with raised wire floors (10 poults per cage, 4 cages per diet). Birds were brooded in a temperature-regulated room with continuous light (24L:0D). Both feed and water were provided *ad libitum*. From 1 to 21 DOA, poults were fed isonitrogenous, crumbled diets (26% CP) consisting of corn-soy (CS, control) with SCB (Levucell SB20®, *Saccharomyces cerevisiae* var. *boulardii*, Agrimerica, Inc., Northbrook, IL.) added to the diets at .01, .02, and .06% (as recommended by the manufacturer of SCB), Table V.1. In the feed pelleting process, the pelleting temperature was maintained at or below 54 C. The poults were weighed individually at 1, 7, 14, 21, and 35 DOA. Feed consumptions were measured by cage at 7, 14, 21, and 35 DOA; and the feed to gain ratios calculated.

At 21 DOA, poults receiving diets containing .02% SCB demonstrated the greatest response in body weight gains. Consequently, birds receiving the diets without (CS) and with .02% SCB (CS + .02% SCB) were randomly sub-sampled and assigned to 6 battery cages (5 poults per cage, with 3 cages per diet). These poults continued to receive the CS and CS + .02% SCB diets from 21 to 35 DOA. At the conclusion of the study (35 DOA), one poult from each cage (3 per diet)

was randomly selected and euthanatized. Since most nutrient absorption occurs in the small intestine, this area was selected for histologic examination. Ileal tissue samples were immediately dissected from the small intestine. One section from each poult was cut just cranial to Meckel's diverticulum to approximately 2.75 cm caudal to Meckel's diverticulum, and placed in 10% neutral buffered formalin. Following histologic fixation, the tissues were processed through a standard alcohol dehydration-toluene sequence, and embedded in paraffin. Longitudinal ileal sections (5 μm) were cut parallel to Meckel's diverticulum and subsequently stained with periodic acid-Shiff (PAS).

The procedures used to quantitate the number of goblet cells, villus height, villus width, and crypt depth are described as follows. Three photomicrographic images were prepared at random from each sample, using a magnification factor of 64X, midway between the villus tip and its base. The number of goblet cells within .01 mm of the apical surface along the perimeter of each of the three randomly selected villi from each photomicrograph were then quantitated. The number of goblet cells per mm of epithelium was then calculated. Using a magnification factor of 16X, three photomicrographic images were recorded at random from each longitudinal tissue section such that one side of the entire section was clearly visible. Three

measurements were randomly taken of the following morphological features: 1) the villus tip to the muscularis mucosae (villus height + crypt depth), 2) the tip of the villus to its base (villus height), and 3) the width of the villus at the point midway between the villus tip and its base. Crypt depth was then calculated as villus height + crypt depth minus villus height. All distances were calibrated using photomicrographs of a stage micrometer recorded at each magnification factor used (64X and 16X). The bird means for each of the histological variables were used for statistical comparison between treatments.

Statistical analysis

Data from poult performance and morphologic measurements were analyzed by one-way ANOVA using the General Linear Models procedure of SAS® (SAS Institute, 1988). Cages and birds were used as the units in the analysis of poult performance and ileal morphology, respectively. Since the data did not appear to be linear, pairwise comparisons were used. Mean values determined to be significant were separated using the Protected Least Significant Difference test (SAS, 1988).

RESULTS AND DISCUSSION

Body weights and cumulative feed to gain ratios of poults from 1 to 21 DOA fed diets without and with .01, .02, or .06% SCB are summarized in Table V.2. Increased ($P<.004$) body weights were observed at 7 and 21 DOA in poults fed the three diets containing SCB, while at 14 DOA only the body weights of poults receiving .02% SCB were greater ($P<.004$) than the control fed birds. Potter *et al.* (1991) has reported increased body weights in poults fed CP deficient diets supplemented with enzymes, therefore, these results may be due to increased enzymatic activity in the birds receiving the supplemental SCB. There were no dietary differences ($P>.05$) in feed to gain ratios from 1 to 21 DOA (Table V.2). The body weights and feed consumptions of the poults studied from 21 to 35 DOA were measured at the conclusion of the experiment (35 DOA). Body weights were greater ($P<.03$) at 35 DOA for poults fed diets containing .02% SCB as compared to those fed the control diet (1411 and 1333 g, respectively). No differences ($P>.05$) in weight gain, feed consumptions, or feed to gain ratios were observed between 21 and 35 DOA.

Mean ileal goblet cell numbers, villus height, and crypt depth of poults (35 DOA) fed diets without and with .02% SCB are summarized in Table V.3. A reduction ($P<.05$) in the number of goblet cells per mm of villus height and a

decrease (P<.02) in crypt depth were apparent in the ileal sections of poults fed the .02% SCB diet, Figures V.1 and V.2. The villus height and width were not influenced by the addition of SCB to the diet.

A decrease in the number of goblet cells in the ileum of poults fed the .02% SCB may be indicative of an alteration in the microbial ecology within the lumen of the small intestine resulting from the presence of SCB (Larson, 1989). Furthermore, because the crypts of Lieberkuhn are responsible for the production of the epithelial cells of the villi through mitosis (Imondi and Bird, 1966; Hodges, 1974), the decreased crypt depth in the ileal portion of the small intestine accompanying the feeding of .02% SCB may indicate a decreased turnover rate (Neutra, 1988). This decreased turnover rate could be resulting from a decrease in the concentration of bacteria producing toxic metabolites (Radecki *et al.*, 1992) or the ability of SCB to suppress the effects of these toxic metabolites (Stanley *et al.*, 1993). The energy conserved by the reduced turnover rate of the epithelial cells may then be utilized by the poult for lean tissue mass (Radecki *et al.*, 1992).

Table V.1. Composition and CP analysis of crumbled diets without (CS) or with supplemental yeast at .01% (CS + .01% SCB), .02% (CS + .02% SCB), and .06% (CS + .06% SCB) fed to male poults from day-old to 35 days of age

Ingredients and analysis	CS	CS + .01% SCB	CS + .02% SCB	CS + .06% SCB
	---------- (%) ----------			
Corn, yellow (8.9% CP)	50.40	50.39	50.39	50.37
Yeast[1] (26.2% CP)	. . .	.01	.02	.06
Soy bean meal (46% CP)	37.25	37.25	37.24	37.22
Meat and bone meal (50% CP)	7.50	7.50	7.50	7.50
Limestone flour	.50	.50	.50	.50
Fat, animal	2.25	2.25	2.25	2.25
Monocalcium phosphate (16% Ca, 21% P)	1.35	1.35	1.35	1.35
Salt, iodized	.05	.05	.05	.05
Vitamin premix[2]	.20	.20	.20	.20
Trace mineral premix[3]	.10	.10	.10	.10
L-lysine (78.4%)	.15	.15	.15	.15
DL-methionine (98%)	.19	.19	.19	.19
Coban-60® premix[4] (132 g/kg)	.05	.05	.05	.05
Calculated analysis				
CP	26	26	26	26
ME, kcal/kg	2908	2907	2905	2902
Analyzed[5]				
CP	26.1	25.7	26.6	24.4

[1] Levucell SB20®, *Saccharomyces cerevisiae* var. *boulardii*, Agrimerica, Inc., Northbrook, IL.

[2] Supplied per kg of diet: vitamin A, 17,220 IU; cholecalciferol, 6,660 ICU; vitamin E, 30 IU; vitamin B_{12}, 0.006 mg; riboflavin, 12 mg; niacin, 115 mg; d-pantothenic acid, 32 mg; menadione, 2 mg; folic acid, 0.9 mg; pyridoxine, 3 mg; thiamine, 1.5 mg; biotin, 0.11 mg; choline, 2,500 mg.

[3] Supplied per kg of diet: Mn, 160 mg; Zn, 150 mg; Fe, 120 mg; Cu, 135 mg; I, 1.5 mg; Se, 0.3 mg.

[4] Gratuitously provided by Elanco, Inc., Indianapolis, IN.

[5] Kjeldahl analysis.

Table V.2. Body weights and feed to gain ratios of poults fed diets without (CS) or with either .01% yeast[1] (CS + .01% SCB), .02% yeast (CS + .02% SCB), or .06% yeast (CS + .06% SCB) from 1 to 21 days of age

	Days of age				
Diet	1	7	14	21	1 to 21
	Body Weight (g)				Feed to Gain Ratio (g:g)
CS	59.5	122[b]	269[b]	464[b]	1.64
CS + .01% SCB	59.8	132[a]	286[ab]	516[a]	1.56
CS + .02% SCB	59.8	141[a]	311[a]	527[a]	1.58
CS + .06% SCB	58.8	138[a]	294[ab]	511[a]	1.58
SEM	.7	4	10	15	.03
Source of variation	Probabilities				
Diet	NS	.001	.004	.004	NS

[1] Levucell SB20®, *Saccharomyces cerevisiae* var. *boulardii*, Agrimerica, Inc., Northbrook, IL.
[a,b] Means within a column with no common superscript differ significantly (P<.004).

Table V.3. Goblet cell numbers, villus height, villus width, and crypt depth of the ileum in poults fed diets without (CS) or with .02% yeast[1] (CS + .02% SCB) from 21 to 35 days of age

Diet	Goblet Cells[2]	Villus Height	Villus Width	Crypt Depth
	number/mm	(mm)		
CS	40.7[a]	1.03	.079	.19[a]
CS + .02% SCB	31.9[b]	1.07	.093	.14[b]
SEM	2.2	.04	.004	.01
Source of variation	Probabilities			
Diet	.05	NS	NS	.02

[1] Levucell SB20®, *Saccharomyces cerevisiae* var. *boulardii*, Agrimerica, Inc., Northbrook, IL.
[2] Goblet cells were enumerated within .01 mm of the apical surface along the perimeter of 9 villi per bird.
[a,b] Means within a column with no common superscripts differ significantly (P<.05).

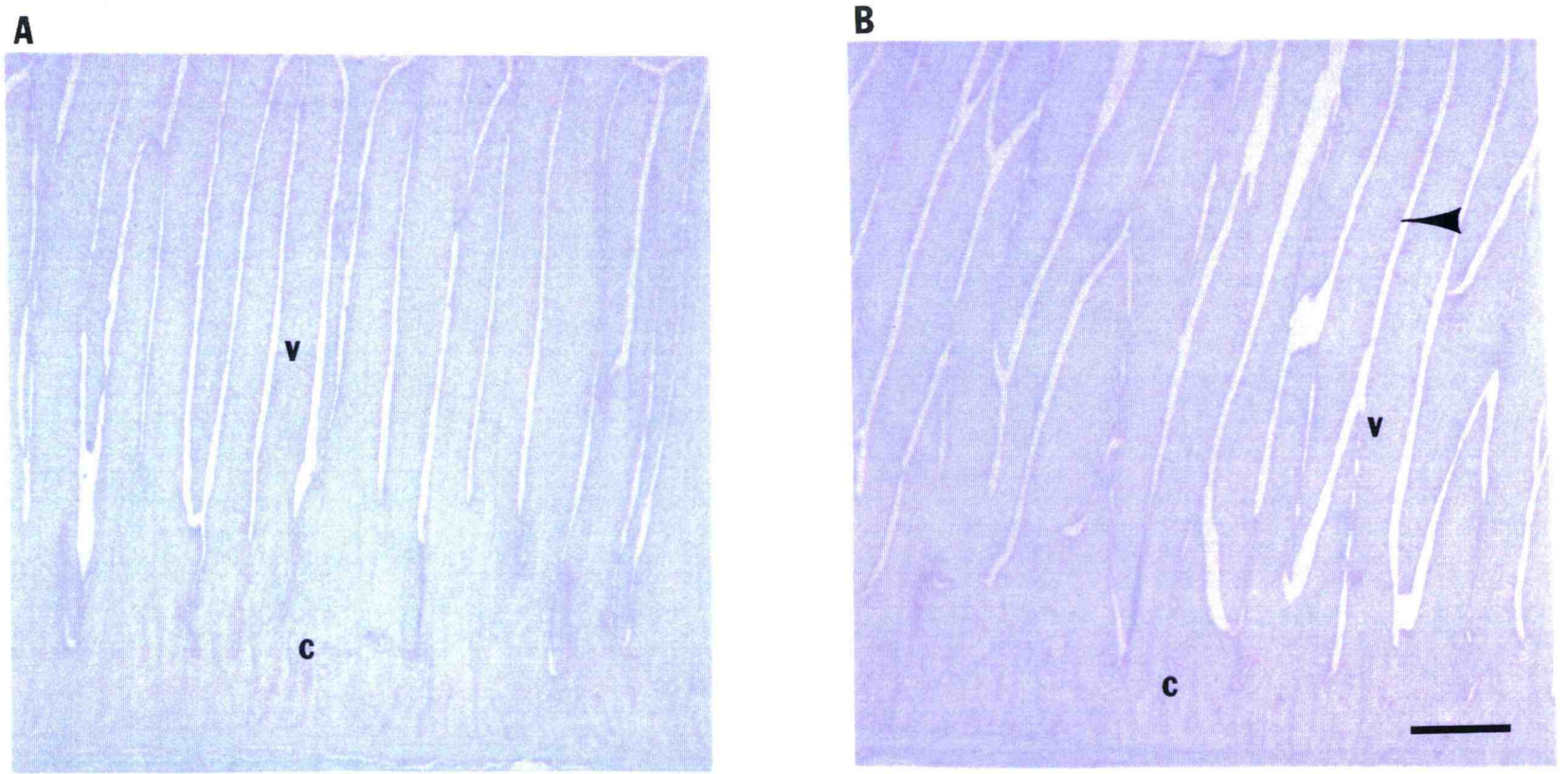

Figure V.1. Ileal mucosa from control (A) and yeast (B) fed poults at 35 days of age. Note the reduced crypt depth (C) in the mucosa of the poults fed .02% yeast (B) as compared to the control group (A). A decreased number of goblet cells (arrow), was also observed in the poults receiving yeast (B). No differences were observed in villus (V) height or width. Bar is .2 mm. Periodic acid-Shiff.

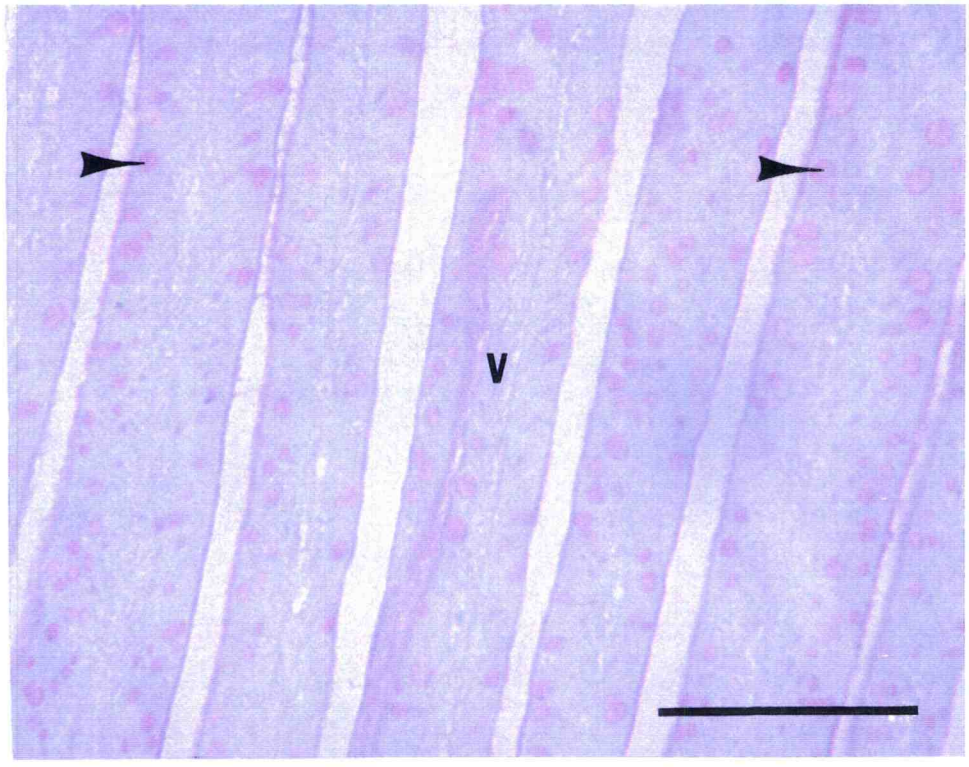

Figure V.2. Villi (V) of the ileal mucosa from poults at 35 days of age fed a control diet. Goblet cells (noted by the arrows) were reduced in the epithelial mucosa of the poults fed .02% yeast as compared to the control group. Bar is 1 mm. Periodic acid-Shiff.

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CHAPTER VI

THE EFFECTS OF FEEDING A DIETARY YEAST CULTURE OF *SACCHAROMYCES CEREVISIAE*[XP] TO LARGE WHITE MALE MARKET TURKEYS OF TWO GENETIC LINE CROSSES[1]

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[1] Southern Regional Research Project, Number S-213.

ABSTRACT

Three feeding experiments were conducted to evaluate the effect of feeding two different genetic line crosses of Nicholas Large White male market turkeys diets containing .25% yeast culture (YC, XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills Inc., Cedar Rapids, IA). Body weights, feed consumption, feed to gain ratios, livability, and the incidence of leg weakness were measured during the grow-out periods (day-old to 17 wks). In each experiment, males were housed in the same room (at different times) and randomly assigned to floor pens covered with pine wood shavings as litter. Birds were weighed at one day of age and body weights, feed consumption, and feed to gain ratios for each pen were determined at 2, 5, 8, 11, 14, and 17 weeks of age (WOA). The numbers of males and genetic line cross used in Experiments 1, 2 and 3 were: 120 of line 50-0602, 120 of line 88-0602, and 156 of line 88-0602, respectively. In Experiment 1, YC increased ($P<.11$) body weights at 5, 8, 11, and 14 WOA. Periodic weight gains were greater for birds fed the YC from 2 to 5 ($P<.001$) and 5 to 8 WOA ($P<.02$). Cumulative feed to gain ratios were improved ($P<.006$) at 5 WOA. Periodic feed conversions were improved between 2 and 5 WOA ($P<.005$) for birds fed supplemental YC. In Experiment 2, no differences ($P>.05$) were observed in the parameters measured. In Experiment 3, birds supplemented

with YC demonstrated lower ($P<.01$) body weights from 2 through 17 WOA and lower ($P<.02$) periodic weight gains between day-old to 2, 2 to 5, 5 to 8, and 8 to 11 WOA. Periodic feed to gain ratios were depressed ($P<.04$) from 2 to 5 WOA in YC supplemented birds. Cumulative feed consumption was also reduced ($P<.05$) in birds fed .25% YC at 2, 8, and 11 WOA while periodic feed consumption was depressed from 5 and 8 WOA ($P<.05$) in birds fed YC. No dietary differences ($P>.05$) were observed among the three experiments in either bird livability or the incidence of leg weakness. Results from these experiments indicate that unelucidated factors may influence the effects observed when dietary YC is fed to male turkeys.

INTRODUCTION

The variability in the results from research conducted to study the supplementation of poultry rations with yeast culture (YC) has been impeding possible benefits of feeding a YC to poultry for years (Miles and Bootwalla, 1991). In 1983, Brewer observed no significant effects of feeding a YC to market turkeys. Similarly, Savage *et al.* (1985) also reported no differences in body weight gains in market males at 20 weeks of age (WOA) or female poults at 16 WOA fed diets containing supplemental YC. On the contrary, reports of improved body weights from 12 to 22 WOA have been observed in Nicholas (genetic line cross 88-0602) and Hybrid Large White market turkeys fed diets containing YC (Anonymous, 1993). Cantor *et al.* (1983) reported improved feed palatability in Nicholas Large White poults to 23 days of age. Increased nutrient retentions have also been reported in poults fed diets containing 1% YC (Bradley and Savage, 1994b). Other researchers have consistently reported improved results in reproductive performance in select genetic lines of Wrolstad Medium White turkey breeder hens fed diets containing a YC (Savage and Mirosh, 1990a,b; Bradley and Savage, 1993; Hayat *et al.*, 1993; Bradley and Savage, 1994a). These reports suggest that some of the variability observed when conducting studies with YC may be

associated with genotype, environment, and management conditions.

The objective of the present study was to evaluate the effect of feeding .25% yeast culture (XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills Inc., Cedar Rapids, IA) diets to Nicholas commercial market turkeys.

MATERIALS AND METHODS

Three experiments were conducted using commercial male market turkeys obtained from the Oregon Turkey Hatchery, Aurora, OR (Experiment 1, May 1992) and Cuddy Farms Inc., Sonoma, CA (Experiment 2, December 1992 and Experiment 3, May 1993). All poults received standard hatchery services: sexed, detoed and beak-trimmed, snood removal, and a subcutaneous injection of B-complex vitamins (except for Experiment 2 where poults were not detoed and beak-trimmed).

Male Nicholas market turkey poults (n = 120) from the 50-0602 line cross (Experiment 1) and the 88-0602 line cross (n = 120, Experiment 2; n = 156, Experiment 3) were housed during different seasons in the same windowless house with cross ventilation. Poults were randomly selected, individually wing banded for identification, and placed into one of 6 pens providing three replicate pens per diet (20 poults per pen in Experiments 1 and 2, and 26 poults per pen in Experiment 3). All poults were provided with continuous incandescent light (24L:0D) for the first 2 WOA using one 100 w bulb per pen (measuring 3 X 3 m). The daily photoperiod was subsequently changed to 16L:8D (Experiment 1) or 12L:12D (Experiments 2 and 3) for the remainder of the grow-out period and birds were provided one 40 w bulb per pen. Mortality was recorded daily: by wing band, bird weight, and cause of death (if determined). The incidence

of leg weaknesses, classified as a deviation in the plane of the leg bones (legs bowing inward or outward, or displaying a rotated tibia), were recorded when observed and re-determined at the conclusion of each study.

The feeding program for each of the three experiments consisted of the same balanced formulations provided in six feeding periods. Feed compositions for each of the six periods are described in Table VI.1. Feed and water were provided *ad libitum*. Commencing at 2 WOA, pelleted-type diets were prepared at a local feed processing facility. The analyzed percent CP (Kjeldahl) are summarized in Table VI.2. The coccidiostat (Coban-60®) was incorporated into the diets (66 g of monensin per ton of finished feed) and fed from day-old to 8 WOA.

Turkeys were weighed individually at day-old, 2, 5, 8, 11, 14 WOA, and at the conclusion of each experiment (Experiments 1 and 3, 17 WOA; Experiment 2, 15 weeks and 6 days). Feed consumptions were determined by pen for each weighing period. Cumulative and periodic feed to gain ratio calculations were adjusted for mortality. At the conclusion of each study, the bird's sex was verified and mis-sexed birds were not included in the calculations of mean body weights or mean weight gains.

Statistical Analysis

Data from each experiment (body weights, periodic weight gains, feed consumptions, and feed to gain ratios) were analyzed separately as completely randomized designs using the General Linear Models procedure of SAS® (SAS, 1988). The experimental units used in this study were individual bird weights for body weight data, and the pen means for feed consumption and feed to gain ratio data. Significant means were separated using the Least Significant Difference test (SAS, 1988).

RESULTS AND DISCUSSION

Experiment 1

Body weights of Nicholas market turkey toms measured from day-old to 17 WOA in Experiment 1 (line cross 50-0602) are summarized in Table VI.3. No differences ($P>.05$) in body weights due to diet were observed until 5 WOA, at which time the birds fed the supplemental YC were heavier ($P<.008$). The improvement in mean body weight of males fed dietary YC was sustained through 14 WOA, and although the significance ($P<.06$) of the body weights were not as pronounced, the turkeys fed dietary YC were heavier at 17 WOA.

Periodic body weight gains were calculated for each period from day-old to 17 WOA, Table VI.4. Increased periodic body weight gains were observed in males fed the diet containing YC from 2 to 5 ($P<.001$) and from 5 to 8 WOA ($P<.02$). Periodic feed to gain ratios were lower in birds supplemented with YC from 2 to 5 ($P<.005$) and 14 to 17 ($P<.06$) WOA, Table VI.5. Cumulative feed to gain ratios were significantly better at 5, 8, and 17 WOA in birds fed the supplemental YC, Table VI.6. Cumulative and periodic feed consumptions did not differ ($P>.05$) as a result of dietary YC from day-old to 17 WOA, Tables VI.7 and VI.8.

Mortality rates from day-old to 14 WOA were 3.3 and 6.7 percent for diets without and with YC, respectively. Between 14 and 17 WOA, mortality resulting from aspergillosis resulted in 15.5 and 8.9 percent for birds fed diets without and with .25% YC, respectively. The incidence of leg weaknesses observed from day-old to 17 WOA were 13.8 and 7.7 for the birds fed diets without and with .25% YC, respectively.

Experiment 2

Body weights of Nicholas (line cross 88-0602) toms determined at day-old, 2, 5, 8, 11, 14 and 15 weeks, 6 days are summarized in Table VI.3. No differences ($P>.05$) in body weights, periodic weight gains, feed intakes, or feed to gain ratios between diets without or with YC were observed from day-old to the conclusion of the experiment (Tables VI.4, 5, and 6).

Percent mortality from day-old to the conclusion of the study was 7.3 and 5.6 for birds fed diets without or with YC, respectively. Leg weaknesses were more pronounced as the bird's body weight increased at 11 WOA, but was numerically lower in the birds supplemented with .25% YC at 15 weeks and 6 days of age as compared to the control diet (2.1 vs 10.0, respectively).

Experiment 3

Body weights of Nicholas market turkeys (line cross 88-0602) fed diets without and with .25% YC from Experiment 3 are summarized in Table VI.3. Depressed ($P<.01$) body weights were observed in birds fed diets containing .25% YC from 2 through 17 WOA. Periodic body weight gains from day-old through 17 WOA (Table VI.4) revealed significantly depressed periodic weight gains for birds fed the YC from day-old to 11 WOA. Feed to gain ratios were highest ($P<.04$) in birds fed diets supplemented with YC from 2 to 5 WOA, Table VI.5. Cumulative feed to gain ratios, determined from day of age to 17 WOA, revealed no differences ($P>.05$) between diets without or with YC, Table VI.6. Feed consumption was reduced ($P<.05$) in birds from 5 to 8 WOA fed diets with the supplemental YC. Cumulative feed consumption was depressed at 2 ($P<.01$), 8 ($P<.05$), 11 ($P<.02$), and 14 WOA ($P<.07$) in birds fed the YC diets.

Mortality from day-old to 17 WOA for birds fed diets without and with YC were 5.1 and 6.4 percent, respectively. Leg weaknesses observed at 17 WOA were 0.0 and 5.6 for the birds fed diets without and with .25% YC, respectively.

The results of these three experiments suggest that the Nicholas line cross 50-0602 responded differently to YC supplementation than did the Nicholas line cross 88-0602 male turkeys suggesting a genotype-dietary YC interaction.

Genotype-dietary YC interactions have been reported by various authors (Bradley and Savage, 1993; Hayat *et al.*, 1993; Bradley and Savage, 1994a) using Wrolstand Medium White turkey breeder hens. These somewhat inconsistent findings indicate that the genotype of the bird must be considered when incorporating a YC into poultry feeds. In addition, the variability between Experiments 2 and 3 also suggest that a large amount of variability in results is associated with feeding a YC to market turkeys of the same genotype. Despite the conscious efforts made to eliminate environmental and managerial differences between Experiments 2 and 3, variations occurred in the analyzed CP of the diets fed in each Experiment. The CP levels in Experiment 3 were consistently higher except in the feed fed from 5 to 8 WOA and could be the result of poor quality control by the feed manufacturer. The season of the year also differed between Experiments 2 and 3. Experiment 2 was conducted during the winter months from December 1992 through April 1993, while Experiment 3 was conducted during a mild summer from May to September 1993.

Table VI.1. Composition of basal corn-soy diets[1] fed to Nicholas Market Turkeys from day-old to 17 weeks of age

Ingredients and analysis	Weeks of Age					
	Day-old to 2	2 to 5	5 to 8	8 to 11	11 to 14	14 to 17
	(%)					
Corn, yellow (8.9% CP)	41.63	50.41	52.14	55.83	63.62	70.62
Yeast culture[2] (12% CP)	.25	.25	.25	.25	.25	.25
Soybean meal (47.5% CP)	47.18	37.25	33.75	28.75	20.75	14.25
Meat and bone meal (50% CP)	6.00	7.50	7.50	7.50	7.50	7.50
Limestone flour	.75	.50	.50	.50	.50	.25
Fat (blended, animal)	1.70	2.25	4.25	5.75	6.25	6.50
Vitamin premix[3]	.30	.20	.15	.15	.15	.15
Monocalcium phosphate (16% Ca, 21% P)	1.90	1.35	1.15	1.05	.80	.15
Salt, iodized	.10	.05	.10	.10	.10	.10
Trace mineral premix[4]	.50	.10	.10	.10	.10	.10
DL-methionine (98%)	.19	.19	.16	.12	.08	.08
L-lysine (78.4%)	.15	.15	.15	.15	.15	.15
Coban-60[5] (132g/kg)	.05	.05	.05	. . .	. . .	. . .
Calculated analysis						
CP (%)	28.13	25.95	24.43	22.36	19.24	16.90
ME, kcal/kg	2,820	2,909	3,057	3,198	3,327	3,446

[1] Manufactured by Kropf Feed and Seed, Inc., Harrisburg, OR.

[2] XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills Inc., Cedar Rapids, IA, was added to CS + YC diets only.

[3] **From 0 to 8 WOA** contained per kg: vitamin A, 19 065 IU; vitamin D_3, 7 350 ICU; riboflavin, 11 g; d-pantothenic acid, 22 g; niacin, 110 g; choline, 847 g; vitamin B_{12}, 6.6 mg; vitamin E, 33 110 IU; vitamin K, 2.2 g; folacin, 1 g; biotin, 121 mg; pyrodoxine, 3.3 g; thiamine, 1.8 g.

From 8 to 17 WOA contained per kg: vitamin A, 14 670 IU; vitamin D_3, 4 400 ICU; riboflavin, 10.8 g; d-pantothenic acid, 29 g; niacin, 110 g; choline, 440 g; vitamin B_{12}, 4.4 mg; vitamin E, 22 000 IU; vitamin K, 1.5 g; folacin, 732 mg; biotin, 112 mg; pyrodoxine, 2.2 g, thiamine, 1.3 mg.

[4] Supplies per kg of feed: Ca, 650 g; Mn, 332 g; Zn, 332 g; Fe 266 g; Cu, 27 g; I, 3.3 g; Se, .66 g.

[5] Gratuitously provided by Elanco Inc., Indianapolis, IN.

Table VI.2. Analyzed[1] CP (%) of corn-soy diets without (CS) and with .25% yeast culture[2] (CS + YC) fed to Nicholas Market Turkeys from day-old to 17 weeks of age

	Weeks of age					
Diet	Day-old to 2	2 to 5	5 to 8	8 to 11	11 to 14	14 to 17[3]
Experiment 1						
CS	29.41	22.48	21.89	20.75	18.38	16.42
CS + YC	28.17	21.78	22.28	20.66	18.37	16.34
Experiment 2						
CS	28.10	22.60	23.30	20.80	17.86	15.92
CS + YC	28.20	22.30	24.03	19.34	18.54	15.52
Experiment 3						
CS	28.65	23.38	22.01	23.08	18.99	18.41
CS + YC	28.98	24.84	22.91	20.43	18.08	18.34

[1] Kjeldahl analysis.
[2] XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills Inc., Cedar Rapids, IA.
[3] Except in Experiment 2, birds were 15 weeks and 6 days of age.

Table VI.3. Body weights (kg) of Nicholas Market Turkeys fed diets without (CS) and with .25% yeast culture[1] (CS + YC) from day-old to 17 weeks of age

		Weeks of Age					
Diet	Day-old	2	5	8	11	14	17[2]
Experiment 1							
CS	.058	.24	1.04[b]	2.80[b]	5.69[b]	8.31[b]	9.98
CS + YC	.059	.24	1.12[a]	3.03[a]	6.04[a]	8.77[a]	10.46
SEM	.001	.01	.02	.06	.09	.12	.18
Source of variation	---------- Probabilities ----------						
Diet	NS	NS	<.001	<.003	<.008	<.008	<.06
Experiment 2							
CS	.055	.26	1.09	3.40	6.57	9.77	11.98
CS + YC	.054	.25	1.06	3.43	6.65	9.94	12.24
SEM	.002	.01	.05	.15	.28	.45	.61
Source of variation	---------- Probabilities ----------						
Diet	NS	NS	NS	NS	NS	NS	NS
Experiment 3							
CS	.061	.25[a]	1.02[a]	3.11[a]	6.38[a]	9.40[a]	12.55[a]
CS + YC	.060	.24[b]	.90[b]	2.82[b]	5.57[b]	8.60[b]	11.85[b]
SEM	.001	.01	.02	.05	.09	.12	.15
Source of variation	---------- Probabilities ----------						
Diet	NS	<.01	<.001	<.001	<.001	<.001	<.001

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.

[2] Experiment 2, birds were 15 weeks and 6 days of age.

[a,b] Means within columns in each experiment with no common superscripts differ significantly (P<.05).

Table VI.4. Periodic body weight gains (kg) of Nicholas Market Turkeys fed diets without (CS) and with .25% yeast culture[1] (CS + YC) from day-old to 17 weeks of age

	Weeks of Age					
Diet	Day-old to 2	2 to 5	5 to 8	8 to 11	11 to 14	14 to 17[2]
Experiment 1						
CS	.18	.80[b]	1.76[b]	2.87	2.62	1.69
CS + YC	.18	.88[a]	1.90[a]	2.98	2.74	1.66
SEM	.01	.01	.04	.05	.07	.14
Source of variation	---------- Probabilities ----------					
Diet	NS	<.001	<.02	NS	NS	NS
Experiment 2						
CS	.20	.83	2.31	3.17	3.20	2.08
CS + YC	.20	.81	2.36	3.23	3.29	2.26
SEM	.01	.04	.10	.14	.23	.30
Source of variation	---------- Probabilities ----------					
Diet	NS	NS	NS	NS	NS	NS
Experiment 3						
CS	.19[a]	.76[a]	2.09[a]	3.27[a]	3.01	3.16
CS + YC	.18[b]	.67[b]	1.91[b]	2.74[b]	3.01	3.27
SEM	.01	.01	.04	.06	.08	.08
Source of variation	---------- Probabilities ----------					
Diet	<.02	<.001	<.003	<.001	NS	NS

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.

[2] Except in Experiment 2, birds were 15 weeks and 6 days of age.

[a,b] Means within experiments in columns with no common superscripts differ significantly (P<.05).

Table VI.5. Periodic feed to gain ratios (g:g) of Nicholas Market Turkeys fed diets without (CS) and with .25% yeast culture[1] (CS + YC) from day-old to 17 weeks of age

	Weeks of age				
Diet	2 to 5	5 to 8	8 to 11	11 to 14	14 to 17[2]
Experiment 1					
CS	1.82[a]	1.96	2.11	2.73	4.26
CS + YC	1.71[b]	1.87	2.12	2.89	3.54
SEM	.01	.04	.05	.07	.20
Source of variation	---------- Probabilities ----------				
Diet	<.005	NS	NS	NS	<.06
Experiment 2					
CS	1.74	1.70	2.23	2.95	3.62
CS + YC	1.72	1.68	2.30	3.02	3.48
SEM	.02	.06	.08	.11	.21
Source of variation	---------- Probabilities ----------				
Diet	NS	NS	NS	NS	NS
Experiment 3					
CS	1.90[b]	1.82	2.02	2.87	3.53
CS + YC	1.98[a]	1.73	1.92	2.82	3.11
SEM	.02	.09	.14	.10	.15
Source of variation	---------- Probabilities ----------				
Diet	<.04	NS	NS	NS	NS

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.

[2] Except in Experiment 2, birds were 15 weeks and 6 days of age.

[a,b] Means within the same experiment in columns having no common superscripts differ significantly (P<.05).

Table VI.6. Cumulative feed to gain ratios (g:g) of Nicholas Market Turkeys fed diets without (CS) and with .25% yeast culture[1] (CS + YC) from day-old to 17 weeks of age

	Weeks of Age					
Diet	2	5	8	11	14	17[2]
Experiment 1						
CS	1.45	1.76[a]	1.89	2.00	2.23	2.56
CS + YC	1.45	1.66[b]	1.80	1.96	2.25	2.48
SEM	.04	.01	.03	.03	.02	.02
Source of variation	---------- Probabilities ----------					
Diet	NS	<.006	<.07	NS	NS	<.07
Experiment 2						
CS	1.52	1.70	1.70	1.96	2.29	2.53
CS + YC	1.56	1.69	1.68	1.98	2.32	2.54
SEM	.20	.04	.04	.03	.05	.04
Source of variation	---------- Probabilities ----------					
Diet	NS	NS	NS	NS	NS	NS
Experiment 3						
CS	1.55	1.83	1.82	1.92	2.23	2.56
CS + YC	1.55	1.89	1.78	1.85	2.18	2.44
SEM	.05	.02	.07	.04	.03	.04
Source of variation	---------- Probabilities ----------					
Diet	NS	NS	NS	NS	NS	NS

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.

[2] Except in Experiment 2, birds were 15 weeks and 6 days of age.

[a,b] Means within columns in each experiment with no common superscripts differ significantly (P<.05).

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CHAPTER VII

GENERAL DISCUSSION

CONCLUSIONS AND POSTULATIONS

The studies conducted demonstrate that the incorporation of a dietary yeast in the form of a yeast culture containing *Saccharomyces cerevisiae*^{XP} (YC) and a dried yeast containing *Saccharomyces cerevisiae* var. *boulardii* (SCB) into turkey diets can improve nutrient retention, improve hatchability of fertile turkey eggs in select genetic lines, alter gut morphology in the small intestine (ileum), and also produce inconsistent results regarding male market turkey performance.

The common ingredient to both the dried yeast (SCB) and the YC are viable yeast cells which are believed to be the essential component. It is hypothesized that when yeast cells are fed to birds, they produce enzymes and vitamins within the bird's gastrointestinal tract (which might otherwise be limited in availability and be suppressing the bird's optimal performance) and provide improved responses.

Chapter III

Increased utilization of dietary gross energy, nitrogen, calcium, phosphorus, boron, potassium, magnesium,

and manganese were observed in poults fed diets supplemented with YC when compared to the control diet and diet containing autoclaved (inactivated) YC. The retentions of copper, iron, and zinc were not statistically improved with the addition 1% YC to the diets of poults although there were numerical differences. The absence of an effect with the autoclaved (inactivated) YC indicates that autoclaving impairs the ability of the YC to increase the utilization of selected nutrients in poults at 28 DOA. Since the process of autoclaving greatly reduces the number of viable yeast cells, the process of feed pelleting may also be detrimental to viable yeast cells. One challenge confronting the poultry industry concerns the use of DFM products and the practice of feed pelleting and its inactivation of the DFM's viable microbial cells (Headen, 1992). Therefore, the development of better feed application procedures and new yeast DFM products must be pursued. In the future, researchers may identify yeast and other viable DFM products that can be added to the feed in a liquid form (such as mixing with cane molasses solubles or blended animal-vegetable fats, etc.) following the pelleting process to insure microbial viability in the finished feed (Mark Richards, 1993, Medipharm Inc., 10215 Dennis Drive, Des Moines, IA, personal communication).

Enzymatic activity

Phytase is an enzyme capable of releasing nutrients bound by phytate, a well known natural anti-nutritional factor present in most common cereal grains. Phytate's strong chelating ability allows it to tightly bind nutrients such as calcium, zinc, and copper in the gastrointestinal tract of poultry, and results in higher nutrient requirements in the ration as well as increased nutrients excreted (Savage *et al.*, 1964; Waldroup *et al.*, 1964; Scott *et al.*, 1982; Erdman, 1989; Cheeke, 1991; Perney *et al.*, 1993; Power, 1993; Ward, 1993). Phytase has been reported to increase the utilization of unavailable nutrients in corn-soybean meal type rations (Mitchell and Edwards, 1994), thereby reducing the nutrients excreted. It is not yet economically advantageous, however, to supplement poultry diets with phytase because of the relatively low cost of phosphorus (Ledoux *et al.*, 1994). Nevertheless, because future regulatory restrictions on the application of poultry waste are deemed inevitable (Patterson and Lorenz, 1993), the enforcement of these regulations may cause phytase supplementation to become more economically feasible (Robert Wagstaff, 1994, Kemin Industries, 2100 Maury Street, Des Moines, IA, personal communication).

Yeast DFM (yeast cultures) possess phytase activity (Thayer *et al.*, 1978) and have been observed to increase the

utilization of nutrients (gross energy, nitrogen, phosphorus, calcium, boron, potassium, magnesium, and manganese) in corn-soybean meal rations fed to turkeys. Therefore, the use of yeast DFM could be a viable and more economical alternative to the use of phytase supplementation. In addition, supplemental enzymes present in yeast and YC may also permit the utilization of low quality grains to a greater extent (Gonzalez, 1993).

Chapter IV

Early embryonic mortality (Days 0-10) was reduced in the three lines of breeder hens when supplemental YC was provided in the maternal diet. Hatch of fertile eggs was consistently increased in one of the lines (C), revealing a significant genotype-dietary YC interaction. Genotype and the duration of pre-incubation egg storage time proved to be important factors to be considered when evaluating the efficacy of feeding breeder hens supplemental YC.

The nutrient requirements of each bird, such as protein, depend on numerous metabolic reactions which are governed by enzymatic systems involved in the catabolism and anabolism of amino acids. Each of these enzyme systems is under genetic regulation which can also be influenced by the environment of the bird (Scott *et al.*, 1982). Hens of two lines (H and C) apparently require an increased amount of a

specific nutrients such as protein or phosphorus which the YC may be providing through increased enzymatic activity in the gut (Thayer *et al.*, 1978).

Chapter V

The addition of SCB to poult diets increased body weights and decreased both goblet cell numbers and crypt depth in the ileum. A decreased crypt depth in the ileal portion of the small intestine accompanying the feeding of .02% SCB may indicate a decreased proliferation of epithelial cells of the ileal mucosa (Neutra, 1988). This decreased turnover rate in the ileum could be a result of a decreased number of toxin producing bacteria (Radecki *et al.*, 1992) or possibly the ability of yeast to suppress the effects of toxic metabolites (Stanley *et al.*, 1993). The energy conserved by a reduction in the epithelial cell proliferation may then be utilized by the poult for growth, thereby improving its feed to gain ratio (Radecki *et al.*, 1992).

To date, literature regarding the incorporation of SCB and YC into poultry diets is inconclusive. Further work is encouraged in order to understand the potential benefits of yeast DFM products in poultry feeds. Potter *et al.* (1991) reported that the addition of enzymes to feed or water of turkey poults fed low protein diets significantly increased

weight gain. The results from this experiment are in agreement with those observations as poults fed 26% CP diets sustained improved body weights when supplemented with SCB at .01, .02, and .06% of the diets. The supplementation of .02% SCB resulted in optimal poult performance. The enzymatic activity (such as phytase) in the gastrointestinal tract should be measured in order to evaluate the enzymatic contribution of SCB in turkeys. If the yeast's enzymes are the chief active component in both yeasts and YC, this factor could explain some of the variation encountered when using yeast DFM products (Potter *et al.*, 1991).

Chapter VI

Three experiments evaluating the effects of .25% YC in the diets of Large White market male turkeys of two different line crosses were conducted and resulted in confounding results. The results of supplementing diets with YC were inconclusive since body weights and feed to gain ratios were improved in one experiment and were either not influenced or suppressed in two subsequent experiments. Different commercial market male turkey line crosses (50-0602 and 88-0602) responded differently to the same level of YC supplementation suggesting the presence of a genotype-dietary YC interaction. Genotype-dietary YC interactions have been reported by various authors (Bradley and Savage,

1993; Hayat *et al.*, 1993; Bradley and Savage, 1994) using Wrolstand Medium White turkey breeder hens. These somewhat inconsistent findings also indicate that the genotype of the market turkey must be considered when incorporating a YC into poultry feeds. In addition, there was variability associated with feeding a YC to market turkeys of the same genotype which may indicate a environmental-dietary YC interaction.

General Conclusion

The responses observed in these studies have identified bird genotype and management as factors that influence the responsiveness of birds fed diets containing yeast (SCB and YC). Increased hatchability in select genetic lines of breeder hens fed diets containing YC were repeatable, while variable results were observed in body weight gain and feed to gain ratios of market male turkeys. The fact that excessive variability was present between market turkeys studies does not discredit the repeatable responses observed in breeder turkey hen studies. The variable results observed in the market turkey studies indicate that further research is required to understand the various factors which affect a bird's responsiveness to dietary inclusion of a YC.

FUTURE USE AND DEVELOPMENT OF DFM PRODUCTS

The possibilities for future uses of DFM products are intriguing. As more products are being developed and evaluated, it is imperative that a clear and detailed identification of each of these products be made instead of simply referring to them as a "DFM". In addition, future genetic and environmental manipulation of the yeast cells should be pursued in order to produce specialized yeast DFM products with very specific poultry feed applications. Currently, a yeast DFM product developed for use in corn-soybean meal type feeds (for all types of poultry) is being pursued (Wiseman, 1991; Miles, 1993; Andrew Morgan and Craig Wyatt, 1994, Finnfeeds International LTD High Street, Marlborough, Wiltshire, SNB 1AA, U.K., personal communications). In the future, a yeast DFM product could be specifically manufactured for use in poultry starter diets containing wheat while a different yeast DFM product could be produced for use in diets containing barley.

NOVEL TECHNOLOGIES IN DFM FEED DELIVERY

Since the viability of DFM products is thought to be important in their effectiveness, new ideas and technologies may facilitate novel ways in which to incorporate DFM in poultry diets. Incorporating a bacterial DFM within a by-

product carrier, such as condensed cane molasses solubles, has been proven to be effective in preserving bacterial viability in mash diets fed to SCWL laying hens (Nahashon *et al.*, 1993). Yeast may also be used to encapsulate micro-nutrients as an encapsulating agent. This technique would allow for the protection and uniform distribution throughout the feed of the yeast as well as the micro-nutrients (Johnson, 1977). Another approach, which has been used to a limited extent, is to blend the DFM product with dietary animal fat which is then applied to the feeds after the pelleting process (John Gauwitz, 1993, 305 W. Ash, Chillicothe, IL, personal communication). In addition, the procedure of top-dressing a feed with a DFM product may prove to be especially valuable when starting birds. The economic benefits of yeast and other DFM products, however, need to be evaluated under field conditions in order to ascertain their practical use in the industry (Jones, 1994).

FUTURE PROSPECTS FOR DFM PRODUCTS

In order to better understand the mode of action of DFM products, Miles (1993) believes future research should emphasize the identification of animals within a population which would benefit from DFM supplementation. This concept may be taken a step further in that future research may need to emphasize the identification of genetic populations of

poultry which may benefit from the use of a specific DFM. Bradley and Savage (1993 and 1994) have reported studies using three genetic lines of turkey breeder hens which have confirmed the hypothesis that YC (a yeast DFM product) may not be suitable for use in certain genetic lines of birds. In addition, further studies designed to evaluate the effects of yeast DFM products in the diets of poultry might emphasize the following:

1) the evaluation of DFM products in stress related situations in poultry production systems,
2) the use of DFM products to increase the availability of nutrients from lower quality feedstuffs,
3) the effects of DFM products on embryo development,
4) the quantitation of morphological changes in the gastrointestinal tract of poultry fed DFM products,
5) the comparison of different yeast DFM products commercially available, and
6) the evaluation of these DFM products using larger flocks raised under industrial management conditions (field trials--in order to determine the efficacy of their use in the poultry industry).

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APPENDICES

APPENDIX 1. EFFECTS OF A YEAST CULTURE AND AUTOCLAVED YEAST CULTURE CONTAINING *SACCHAROMYCES CEREVISIAE*[XP] ON THE RETENTION OF DIETARY CALCIUM AND PHOSPHORUS MEASURED COLORIMETRICALLY

Introduction

Chapter III reported the effect of yeast culture (YC) and autoclaved YC on select mineral retention measured using an Inductively Coupled Argon Plasma Analyzer. The purpose of this appendix is to present the preliminary calcium and phosphorus retention data obtained using colimetric procedures. This brief report substantiates the results described in Chapter III in establishing a possible mode of action for YC in poultry diets.

The percent nutrient retention of dietary calcium (Ca) was measured using atomic absorption spectrophotometry (AOAC, 1980). Total P was determined colorimetrically by procedures previously described by Fiske and SubbaRow (1925). Calculations used to determine the percent retentions of Ca and P from the diets were performed utilizing the equation given by Edwards and Gillis (1959).

Results

Results of Ca and P retained from poults fed diets without and with YC and autoclaved YC are summarized in Table A1.1. Increases in the percent utilization of dietary

Ca ($P<.01$) and P ($P<.004$) were observed in poults fed the supplemental YC when compared to the control and control plus autoclaved YC diets. These results are similar to those of Chapter III which indicated that autoclaving the YC impairs the factors which increase the utilization of dietary Ca and P of poults at 28 days of age.

Table A1.1. Percent retention[1] of calcium and phosphorus in poults fed corn-soy diets without (CS) and with 1% yeast culture[2] (CS + YC) or 1% autoclaved[3] yeast culture (CS + AYC) which were measured using colorimetric procedures

Diet	Ca	P
	---------- % ----------	
CS	45.17[b]	46.35[b]
CS + YC	56.26[a]	62.71[a]
CS + AYC	45.15[b]	44.28[b]
SEM	1.90	2.91
Source of variation	---------- Probabilities ----------	
Diet	.01	.004

[1] Data are from 3 pens per diet (3 composite samples from 3 days of collection, 5 poults per pen).

[2] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.

[3] XP yeast culture® autoclaved at 121 C at 12 psi for 35 min.

[a,b] Means within a column with no common superscripts differ significantly (P<.01).

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APPENDIX 2. THE INFLUENCES OF PRE-INCUBATION EGG STORAGE TIMES AND GENOTYPE ON HATCHABILITY, AND THE EFFECTS FEEDING MEDIUM WHITE TURKEY HENS A DIET CONTAINING A YEAST CULTURE OF *SACCHAROMYCES CEREVISIAE*^{XP} ON HATCHABILITY OF EGGS STORED 0-14 D, BLOOD CHEMISTRIES, EGG CHARACTERISTICS, AND ILEAL MORPHOLOGY

Introduction

The main focus of Chapter IV was to report the effects of yeast culture (YC, XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills, Inc., Cedar Rapids, IA) on the hatchability of fertile eggs stored for different periods of pre-incubation egg storage (PIES) time. The focus of this appendix is to report the effects of PIES time on the hatchability of fertile eggs among the three different genetic lines and to provide the results of the dietary YC on incubation parameters for the entire PIES (0-14 d) of each hatch. In addition, the effects of a dietary YC on blood plasma chemistries and egg characteristics among three different genetic lines of breeder hens are presented.

Effects of PIES Times: 0-7 and 8-14 d

The effects of PIES times (0-7 and 8-14 d) among three differing genetic lines (L, H, and C) fed diets without and with .5% yeast culture (YC) for Experiment 1 are summarized in Table A2.1. PIES duration increased ($P<.02$) early

embryonic mortality (0 to 10 d) in eggs stored from 8-14 d prior to incubation. The incidence of late embryonic mortality (21 to 28 d) was increased ($P<.01$) in lines L and H in eggs stored greater than 7 d prior to incubation. In the C line, increased ($P<.01$) incidence of pipped embryos was observed in eggs stored greater than 7 d while hatchability was not affected. In the L and H lines, PIES times greater than 7 d resulted in reduced ($P<.02$) hatchability as compared to eggs stored less than 8 d. In addition, an increase ($P<.02$) in the incidence of late embryonic mortality was a prelude to reducing the hatchability of eggs from L and H line hens stored greater than 7 d prior to incubation. These results support the generally accepted hypothesis that as PIES time increases, the hatchability of fertile eggs decrease (Landauer, 1967).

Genetic line differences were apparent for the incidences of early embryonic mortality, late embryonic mortality, pipped embryos, and hatchability. Early embryonic mortality was greatest ($P<.001$) in the H line. All three lines (L, H, and C) differed ($P<.001$) in the incidence of late embryonic mortality with the H line being the highest and the L line exhibiting the lowest percent mortality. The L line is traditionally characterized by a higher incidence of pipped embryos, and this situation was again observed in the present study ($P<.001$). The L and C

lines demonstrated the highest (P<.001) hatchability of fertile eggs of the three genetically dissimilar lines. The duration of PIES time was also significant for the incidences of early embryonic mortality, late embryonic mortality, and hatchability. The incidences of early and late embryonic mortality were greater (P<.01) in eggs stored greater than 7 d. This greater embryonic mortality culminated in a reduced (P<.001) hatchability for eggs stored greater than 7 d. A genetic line by PIES time interaction was also significant (P<.04) for the incidence of late embryonic mortality and was caused by the C line displaying a decreased mortality as egg storage time increased. These results confirm the report of Yoo and Wientjes (1991) that genetic line differences as well as PIES time affect hatchability results. These results also suggest that even under ideal conditions, eggs should not be stored greater than 7 d prior to incubation for optimal hatchability. Further research is required to determine the optimal PIES time.

Effects of PIES Times: 0-4, 5-9, and 10-14 d

The effect of PIES times (0-4, 5-9, and 10-14 d) among the three genetic lines (L, H, and C) fed diets containing a YC (Chapter IV, Experiment 2) are summarized in Table A2.2. There were no differences (P>.05) in fertility or the

incidence of early embryonic mortality (0 to 10 d) due to PIES time. The duration of PIES increased ($P<.05$) mid-embryonic mortality (11 to 20 d) in eggs from H line hens stored from 0-4 d as compared to eggs stored from 5-9 d prior to incubation. The incidence of late embryonic mortality was also increased ($P<.01$) in eggs from C and L line hens stored greater than 5 and 10 d prior to incubation, respectively. In the H line, a significant ($P<.05$) increase in the number of pipped embryos was observed in eggs stored from 10-14 d as compared to eggs stored from 5-9 d; however, hatchability was not affected although it was numerically reduced in eggs stored from 0-4, 10-14 and 0-14 d. In the L line, eggs stored less than 5 d resulted in reduced ($P<.01$) hatchability. In line C, the hatchability of fertile eggs was higher ($P<.01$) in eggs stored from 0-4 d as compared to eggs stored from 10-14 d prior to incubation. This data suggests that the optimal PIES time is between 5-9 d.

Differences among genetic lines were observed in the incubation parameters measured. The L and C lines maintained overall greater ($P<.001$) fertility and hatchability. Early embryonic mortality was highest ($P<.001$) in the H line, while the incidences of late ($P<.001$) and mid-embryonic ($P<.03$) mortality were lower in the L line than lines H and C. The L and H line experienced

a higher (P<.003) incidence of pipped embryos than the C line hens. These findings confirm those of Experiment 1 as well as Yoo and Wientjes's (1991) observations that genetic lines differ in the decline of hatchability as PIES time increases. Future research is encouraged which focuses on the correlation of these genetic characteristics and the subsequent responses in hatchability obtained when YC is included in the diet of the breeder hen.

Effects of Dietary Yeast Culture on Incubation Performance

In Chapter IV (Experiments 1 and 2) incubation performance was studied by PIES duration in order to better understand the affects of dietary YC on egg storage. However, valuable information exists in the overall (PIES duration of 0-14 d) incubation performances of the three genetic lines fed diets without and with .5% YC. Consequently, incubation performance of eggs stored from 0-14 d prior to incubation in Experiments 1 and 2 of Chapter IV are summarized in Tables A2.3 and A2.4, respectively.

In Experiment 1, the incidence of early embryonic mortality was reduced in lines L (P<.09) and C (P<.08). The addition of yeast culture to the feed increased (P<.04) the incidence of pipped embryos in the L line, had no affect in the H line, and reduced the incidence of pipped embryos in

the C line causing a significant (P<.01) genetic line by dietary yeast culture interaction.

The percent hatchability of fertile eggs was decreased (P<.08) in line L and increased (P<.05) in line C which consequently caused the line by dietary yeast culture interaction to become significant (P<.01). Overall, a decreased (P<.02) early embryonic mortality and an increased (P<.05) incidence of pipped embryos resulted from the incorporation of yeast culture into the feed. In addition, overall line differences, similar to those already reported, were apparent in early embryonic mortality, the incidence of pipped embryos, and hatchability of fertile eggs.

In Experiment 2, similar results to those of Experiment 1 were observed, reduced (P<.02) fertility in the L line and increased (P<.03) fertility in the H line resulted in a significant (P<.01) line-dietary YC interaction. Early embryonic mortality was reduced in lines H (P<.05) and C (P<.10), while in the L line, it was numerically increased in the .5% YC breeder hen diet. Consequently, an additional line-dietary YC interaction was observed (P<.01). Late embryonic mortality was reduced only in line C when YC was included in the hen's diet. The addition of YC to the breeder hen diet increased the hatchability of fertile eggs stored 0-14 d in lines H (P<.05) and C (P<.09), while no dietary differences were

experienced in the L line. Line differences similar to Experiment 1 were observed in each of the incubation parameters recorded. Since the supplementation of YC numerically reduced hatchability in the L line and increased it in lines H and C, the genotype-dietary YC interaction was significant ($P<.01$). These findings indicate that the genotype of the bird is a factor when evaluating the efficacy of utilizing a supplemental YC in the diet of breeder hens.

Effects of Dietary Yeast Culture on Select Blood Plasma Chemistries

In Experiment 1 (Chapter IV), blood plasma chemistries were determined from each of the three genetic lines fed diets without and with YC. Blood samples were collected from 6 hens selected at random in each line-diet combination at 34, 41, and 48 WOA (C line); and at 36, 43, and 50 WOA (L and H lines). All hens selected were in active egg production at the time of sampling. The blood samples were collected into heparinized vacutainers (via brachial venipuncture), followed by separation of the blood plasma. The whole blood samples were centrifuged at 3,000 x g for 10 min at 5 C, and the serum harvested in plastic containers and stored on ice. The plasma samples were analyzed within 24 hours of collection at the Clinical Chemistry Laboratory located in the Good Samaritan Hospital, Corvallis, OR. No pre-dietary differences ($P>.05$) were observed in blood chemistries collected prior to feeding the hens breeder diets without and with .5% YC (34 and 36 WOA).

Statistical analysis

The $\log_{10}$ transformation of selected blood plasma chemistries (creatine phosphokinase, lactate dehydrogenase, triglyceride) did not reveal differences from untransformed data, thus, the data was analyzed without transformations.

Blood samples collected at 41 and 48 WOA (C line) and 43 and 50 WOA (L and H lines) represent the effect of dietary YC supplementation on the physiological process of egg formation, Table A2.5 and Table A2.6. Since the effect of diet on blood plasma chemistries was most important to the authors (G. L. Bradley and T. F. Savage), and there was not a time-diet interaction, the data for each of the two time periods was pooled and the effect of time was used as a blocking factor in the analysis.

Results

The influence of dietary YC supplementation on hen blood plasma chemistries in the three genetically dissimilar lines (L, H, and C) are summarized in Table A2.5 and Table A2.6. Dietary YC supplementation increased ($P<.06$) plasma cholesterol in the L line and numerically increased it in the H line hens, making the overall dietary effect significant ($P<.01$). No dietary differences ($P>.10$) were observed in the other blood plasma chemistries in the H line hens. Dietary supplementation of YC to the breeder hen diet of the C line hens resulted in increased plasma protein ($P<.06$), cholesterol ($P<.02$), potassium ($P<.05$), and triglycerides ($P<.06$). Genetic line differences in blood plasma chemistries were apparent for several of the variables measured. The L line demonstrated significantly

greater mean plasma values for creatine phosphokinase ($P<.01$), lactate dehydrogenase ($P<.01$), uric acid ($P<.01$), cholesterol ($P<.001$), sodium ($P<.001$), and potassium ($P<.001$) than hens of lines H and C. A higher ($P<.03$) level of aspartate aminotransferase was observed in the L line ($P<.03$) when compared to the H line hens. These findings support the distinct genetic characteristics of the L, H, and C lines. There were no ($P>.05$) genetic line by dietary YC interactions for any of the chemistries measured.

Effects of Dietary Yeast Culture on Egg Characteristics

The effects of adding supplemental YC to the diet of the three genetic lines of breeder hens (L, H, and C) on egg weight, percent yolk weight, and breaking strength (kg) were measured from hens described in Chapter IV (Experiments 1 and 2) and are summarized in Table A2.7. No differences due to the incorporation of YC in the diet of hens on egg weight, percent yolk weight, or breaking strength were observed (Experiment 1--birds were 50 weeks of age (WOA) in the L an H lines and 28 WOA in the C line, Experiment 2--birds were 51 WOA). In Experiment 1, however, eggs from L line hens demonstrated a greater ($P<.01$) shell breaking strength (kg) than those from H and C line hens, Table A2.7. A genetic line difference ($P<.02$) for egg weight was observed in Experiments 1 and 2. The line L hens have heavier egg weights than the H line hens. These data demonstrate that individual genetic line characteristics in egg quality are important factors in the understanding of genetic variation and in the evaluation and interpretation of dietary treatments given to genetically distinct hens.

Effects of Dietary Yeast Culture on Goblet Cell Numbers and Ileal Morphology of Line C Hens

The effects of .5% supplemental YC in diets fed to C line Wrolstad Medium White turkey breeder hens (Chapter IV, Experiment 2) on goblet cell numbers, villus height, villus width, and crypt depth was evaluated using the methodology outlined in Chapter V. However, a total of 16 counts and measurements (instead of 9 as in Chapter V) were made in the determinations of the mean of each individual bird for the number of goblet cells, villus height and width, and crypt depth.

Results

Goblet cell numbers, villus height, villus width, and crypt depth for hens fed diets without and with dietary YC are summarized in Table A2.8. Goblet cell numbers (per mm of villus height) were reduced ($P<.13$) from 65.8 to 51.8 in hens fed .5% supplemental YC. Although the reduction in the number of goblet cells was not statistically significant ($P>.10$), perhaps because of an insufficient number of hens sampled (replications), a reduction in the number of goblet cells of turkeys fed diets supplemented with yeast (*Saccharomyces cerevisiae* var. *boulardii*) has been observed in a previous study (Chapter V). Villus height was increased ($P<.08$) with the addition of YC to the diet of the

breeder hens. A significant ($P<.003$) increase in villus width was also observed in hens fed diets containing .5% YC, however, no dietary differences ($P>.05$) were observed in mean crypt depth.

The increases in villus height and width observed in hens fed YC indicate a larger surface area for absorption of nutrients in the intestine. This factor may be correlated with the increased hatchability of fertile eggs reported in Chapter IV, Experiment 2; however, morphological analyses of the other to genetic lines (L and H) would be required in order for a definitive conclusion to be established. Future experiments which study the morphology differences among the three lines of breeder hens fed YC are required.

Table A2.1. Incubation performance (%) of eggs stored from 0-7, 8-14, and 0-14 d prior to incubation in three lines of Wrolstad Medium White hens during the 1992 breeding season (Experiment 1)

Line	Pre-incubation Egg Storage Time (d)	Fertility	Early Embryonic Mortality (0-10 d)	Mid-Embryonic Mortality (11-20 d)	Late Embryonic Mortality (21-28 d)	Pipped Embryos[1]	Hatchability
Low	0-7	95.33	3.85	.39	9.20[b]	15.15	72.60[a]
	8-14	94.33	4.62	.42	14.66[a]	16.83	65.34[b]
	0-14	94.48	4.56	.40	11.29[b]	15.45	69.78[ab]
	SEM	.59	.73	.15	.99	1.71	1.82
High	0-7	95.21	7.27[b]	.19	15.24[b]	14.38	63.91[a]
	8-14	95.26	10.20[a]	.37	18.44[a]	12.30	59.51[b]
	0-14	95.37	9.11[ab]	.40	15.90[ab]	13.42	62.17[ab]
	SEM	.84	.72	.14	1.14	1.15	1.10
Cross	0-7	95.54	5.15	.43	15.30	9.95[c]	70.35
	8-14	94.58	4.77	.46	14.23	13.86[a]	67.91
	0-14	93.30	5.16	.60	13.95	11.96[b]	69.53
	SEM	.84	.49	.20	1.02	.70	1.56
Source of variation		---------- Probabilities ----------					
	Line	NS	<.001	NS	<.001	<.001	<.001
	Time	NS	<.01	NS	<.01	NS	<.001
	Interaction	NS	NS	NS	<.04	NS	NS

[1] Embryos which externally pipped the shell, but did not hatch.
[a,b] Means in columns within lines without a common superscript differ significantly (P<.05).

Table A2.2. Incubation performance (%) of eggs stored from 0-4, 5-9, 10-14, and 0-14 d prior to incubation in three lines of Wrolstad Medium White hens during the 1993 breeding season (Experiment 2)

Line	Pre-incubation Egg Storage Time (d)	Fertility	Early Embryonic Mortality (0-10 d)	Mid-embryonic Mortality (11-20 d)	Late Embryonic Mortality (21-28 d)	Pipped Embryos[1]	Hatchability
Low	0-4	93.83	6.46	.92	8.04[b]	12.27	72.30[a]
	5-9	92.62	8.10	1.37	9.47[b]	14.98	66.09[b]
	10-14	95.80	8.33	1.38	12.04[a]	15.14	63.11[b]
	0-14	93.51	7.56	1.37	9.85[ab]	14.68	66.55[b]
	SEM	1.04	1.05	.39	.98	1.21	1.76
High	0-4	87.98	10.73	2.68[a]	13.51	13.33[ab]	59.75
	5-9	87.66	13.40	1.28[b]	12.18	11.81[b]	61.35
	10-14	90.30	11.00	2.13[ab]	12.95	16.54[a]	57.39
	0-14	87.40	12.03	1.86[ab]	13.01	13.30[ab]	59.81
	SEM	2.15	1.61	.49	1.64	1.64	2.33
Cross	0-4	93.86	7.83	2.31	9.74[b]	9.65	70.48[a]
	5-9	91.54	7.86	1.56	13.56[a]	11.57	65.43[ab]
	10-14	93.54	11.01	2.52	15.44[a]	12.78	58.24[b]
	0-14	90.88	8.83	1.87	13.44[a]	11.56	64.31[ab]
	SEM	1.20	1.24	.53	1.41	1.39	2.99
Source of variation		---------- Probabilities ----------					
	Line	<.001	<.001	<.03	<.001	<.003	<.001
	Time	NS	NS	NS	<.01	<.01	<.001
	Interaction	NS	NS	NS	NS	NS	NS

[1] Embryos which externally pipped the shell, but did not hatch.
[a,b] Means in columns within lines with no common superscripts differ significantly (P<.05).

Table A2.3. Incubation performance (%) of eggs stored from 0-14 d prior to incubation in three lines of Wrolstad Medium White hens fed diets without (CS) or with .5% yeast culture[1] (CS + YC) during the 1992 breeding season (Experiment 1)

Line	Diet	Fertility	Early Embryonic Mortality[2]	Late Embryonic Mortality[3]	Pipped Embryos[4]	Hatchability
Low	CS	94.68	5.41	10.26	12.27[b]	72.75
	CS + YC	94.28	3.70	12.32	18.62[a]	66.82
	SEM	.51	.57	1.08	1.62	1.92
High	CS	92.36	9.31	15.50	13.31	62.39
	CS + YC	92.38	8.91	16.30	13.54	61.94
	SEM	1.32	.42	.80	.78	.88
Cross	CS	94.34	5.57	14.65	12.85	67.33[b]
	CS + YC	92.26	4.75	13.24	11.06	71.73[a]
	SEM	1.19	.27	.94	.69	1.24
Source of variation		----------	---------- Probabilities ----------			
	Line	NS	<.01	<.01	<.01	<.01
	Diet	NS	<.02	NS	<.05	NS
	Interaction	NS	NS	NS	<.01	<.01

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills, Inc., Cedar Rapids, IA.
[2] 0 to 10 d of incubation.
[3] 21 to 28 d of incubation.
[4] Embryos which externally pipped the shell, but did not hatch.
[a,b] Means in columns within lines with no common superscripts differ significantly (P<.05).

Table A2.4. Incubation performance (%) of eggs stored from 0-14 d prior to incubation in three lines of Wrolstad Medium White hens fed diets without (CS) or with .5% yeast culture[1] (CS + YC) during the 1993 breeding season (Experiment 2)

Line	Diet	Fertility	Early Embryonic Mortality[2]	Late Embryonic Mortality[3]	Pipped Embryos[4]	Hatchability
Low	CS	95.50[a]	6.60	9.43	15.11	67.94
	CS + YC	91.50[b]	8.51	10.27	14.26	65.16
	SEM	.92	.83	.78	1.00	1.43
High	CS	83.80[b]	14.72[a]	13.25	13.24	56.54[b]
	CS + YC	91.00[a]	9.35[b]	12.77	13.36	63.07[a]
	SEM	1.88	1.54	1.70	1.63	1.93
Cross	CS	91.30	10.64	15.66[a]	13.41	59.00
	CS + YC	90.40	7.01	11.19[b]	9.73	69.61
	SEM	1.36	1.35	1.30	1.45	3.68
Source of variation		----------	----------	Probabilities ----------	----------	----------
	Line	<.01	<.01	<.02	<.06	<.01
	Diet	NS	<.02	NS	NS	<.01
	Interaction	<.01	<.01	NS	NS	<.01

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills, Inc., Cedar Rapids, IA.
[2] 0 to 10 d of incubation.
[3] 21 to 28 d of incubation.
[4] Embryos which externally pipped the shell, but did not hatch.
[a,b] Means in columns within lines with no common superscripts differ significantly (P<.05).

Table A2.5. The effect of corn-soy bean diets without (CS) and with .5% yeast culture[1] (CS + YC) on selected blood plasma chemistries of Wrolstad Medium White turkey breeder hens[2] during the 1992 breeder season (Experiment 1)

Line	Diet	Creatine Phosphokinase	Lactate Dehydrogenase	Aspartate Aminotransferase	Uric Acid	Total Protein
		(IU/l)	(IU/l)	(IU/l)	(mg/dl)	(mg/dl)
Low	CS	6199	359	572	7.5	4.9
	CS + YC	5873	336	536	6.7	4.9
	SEM	620	35	31	.7	.1
High	CS	3793	266	463	5.6	4.8
	CS + YC	3584	259	492	5.5	5.0
	SEM	636	24	23	.5	.1
Cross	CS	5052	276	527	5.7	4.8
	CS + YC	4199	265	514	5.3	5.0
	SEM	703	35	34	.4	.1
Source of variation		---------- Probabilities ----------				
Line		<.01	<.01	<.03	<.01	NS
Diet		NS	NS	NS	NS	NS
Interaction		NS	NS	NS	NS	NS

[1] XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills, Inc., Cedar Rapids, IA.
[2] Hens were 36, 43, and 50 weeks of age (WOA) in the L and H lines and 34, 41, and 48 WOA in the C line.
[a,b] Means in columns within lines with no common superscripts differ significantly (P<.05).

Table A2.6. The effect of corn-soy bean diets without (CS) and with .5% yeast culture[1] (CS + YC) on selected blood plasma chemistries of Wrolstad Medium White turkey breeder hens[2] during the 1992 breeder season (Experiment 1)

Line	Diet	Phosphorus	Cholesterol	Sodium	Potassium	Triglyceride
		(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)
Low	CS	6.0	200	161	5.1[a]	1043[a]
	CS + YC	5.9	238	160	5.0[a]	1229[a]
	SEM	.2	14	1	0.1	201
High	CS	5.2	181	159	4.6[a]	885[a]
	CS + YC	5.5	186	158	4.7[a]	941[a]
	SEM	.3	13	1	0.1	134
Cross	CS	5.8	150[b]	157	4.5[b]	895[b]
	CS + YC	6.3	206[a]	158	4.7[a]	1331[a]
	SEM	.6	17	1	.1	165
Source of variation		----------	----------	Probabilities	----------	----------
Line		NS	<.02	<.001	<.001	NS
Diet		NS	<.01	NS	NS	NS
Interaction		NS	NS	NS	NS	NS

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills, Inc., Cedar Rapids, IA.
[2] Hens were 36, 43, and 50 weeks of age (WOA) in the L and H lines and 34, 41, and 48 WOA in the C line.
[a,b] Means in columns within lines with no common superscripts differ significantly (P<.05).

Table A2.7. Egg weight, percent yolk weight, and shell breaking strength of eggs from hens[1] fed corn-soy diets without (CS) or with yeast culture[2] (CS + YC) during the 1992 (Experiment 1) and 1993 (Experiment 2) breeding seasons

Line	Diet	Egg Weight (g)	Percent Egg Yolk	Breaking Strength (kg)	Egg Weight (g)	Percent Egg Yolk	Breaking Strength (kg)
		---------- Experiment 1 ----------			---------- Experiment 2 ----------		
Low	CS	83	35.0	4.7	73	31.8	5.0
	CS + YC	82	35.1	4.4	73	30.7	5.3
	SEM	1	.5	.1	1	.6	.2
High	CS	80	35.4	4.1	71	30.4	5.0
	CS + YC	79	35.7	4.0	72	30.4	4.8
	SEM	1	.6	.2	1	.4	.1
Cross	CS	80	35.1	4.0	71	30.7	5.6
	CS + YC	80	36.3	4.2	72	30.4	4.9
	SEM	1	.7	.2	1	.7	.6
Source of variation		---------- Probabilities ----------					
	Line	<.01	NS	<.01	<.02	NS	NS
	Diet	NS	NS	NS	NS	NS	NS
	Interaction	NS	NS	NS	NS	NS	NS

[1] L and H line hens were 50 weeks of age (WOA) and C line hens were 48 WOA (Experiment 1), and hens were 51 WOA (Experiment 2).

[2] XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills, Inc., Cedar Rapids, IA.

Table A2.8. Goblet cell count per mm of villus, mean villus height, villus width and area, and crypt depth in line C hens fed diets without (CS) or with .5% yeast culture[1] (CS + YC) at 51 weeks of age

Diet	Goblet cells[2]	Villus Height	Villus Width	Crypt Depth
	number/mm		(mm)	
CS	66	1.22	.068[b]	.13
CS + YC	52	1.49	.088[a]	.15
SEM	5	.08	.003	.01
Source of variation		Probabilities		
Diet	<.13	<.08	<.008	<.32

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills, Inc., Cedar Rapids, IA.
[2] Goblet cells were enumerated within .01 mm of the apical surface along the perimeter of 9 villi per bird.
[a,b] Means within a column with no common superscripts differ significantly (P<.01).

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APPENDIX 3. EFFECTS OF MATERNAL DIETS SUPPLEMENTED WITH YEAST CULTURE (YC), FEEDING DIETS CONTAINING AUTOCLAVED YC (TRIAL 1) AND FEEDING DIETS SUPPLEMENTED WITH YC (TRIAL 2) ON POULT PERFORMANCE

Introduction

Two trials were conducted to evaluate the effects of maternal diets without and with .5% YC (XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills, Inc., Cedar Rapids, IA) on poult livability and general performance and to evaluate the effects of additional dietary treatments. The breeder hens described previously in Chapter IV, Experiments 1 and 2 were the source of the poults in Trials 1 and 2, respectively.

The objective of Trial 1 was to evaluate the effect of maternal diets containing .5% YC (Experiment 1) and the effects of feeding poults .5% biologically inactivated (autoclaved) YC on body weights, feed to gain ratios, and mortality at 28 and 56 days of age (DOA). The YC in this trial was autoclaved as a preliminary study conducted to investigate the possible lack of YC activity after heat treatment (autoclaving being an exaggerated model of feed pelleting).

The objectives of Trial 2 were to determine the effects of maternal diets with .5% supplemental YC (Experiment 2) and the feeding of diets without and with .5% YC to poults

of three genetic lines (L, H, and C) on body weights, feed consumptions, feed to gain ratios, and mortality (1 to 29 DOA). In addition, percentage gizzard, liver, and small intestine weights were determined at 29 DOA.

Materials and Methods

In Trial 1, 160 poults from each of the two maternal diets were randomized among 8 pens (4 pens per maternal diet, 40 poults per pen). Poults were fed 28% CP corn-soybean meal diets without (CS) and with .5% autoclaved YC (CS + AYC, autoclaved at 121 C for 35 min at 12 psi). Body weights were measured at 1, 28, and 35 DOA. Feed to gain ratios, and percent mortality were determined at 28 and 56 DOA.

In Trial 2, 120 poults from each of the two maternal diets were randomized within maternal diet and three genetic lines (2 X 2 X 3 factor experiment) among 24 battery cages (4 pens per maternal diet within line, 4 pens per poult diet within line, 10 poults per pen). The poults were fed 28% CP corn-soy diets without (CS) and with .5% YC (CS + YC). Poult body weights were measured at 1, 15, and 29 DOA. Feed consumption, feed to gain ratios, percent mortality, and the percent gizzard, liver and small intestine (as a percent of live body weight) were determined at 29 DOA.

Results

Trial 1

The effects of maternal diet (.5% dietary YC) and feeding .5% autoclaved YC to poults from 1 to 56 DOA are summarized in Table A3.1. There were no differences ($P<.05$) in body weight, feed to gain ratios, or percent mortality at 4 and 8 WOA due to either .5% autoclaved YC or maternal diets containing .5% YC. These results suggest that autoclaved YC and maternal dietary YC do not affect body weight, feed to gain ratios, or percent mortality at 4 and 8 WOA.

Trial 2

The effects of maternal diets (.5% YC) and feeding poults .5% dietary YC from 1 to 28 DOA on initial body weights, body weight gains, feed consumptions, feed to gain ratios, percent mortality, and the percentage of gizzard, liver, and small intestine weights of L, H, and C line poults for each sex were determined. A sex difference ($P<.01$) in body weight gains at 15 and 29 DOA was observed; consequently, body weight and the percent gizzard, liver and small intestine weights in L, H, and C line poults are summarized for females and males in Tables A3.2 and A3.3,

respectively. The initial female body weights were lower ($P<.05$) in poults assigned to the diet containing YC, however, the poults receiving YC surpassed ($P<.05$) the control fed poults in body weight gain at 15 d. The addition of supplemental YC to the diet of female poults also reduced ($P<.05$) percent liver and small intestine weights in the line C poults and increased ($P<.05$) the percent weight of the small intestine in the H line poults at 29 DOA, Table A3.2. The initial male body weights and subsequent body weight gains at 15 and 29 DOA were not different ($P>.05$) in poults fed diets containing YC in any of the three lines. The addition of supplemental YC to the diet of male poults also had no effect ($P>.05$) on the percent gizzard, liver and small intestine weights among the three lines of poults at 29 DOA, Table A3.3.

The effects of .5% supplemental YC on feed consumptions, feed to gain ratios, and percent mortality in L, H, and C line poults at 29 DOA are summarized in Table A3.4. No dietary differences ($P>.05$) were apparent among any of the three genetic lines of poults; however, there was a line effect ($P<.02$) as the H line poults experienced an increase in feed to gain ratios and increased ($P<.07$) percent mortality when compared to the L and C line poults at 29 DOA. The effects of .5% YC in the maternal diet on feed consumptions, feed to gain ratios, and percent

mortality in L, H, and C line poults at 29 DOA are summarized in Table A3.5. No differences (P>.05) due to maternal dietary YC were observed among the three lines of poults; however, there was a consistent (P<.04) increase in feed to gain ratios and an increased (P<.08) percent mortality in the H line poults when compared to the L and C line poults at 29 DOA. These results suggest that the addition of .5% YC to the maternal diet does not affect poult growth performance or livability. The varied poult responses (between lines and sex) beginning at 15 DOA, however, demonstrated that genetic and sex-related variation is important in evaluating diets containing a YC. Consequently, the sex and genotype of poults may be an important aspect in the design of future research involving the influences of feeding diets containing a YC.

Table A3.1. Effects of diets without (CS) and with .5% yeast culture[1] (CS + YC) and maternal diets without (MCS) and with .5% yeast culture (MCS + YC) on body weights, feed to gain ratios, and mortality of straight-run poults[2] at 4 and 8 weeks of age (WOA)

Factor	4 WOA			8 WOA		
	Mean Body Weight	Feed to Gain Ratio	Mortality	Mean Body Weight	Feed to Gain Ratio	Mortality
Diet	(g)	(g:g)	(%)	(g)	(g:g)	(%)
CS	1.50	1.42	.8	4.56	1.88	2.0
CS + YC	1.47	1.42	2.3	4.66	1.88	2.5
SEM	.02	.01	.8	.06	.04	1.1
Maternal Diet						
MCS	1.48	1.42	1.0	4.62	1.84	1.8
MCS + YC	1.49	1.41	2.0	4.60	1.92	2.8
SEM	.01	.01	.9	.06	.04	1.1
Source of variation	---------- Probabilities ----------					
Diet	NS	NS	NS	NS	NS	NS
Maternal Diet	NS	NS	NS	NS	NS	NS

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.
[2] Poults were from L, H, and C line hens described in Chapter IV, Experiment 1.

Table A3.2. Individual mean body weights of female poults[1] fed diets without (CS) and with .5% yeast culture[2] (CS + YC) on body weight gains and percent gizzard, liver and SI[3] weights at 29 days of age

Line	Diet	Day-old Body Weight (1 d)	Body Weight Gain (15 d)	Body Weight Gain (29 d)	Gizzard	Liver	SI
		(g)			(%)		
Low	CS	48.6	173	519	2.96	1.82	6.51
	CS + YC	47.9	164	481	2.99	1.85	6.83
	SEM	1.8	9	21	.16	.04	.16
High	CS	47.9[a]	144[b]	447	3.02	1.91	6.01[b]
	CS + YC	45.9[b]	176[a]	497	3.16	1.94	6.79[a]
	SEM	.7	9	24	.16	.14	.23
Cross	CS	46.7	160	491	2.86	1.86[a]	6.54[a]
	CS + YC	46.6	158	508	2.66	1.69[b]	5.83[b]
	SEM	1.2	10	34	.13	.06	.21
Source of variation		Probabilities					
Sex		NS	<.01	<.001	NS	<.01	NS
Line (L)		NS	NS	NS	NS	<.05	<.01
Diet (D)		NS	NS	NS	NS	NS	NS
Maternal Diet (MD)		NS	<.07	<.01	NS	NS	NS
L X D		NS	<.02	NS	NS	NS	<.02
L X MD		NS	NS	NS	<.05	NS	<.08

[1] Poults were from L, H, and C line hens described in Chapter IV, Experiment 2.
[2] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.
[3] Small intestine (from the end of the duodenal loop to the ileocecocolic junction).

Table A3.3. Individual mean body weights of male poults[1] fed diets without (CS) and with .5% yeast culture[2] (CS + YC) on body weight gains and percent gizzard, liver and SI[3] weights at 29 days of age

Line	Diet	Day-old Body Weight (1 d)	Body Weight Gain (15 d)	Body Weight Gain (29 d)	Gizzard	Liver	SI
		(g)			(%)		
Low	CS	46.6	182	566	2.75	1.79	6.55
	CS + YC	49.7	172	546	2.87	1.79	6.47
	SEM	1.3	8	26	.15	.04	.28
High	CS	47.3	164	538	2.97	1.80	6.18
	CS + YC	46.7	180	564	3.14	1.71	6.17
	SEM	.8	9	23	.14	.04	.19
Cross	CS	48.0	186	604	2.97	1.68	6.29
	CS + YC	45.8	166	554	3.05	1.69	6.03
	SEM	1.0	9	23	.13	.06	.24
Source of variation		Probabilities					
Sex		NS	<.01	<.001	NS	<.01	NS
Line (L)		NS	NS	NS	NS	<.05	<.01
Diet (D)		NS	NS	NS	NS	NS	NS
Maternal Diet (MD)		NS	<.07	<.01	NS	NS	NS
L X D		NS	.02	NS	NS	NS	<.02
L X MD		NS	NS	NS	<.05	NS	<.08

[1] Poults were from L, H, and C line hens described in Chapter IV, Experiment 2.
[2] XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills Inc., Cedar Rapids, IA.
[3] Small intestine (from the end of the duodenal loop to the ileocecocolic junction).

Table A3.4. Feed consumptions, feed to gain ratios, and percent mortality of straight-run poults[1] fed diets without (CS) and with .5% yeast culture[2] (CS + YC) at 29 days of age

Line	Diet	Feed Consumption	Feed to Gain Ratio	Mortality
		--- (g) ---	-- (g:g) --	--- (%) ---
Low	CS	963	1.83	0
	CS + YC	981	1.81	0
	SEM	31	.02	0
High	CS	945	1.89	10.50
	CS + YC	975	2.01	13.00
	SEM	64	.04	7.21
Cross	CS	1019	1.86	7.75
	CS + YC	947	1.86	3.25
	SEM	30	.05	3.93
Source of variation		---------- Probabilities ----------		
Line		NS	<.02	<.07
Diet		NS	NS	NS
Interaction		NS	NS	NS

[1] Poults were from L, H, and C line hens described in Chapter IV, Experiment 2.

[2] XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills Inc., Cedar Rapids, IA.

Table A3.5. Feed consumptions, feed to gain ratios, and percent mortality of straight-run poults[1] from hens fed diets without (MCS) and with .5% yeast culture[2] (CS + YC) at 29 days of age

Line	Maternal Diet	Feed Consumption	Feed to Gain Ratio	Mortality
		--- (g) ---	- (g:g) -	-- (%) --
Low	MCS	979	1.80	0
	MCS + YC	965	1.84	0
	SEM	32	.02	0
High	MCS	958	1.93	9.67
	MCS + YC	961	1.96	13.00
	SEM	74	.06	8.30
Cross	MCS	983	1.85	6.75
	MCS + YC	983	1.87	4.25
	SEM	36	.05	4.08
Source of variation		---------- Probabilities ----------		
Line		NS	<.04	<.08
Maternal Diet		NS	NS	NS
Interaction		NS	NS	NS

[1] Poults were from L, H, and C line hens described in Chapter IV, Experiment 2.

[2] XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills Inc., Cedar Rapids, IA.

APPENDIX 4. THE EFFECTS OF .02% SUPPLEMENTAL YEAST (*SACCHAROMYCESCEREVISIAE* VAR. *BOULARDII*) AND .05% COPPER SULFATE ON POULT PERFORMANCE AND ILEAL MORPHOLOGY[1]

Introduction

The effects of a newly marketed yeast, *Saccharomyces cerevisiae* var. *boulardii* (SCB, Levucell SB20®, Agrimerica, Inc., Northbrook, IL), on poult performance and ileal morphology was described in Chapter V. The effects of copper sulfate in the diets of poults fed SCB was also studied at that time since in preliminary studies (*in vitro*), diets containing .05% copper sulfate (CU) inhibited gas production of a yeast culture (XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills Inc., Cedar Rapids, IA). The *in vivo* effects of this phenomena were investigated by using a 2 X 2 factorial design. Since the focus of this thesis is on the effects of a yeast and a yeast culture, partial results of this factorial study were presented in Chapter V and the complete results are summarized in this Appendix 4. The effects of SCB and exogenous amounts of dietary CU on poult performance and ileal morphology from 21 to 35 days of age (DOA) are summarized as follows.

Materials and Methods

The management and manner in which the influences of supplemental SCB (.02%) and CU (.05%) fed to Nicholas poults (line cross 50-0602) on the effects of weight gain, feed consumption, feed to gain ratios, goblet cell numbers, villus height and width, and crypt depth at 35 DOA were studied have been described in Chapter V.

Statistical Analysis

A factorial design of treatments was used in this analysis with the model: $Y_{ijk} = \mu + SCB_i + CU_j + (SCB*CU)_{ij} + \varepsilon_{ijk}$, where; Y_{ijk} = the k^{th} observation (k = 1, 2, ..., n), on the i^{th} class of SCB (i = 1, 2), on the j^{th} class of CU (j = 1, 2); μ = overall mean; SCB_i = the effect of i^{th} diets and CU_j = the effect of j^{th} CU; $(SCB*CU)_{ij}$ = the interaction between SCB and CU; ε_{ijk} = the random error term. In order to maximize the information contained in this experiment, the mean of the simple effects are given in the tables and are explained in the text while the probabilities of the factorial analysis are given at the bottom of each table. Means were separated by the Protected Least Significant Difference test similar to Chapter V.

Results

Body weight gains, feed consumptions, and feed to gain ratios from 21 to 35 DOA are summarized in Table A4.1. No differences (P>.10) in body weight gains, feed consumptions, and feed to gain ratios were observed between 21 and 35 DOA; however, a numerically higher (100 g) periodic weight gain was observed in birds fed SCB when compared to the control (CS) fed birds. Those birds fed both SCB and CU demonstrated the poorest response in weight gains, feed consumptions and feed to gain ratios. No differences (P>.05) were observed in percent mortality or the incidence of leg weaknesses (data not shown).

Morphology changes (goblet cell numbers, villus height, villus width, and crypt depth) in poults fed the four experimental diets are summarized in Table A4.2. Goblet cell counts were lowest (P<.001) in poults fed SCB. When CU was added to the control and SCB diets, however, goblet cell counts increased (P<.001) beyond those of poults fed the control or SCB. There was also a slight SCB-CU interaction (P<.09) for goblet cell numbers as the influence of SCB was negated by the addition of CU to the diet. Villus height in poults fed SCB + CU was increased (P<.03) when compared with those of poults fed either the control or SCB diets. Although not as apparent as the other morphology changes, a small increase (P<.12) in villus width was observed in

poults fed SCB + CU when compared to the control fed birds. An increased ($P<.02$) crypt depth was also observed in birds fed diets containing SCB + CU as compared to birds fed only the SCB or CU diets, but was not different from the poults fed the control diet. The increased crypt depth observed in the birds receiving the diet containing SCB + CU resulted in a SCB-CU interaction ($P<.006$) and suggests that an increased rate of epithelial cell proliferation is occurring in birds fed this diet as compared to those fed only SCB or CU diets.

The results of this study indicate that CU can eliminate the reduction in goblet cells observed in poults fed SCB. These results also reveal that SCB and CU fed separately do not affect crypt depth, but when they are combined (SCB + CU), they can cause increased crypt depth which may indicate a greater demand for intestinal epithelial maintenance (Neutra, 1988). Furthermore, SCB + CU diets also increased villus height thereby providing a greater surface area for subsequent nutrient absorption. This increased surface area may be an indication that a lower nutrient density exists within the lumen of birds fed CU diets, and thus, the poult is required to maintain a higher villus length (Neutra, 1988). These findings also suggest that the microbial ecology of the ileum is altered when poults are fed diets with SCB, CU, and SCB + CU (Larson, 1989). Consequently, further research

evaluating the effects of SCB, CU, SCB + CU, and the SCB-CU interactions observed on poult performance and gastrointestinal morphology are essential.

Table A4.1. Periodic weight gains, periodic feed consumption and feed to gain ratios in poults[1] fed diets without (CS) and with either .02% yeast[2] (CS + SCB), .05% copper sulfate[3] (CS + CU), or .05% copper sulfate with yeast (CS + CU + SCB) from 21 to 35 days of age

Diet	Weight Gain	Feed Consumption	Feed to Gain Ratio
	---------- (g) ----------		-- (g:g) --
CS	835	1416	1.75
CS + SCB	938	1609	1.73
CS + CU	846	1527	1.81
CS + CU + SCB	745	1353	1.86
SEM	77	115	.11
Source of variation	---------- Probabilities ----------		
SCB	NS	NS	NS
CU	NS	NS	NS
Interaction	NS	NS	NS

[1] Nicholas line (50-0602) described in Chapter V.

[2] Levucell SB20®, *Saccharomyces cerevisiae* var. *boulardii*, Agrimerica, Inc., Northbrook, IL.

[3] Copper sulfate containied .25% copper as $CuSO_4 \bullet 5H_2O$.

Table A4.2. Goblet cell count per mm of villus, mean villus height, width, and crypt depth in poults[1] at 35 days of age (DOA) fed diets without (CS) and with either .02% yeast[2] (CS + SCB), .05% copper sulfate[3] (CS + CU), or .05% copper sulfate with yeast (CS + CU + SCB) from 21 to 35 DOA

Diet	Goblet Cells[4]	Villus Height	Villus Width	Crypt Depth
	number/mm	(mm)		
CS	40.7[b]	1.03[b]	.079	.19[ab]
CS + SCB	31.9[c]	1.07[b]	.093	.14[b]
CS + CU	51.0[a]	1.25[ab]	.092	.14[b]
CS + CU + SCB	51.0[a]	1.39[a]	.011	.24[a]
SEM	2.30	.07	.007	.02
Source of variation	Probabilities			
SCB	<.09	NS	<.07	NS
CU	<.001	<.007	<.09	NS
Interaction	<.09	NS	NS	<.006

[1] Nicholas line (50-0602) described in Chapter V.

[2] Levucell SB20®, *Saccharomyces cerevisiae* var. *boulardii*, Agrimerica, Inc., Northbrook, IL.

[3] Copper sulfate contained .25% copper as $CuSO_4 \bullet 5H_2O$.

[4] Goblet cells were enumerated within .01 mm of the apical surface along the perimeter of 9 villi per bird.

[a,b] Means within a column with no common superscripts differ significantly ($P<.05$).

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APPENDIX 5. EFFECTS OF FEEDING .25% YEAST CULTURE (*SACCHAROMYCES CEREVISIAE*[XP]) TO MARKET TURKEYS ON ILEAL MORPHOLOGY

Introduction

Direct-fed microbials (DFM) have been implicated in altering viscera weights through changes in the gut microbiota. Yeast have also affected viscera weights in broilers fed diets containing aflatoxin (Stanley *et al.*, 1993). In addition, microanatomical changes in duodenal sections have also been observed with the use of bacterial DFM (Samuel Nahashon, 1994, Withycombe Hall 112, Oregon State University, Corvallis, OR 97331, unpublished data). Consequently, the hypothesis that supplemental YC could also influence the percent viscera weights of Large White turkeys was investigated.

The effects of .25% supplemental yeast culture (YC) on selected percent wet viscera weights and ileal microscopic morphology were measured from birds described in Chapter VI, Experiment 3. At each weighing period, one bird (whose weight was close to that of the representative mean pen body weight) was selected from each pen. Select wet viscera weights were measured immediately after dissection at 2, 5, 8, 11, 14, and 17 weeks of age (WOA). The percent wet viscera weights were calculated as the wet viscera weight divided by the live body weight, multiplied by 100.

Histological tissue examinations were performed as described in Chapter IV at 2, 8, 14, and 17 WOA (except 4 photomicrographs were recorded from each bird and 4 counts were taken randomly form each photomicrograph, a total of 16 measurements per bird).

Results

The data summarized in Table A5.1 demonstrates the effects of feeding .25% dietary YC on percent weights of gizzard, spleen, liver ceca, small intestine, crop and the bursa of Fabricius. Because of labor time constraints, the ceca, small intestine and bursa of Fabricius were measured at 2, 5, 8, and 11 WOA; and the crop was weighed at 2, 5, and 11 WOA. No differences ($P>.05$) were apparent during any of the weekly intervals measured, however, numerical trends were observed as the percent gizzard weights were greater in turkeys fed supplemental YC. There was an overall (2 through 17 WOA) increase ($P<.04$) of .37% in gizzard weight in turkeys receiving YC. As expected, the normal growth and development of the turkeys caused decreases ($P<.0001$) in the percent gizzard, spleen, liver, ceca, and bursa weights, and increases ($P<.0001$) in the percent small intestine and crop, Table A5.2.

The effects of diets without and with .25% YC on the number of goblet cells per mm of villus height, villus

height and width, and crypt depth measured at 2, 8, 14, and 17 WOA are summarized in Table A5.3. At 2 WOA, increases in villus width ($P<.006$) and crypt depth ($P<.05$) were apparent for birds fed diets containing .25% dietary YC. Although not significant ($P>.10$), the number of goblet cells were reduced by 9.13 per mm of villus height in birds fed supplemental YC at 2 WOA. No dietary differences ($P>.10$) were observed in the morphologic features measured at 8 WOA. At 14 WOA, there were increases ($P<.03$) in the number of goblet cells and the crypt depth in turkeys fed YC. At 17 WOA, turkeys fed the dietary YC demonstrated increased ($P<.08$) villus heights and reduced ($P<.05$) villus widths.

The number of goblet cells per mm of villus height, villus height and width, and crypt depth measured at 2, 8, 14, and 17 WOA are summarized in Table A5.4. No differences ($P>.05$) were observed in the number of goblet cells between weeks 2, 8, 14, and 17 WOA. A significant ($P<.02$) increase in villus height was observed at 2 WOA as compared to 8 and 14 WOA, however, this trend was not sustained through 17 WOA. The reason for this variation at 17 WOA remains unknown. Although differences ($P<.0002$) in villus width were apparent at 2 and 14 WOA vs 8 and 17 WOA, no plausible explanation can be given at this time to explain these differences. A significant ($P<.01$) decrease in crypt depth was observed between weeks 2 and 8 vs weeks 14 and 17 WOA.

These data indicate that the ileal micromorphology of the growing turkey is constantly changing and although dietary differences do appear, they are not entirely consistent over time. In addition, the responses in the percent weights of gizzard, spleen, liver, small intestine, crop, bursa, and ileal morphology observed in this study suggest that a large amount of variation may be expected when evaluating the effects of YC in the diets of market turkey toms.

Table A5.1. The effects of diets without (CS) and with .25% additional yeast culture[1] (CS + YC) in market turkeys[2] on wet viscera weights (expressed as a percent of live body weight) measured at 2, 5, 8, 11, 14, and 17 weeks of age (WOA)

WOA	Diet	Gizzard	Spleen	Liver	Ceca	SI[3]	Crop	Bursa
2	CS	5.73	3.14	4.60	1.05	.62	.09	.15
	CS + YC	5.89	2.88	5.02	1.17	.73	.08	.19
	SEM	.09	.26	.45	.09	.03	.01	.02
5	CS	4.32	.11	1.82	.96	3.41	.39	.14
	CS + YC	4.34	.10	2.01	1.02	3.38	.35	.16
	SEM	.20	.01	.22	.14	.13	.03	.02
8	CS	4.05	.11	2.10	.98	3.68	NM	.13
	CS + YC	4.33	.13	1.82	1.00	3.67	NM	.15
	SEM	.50	.01	.10	.09	.26	NM	.01
11	CS	2.76	.10	1.40	.68	2.44	.37	.10
	CS + YC	3.09	.10	1.28	.59	2.27	.34	.10
	SEM	.40	.01	.09	.05	.16	.03	.01
14	CS	1.61	.07	1.52	NM	NM	NM	NM
	CS + YC	1.75	.08	1.72	NM	NM	NM	NM
	SEM	.25	.01	.14	NM	NM	NM	NM
17	CS	1.06	.07	1.47	NM	NM	NM	NM
	CS + YC	1.38	.08	1.43	NM	NM	NM	NM
	SEM	15	.01	.10	NM	NM	NM	NM
Source of variation		---------- Probabilities ----------						
	WOA	<.0001	<.0001	<.001	<.01	<.01	<.01	<.004
	Diet	<.04	NS	NS	NS	NS	NS	NS

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills, Inc., Cedar Rapids, IA.

[2] Chapter VI, Experiment 3.

[3] Small intestine (from the end of the duodenal loop to the ileocecocolic junction).

NM = Not measured.

Table A5.2. The effect of age on market turkey[1] wet viscera weights (expressed as a percent of live body weight) measured at 2, 5, 8, 11, 14, and 17 weeks of age (WOA)

WOA	Gizzard	Spleen	Liver	Ceca	Small Intestine[2]	Crop	Bursa
2	5.81[a]	3.01[a]	4.81[a]	1.11[a]	.67[c]	.09[b]	.17[a]
5	4.33[b]	.12[b]	1.91[b]	.99[a]	3.39[a]	.37[a]	.15[a]
8	4.19[b]	.10[b]	1.96[b]	.99[a]	3.68[a]	NM	.14[a]
11	2.43[c]	.10[b]	1.34[c]	.64[b]	2.35[b]	.35[a]	.10[b]
14	1.68[d]	.08[b]	1.62[c]	NM	NM	NM	NM
17	1.22[d]	.07[b]	1.45[c]	NM	NM	NM	NM
SEM	.19	.06	.10	.06	.10	.01	.01
Source of variation	----------	----------	----------	Probabilities	----------	----------	----------
WOA	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Diet	NS	NS	NS	NS	NS	NS	NS

[1] Chapter VI, Experiment 3.
[2] Small intestine was measured from the termination of the duodenal loop where the bile duct intersects the ileocecocolic junction.
[a,b] Means within a column with no common superscripts differ significantly (P<.05).
NM = Not measured.

Table A5.3. The effects of diets without (CS) and with .25% additional yeast culture[1] (CS + YC) in market turkeys[2] on ileal microscopic morphology[3] measured at 2, 8, 14, and 17 weeks of age (WOA)

WOA	Diet	Goblet Cells[4]	Villus Height	Villus Width	Crypt Depth
		number/mm	---------- (mm)	----------	
2	CS	50.22	.88	.073[b]	.13[b]
	CS + YC	41.09	.86	.113[a]	.19[a]
	SEM	5.17	.02	.005	.01
8	CS	44.10	1.20	.138	.20
	CS + YC	48.62	1.04	.145	.19
	SEM	2.37	.11	.020	.03
14	CS	35.19[b]	1.00	.091	.10[b]
	CS + YC	44.33[a]	1.05	.089	.14[a]
	SEM	1.98	.06	.010	.01
17	CS	49.03	.91	.159[a]	.10
	CS + YC	44.71	1.07	.101[b]	.10
	SEM	5.96	.05	.010	.01
Source of variation		---------- Probabilities ----------			
WOA		NS	<.01	<.008	<.001
Diet		NS	NS	NS	<.07
Interaction		NS	NS	NS	NS

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills, Inc., Cedar Rapids, IA.

[2] Nicholas male line crosses (Chapter VI, Experiment 3).

[3] A total of 16 histological measurements were used in estimating the mean for each bird.

[4] Goblet cells were enumerated within .01 mm of the apical surface along the perimeter of 16 villi per bird.

[a,b] Means within a column with no common superscripts differ significantly (P<.05).

Table A5.4. The effect of age on market turkey[1] ileal microscopic morphology[2] measured at 2, 8, 14, and 17 weeks of age (WOA)

WOA	Goblet Cells[2]	Villus Height	Villus Width	Crypt Depth
	number/mm	(mm)		
2	45.7	.87[b]	.09[b]	.16[a]
8	46.4	1.12[a]	.14[a]	.19[a]
14	39.8	1.03[a]	.09[b]	.12[b]
17	46.9	.99[ab]	.13[a]	.10[b]
SEM	2.3	.04	.01	.01
Source of variation	Probabilities			
WOA	NS	<.02	<.0002	<.01
Diet	NS	NS	<.07	NS

[1] Nicholas male line crosses (Chapter VI, Experiment 3).

[2] A total of 16 histological measurements were used in estimating the mean for each bird.

[3] Goblet cells were enumerated within .01 mm from the apical surface in 16 villi per bird.

[a,b] Means within a column with no common superscripts differ significantly (P<.05).

REFERENCE

Stanley, V. G., R. Ojo, S. Woldesenbet, D. Hutchinson, and L. F. Kubena, 1993. The use of *Saccharomyces cerevisiae* to suppress the effects of aflatoxicosis in broiler chicks. Poultry Sci. 72:1867-1872.

APPENDIX 6. EFFECTS OF FEEDING HYBRID MARKET MALE TURKEYS DIETS CONTAINING .5% YEAST CULTURE (*SACCHAROMYCES CEREVISIAE*^{XP} ON PRODUCTION PERFORMANCE

Introduction

This appendix details a feeding trial conducted as a preliminary experiment to determine the effects of feeding commercial Hybrid Large White market male turkeys corn-soybean meal diets without and with .5% yeast culture (YC, XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills Inc., Cedar Rapids, IA) on growth rate, feed consumption, feed to gain ratios, livability, leg weaknesses, and cannibalism. Similar experiments have been described using Nicholas Large White male turkeys in Chapter VI.

Materials and Methods

A total of 240 commercial Hybrid Large White day-old poults were obtained from the Oregon Turkey Hatchery, Aurora, OR (January, 1991). The poults were serviced (sexed, toe-trimmed, desnooded and provided with a subcutaneous injection of B-complex vitamins) at the hatchery. Poults were wing banded for identification, placed in one of 12 pens with pine wood shavings as litter, and fed corn-soy diets without or with .5% YC. The 12 pens

were located in the same room to provide a common environment (temperature, ventilation and lighting). The poults were provided continuous (24L:0D) incandescent light until 1 week of age (WOA) and were then provided with a photoperiod of 12L:12D for the remainder of the study.

The parameters measured were described previously in Chapter VI. The feeding program, feed form, and feeding intervals are identical to those described in Chapter VI. The analyzed CP values of each diet and feeding interval are given in Table A6.1.

The same procedures used to analyze data in Chapter VI were used with the exception that pen means were used as the experimental units for all variables.

Results and Discussion

Body weights of the poults fed diets without and with YC were measured at day-old, 2, 5, 8, 11, 14 and 17 WOA and are summarized in Table A6.2. Although complete randomization procedures were used, there was an unexplained dietary difference ($P<.02$) between diets without and with YC at day-old. The initial trend in body weight at one d of age was reversed at 2 WOA and the turkeys fed YC were lighter at 5 ($P<.001$), 8 ($P<.002$), 14 ($P<.02$), and 17 ($P<.005$) WOA.

Periodic body weight gains were maintained or were consistently lower for birds fed diets containing YC, Table A6.3. Periodic weight gains were less for birds fed supplemental YC from 2 to 5 ($P<.001$), 5 to 8 ($P<.005$), and 14 to 17 ($P<.005$) WOA. A slight compensatory weight gain was noted from 8 to 11 WOA in birds fed YC. This same body weight gain from 8 to 11 WOA has been observed in a previous study utilizing the same feed formulations and a different commercially available YC. Thus, the 8 to 11 week improvement suggests that there may be a limiting factor associated with the diets prior to this time. The hypothesis of the author, which has been studied in preliminary studies, is that the coccidiostat (Coban-60®) used in the feed from day-old to 8 WOA adversely interacted with the dietary YC, depressing turkey performance.

Cumulative feed to gain ratios of Hybrid market male turkeys fed dietary YC were greater at 5 ($P<.01$) and 8 ($P<.02$) WOA and are summarized at each interval in Table A6.4. Periodic feed to gain ratios of male turkeys fed dietary YC were greater from 2 to 5 ($P<.01$) WOA, Table A6.5. Cumulative feed consumptions of Hybrid market male turkeys fed dietary YC were lower at 5 ($P<.05$), and 8 ($P<.004$) WOA. The percent incidences of mortality, inter-bird aggression, and leg weakness of male turkeys fed diets without and with YC are summarized in Table A6.6. Although

no statistical analysis was performed, the incidences of leg weaknesses and inter-bird aggression was 10.5 and 1.74% higher in the birds fed YC, respectively. These results indicate that .5% supplemental YC appeared to be detrimental to the performance of Hybrid Large White male turkeys. The depressed feed consumption of birds fed dietary YC may explain the reduced weight gain. The body weight and leg weakness observed may indicate a negative response to the dietary YC supplementation which was magnified from day-old to 8 WOA and which could possibly be a result of the added coccidiostat, Coban.

Table A6.1. Analyzed[1] crude protein (CP) of market turkey corn-soy diets without (CS) and with .5% yeast culture[2] (CS + YC) fed from day-old to 17 weeks of age

	Weeks of Age					
Diet	1 to 2	2 to 5	5 to 8	8 to 11	11 to 14	14 to 17
CS	28.7	25.0	25.3	21.4	18.2	16.9
CS + YC	29.6	25.2	24.9	21.7	19.4	16.7
Calculated CP						
	28.1	25.9	24.4	22.4	19.2	16.9

[1] Kjeldahl analysis.
[2] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.

Table A6.2. Body weights (kg) of Hybrid market turkeys fed diets without (CS) and with .5% yeast culture[1] (CS + YC) from day-old to 17 weeks of age

		Weeks of age					
Diet	Day-old	2	5	8	11	14	17
CS	.057[b]	.230	.95[a]	2.92[a]	5.9	8.9[a]	12.3[a]
CS + YC	.059[a]	.224	.84[b]	2.51[b]	5.5	8.3[b]	11.4[b]
SEM	.001	.003	.02	.07	.2	.15	.2
Source of variation	---------- Probabilities ----------						
Diet	<.02	NS	<.001	<.002	NS	<.02	<.005
Breeder Standard[2]							
	NA	.308	1.50	3.71	6.4	9.3	12.5

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.

[2] Hybrid turkey standards of 1990.

[a,b] Means within columns in each experiment with no common superscripts differ significantly (P<.05).

Table A6.3. Periodic body weight gains (kg) of Hybrid market turkeys fed diets without (CS) and with .5% yeast culture[1] (CS + YC) from day-old to 17 weeks of age

	Weeks of age					
Diet	0 to 2	2 to 5	5 to 8	8 to 11	11 to 14	14 to 17
CS	.173	.72[a]	1.97[a]	2.9	3.1	3.4[a]
CS + YC	.166	.62[b]	1.67[b]	3.0	2.8	3.0[b]
SEM	.003	.02	.06	.1	.1	.1
Source of variation	---------- Probabilities ----------					
Diet	NS	<.001	<.005	NS	NS	<.005
Breeder Standard[2]						
	.410	.92	2.21	2.7	2.9	3.2

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.

[2] Hybrid turkey standards of 1990.

[a,b] Means within experiments in columns with no common superscripts differ significantly (P<.005).

Table A6.4. Cumulative feed to gain ratio of Hybrid Market Turkeys fed diets without (CS) and with .5% yeast culture[1] (CS + YC) from day-old to 17 weeks of age

	Weeks of age					
Diet	0 to 2	0 to 5	0 to 8	0 to 11	0 to 14	0 to 17
CS	1.83	1.95[b]	1.81[b]	2.01	2.26	2.49
CS + YC	1.90	2.13[a]	1.89[a]	2.01	2.30	2.59
SEM	.03	.04	.02	.03	.03	.06
Source of variation	---------- Probabilities ----------					
Diet	NS	<.01	<.02	NS	NS	NS
Breeder Standard[2]						
	NA	1.40	1.64	1.91	2.18	2.47

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.

[2] Hybrid turkey standards of 1990.

[a,b] Means within experiments in columns with no common superscripts differ significantly (P<.02).

Table A6.5. Periodic feed to gain ratios of Hybrid market turkeys fed diets without (CS) and with .5% yeast culture[1] (CS + YC) from day-old to 17 weeks of age

	Weeks of age				
Diet	2 to 5	5 to 8	8 to 11	11 to 14	14 to 17
CS	1.98[b]	1.74	2.22	2.76	3.10
CS + YC	2.20[a]	1.77	2.12	2.87	3.41
SEM	.04	.02	.04	.12	.15
Source of variation					
Diet	<.01	NS	NS	NS	NS

[1] XP yeast culture®, *Saccharomyces cerevisiae*[XP], Diamond V. Mills Inc., Cedar Rapids, IA.

[a,b] Means within experiments in columns with no common superscripts differ significantly (P<.01).

Table A6.6. Mortality, incidence of aggression (cannibalism), and leg weaknesses of Hybrid market turkeys fed diets without (CS) and with .5% yeast culture[1] (CS + YC) from day-old to 17 weeks of age[2]

Diet	Mortality	Incidence of Aggression	Leg Weaknesses
	---------- % ----------		
CS	6.67	2.86	6.25
CS + YC	4.17	4.60	16.82

[1] XP yeast culture®, *Saccharomyces cerevisiae*^{XP}, Diamond V. Mills Inc., Cedar Rapids, IA.

[2] No statistical analysis was performed.