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Six experiments were carried out with Single Comb White Leghorn laying chickens to assess the effect of feeding a source of direct-fed microbials (*Lactobacillus*; Lacto) and its carrier [condensed cane molasses solubles (CCMS)] on the retentions of fat, nitrogen and several minerals; on the status of the pH of the gastrointestinal (GI) tract; on the phytase activities in the Lacto and in the crop and in the intestinal contents and intestinal, pancreatic and liver tissues; on the histological and anatomical changes of the GI tract and on the production performance.

Feeding 1,100 mg Lacto/kg diet (ppm) and 2,200 ppm Lacto in corn-soya bean meal (C-S) diets to layers stimulated appetite, improved egg production (in Experiment 1 only), egg mass, egg weight, egg size, internal egg quality and fat, nitrogen, calcium and phosphorus retentions ($P < .05$). Production performances were not different between the layers fed the 1,100 ppm diet and those fed the 2,200 ppm Lacto diet. Supplementing Lacto diets with 1 and 3% fat reduced feed consumption, provided better feed conversion, egg production, egg masses, egg size, body weight gains, and nitrogen, calcium and phosphorus retentions.

Feeding 1,100 ppm Lacto barley-corn-soya bean (B-C-S) layer diets improved body weight gains and the retentions of fat, phosphorus and manganese and increased the rate of

passage of digesta ($P < .05$). Feeding Lacto C-S and Lacto B-C-S layer diets increased cellularity of Peyer's patches in the ileums of the layers which may stimulate the mucosal immune system. No changes in length and weight of the intestine were observed.

Daily feed consumption and body weight gains were improved when pullets were fed 1,100 ppm Lacto from 7 to 19 wk of age (WOA). When these pullets were continued on the Lacto feed during the laying period (20 to 59 WOA), increased feed consumption, egg size, nitrogen and calcium retentions, increased cellularity of Peyer's patches, decreased length and weight of intestine were observed ($P < .05$).

Presence of phytase activity was higher in condensed cane molasses solubles (CCMS)-*Lactobacillus* premix than the carrier (CCMS). Feeding the CCMS-Lacto diets to layers decreased the pH of the GI tract, increased phytase activities in the GI tract and intestinal tissues and improved shell thickness and phosphorus retention ($P<.05$). The production performance of layers fed .45% and .25% available phosphorus (AP) diets were not different except for body weight gain. Phosphorus retention was better for layers fed diets containing .25% AP with CCMS-Lacto than the .45% AP control diet.

According to these studies, feeding Lacto to pullets and layers improved their performance and the retention of nutrients such as calcium, phosphorus and nitrogen which subsequently reduced the cost of feeding.

Effect of a Direct-fed Microbial on Performance of Single Comb White Leghorn Chickens

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DEDICATION

This Doctoral Dissertation is dedicated to my parents,
Nahashon and Mwikali Kisyua Itumo

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CHAPTER I

INTRODUCTION

The use of probiotics dates back to ancient times when preservation of food/feed such as milk and milk-products by souring was a common practice. Metchnikoff (1908) proposed that *Lactobacillus* in fermented milk displaced from the alimentary tract, microorganisms that produced noxious substances which result in improved human health and longevity. Probiotics are live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989) and is derived from the Greek word, "for life". Probiotics are synonymous to direct-fed microbials. This change in name was brought about by the United States Food and Drug Administration (FDA, 1989) directive requiring manufacturers to use the term direct-fed microbials instead of probiotics. Direct-fed microbials may be bacteria or fungi and are classified as feed additives which are non-nutritive components of animal feeds. They improve feed acceptance, feed efficiency, health and metabolism of the animal (Cheeke, 1991).

Poultry feeds consist largely of cereal grains (corn, milo), oil seeds (soybean meal) and plant derived by-products. The nutritional values of poultry feeds, in addition to the chemical composition, depend on the extent to which nutrients are digested, absorbed and utilized. Two major factors which can alter digestibility are lack of digestive enzymes in the gastrointestinal (GI) tract of poultry and the presence of anti-nutritional factors in the feedstuffs which interfere with digestion, absorption and utilization of nutrients. Inefficiency in the absorption of carbohydrates, nitrogen and phosphorus results from the components of

the feed ingredients such as fiber and β-glucans found in barley which are not digested by the bird. The lack of adequate levels of the phytase enzyme in most monogastric animals contributes to the low utilization of the phytin phosphorus found in plant feedstuffs.

Poultry feeds are routinely supplemented with amino acids, calcium, phosphorus and trace minerals. The low availability of phosphorus and excessive levels of protein (nitrogen) in practical poultry feeds lead to high concentrations of these elements in the poultry waste. Application of large quantities of poultry manure can lead to accumulation of phosphorus and nitrogen in the soil and together with runoff result in polluting ground and surface water sources and the soil.

The objectives of the poultry producers are to increase production at minimal cost and to maintain a clean environment. Improving the utilization of nutrients such as phytin phosphorus and nitrogen will reduce the level of these nutrients in the feeds, lower the excretion of the nutrients in the manure and subsequently reduce feed cost and pollution problems. One way to attain these objectives is by supplementing poultry feeds with enzymes or direct-fed microbials to improve the utilization of the nutrients in the feeds.

The mechanisms by which direct-fed microbials improve poultry performance are not fully understood. However, *Lactobacillus* species are known to produce lactic acid, which reduces the pH of the GI tract of the host animal. The low pH of the GI tract may enhance the solubilization of minerals such as calcium, phosphorus, magnesium, zinc and iron (Ashmead *et al.*, 1985) which can lead to better utilization of these elements by the host animal.

Phytase activities have been reported in microbials such as *Pseudomonas*, *Bacillus subtilis* and *Saccharomyces cerevisiae* sources. There is no report indicating whether direct-fed microbials possess enzymes such as phytase that enhance phosphorus utilization in

animals. However, evidence suggests that microbial cultures containing *Lactobacillus* were successfully used to suppress pathogenic *E. coli* in the gut wall of chickens (Fuller, 1989, Watkins *et al.*, 1982, and Baba *et al.*, 1991) and *Salmonellae* (Dunham *et al.*, 1993). Therefore, the objectives of these studies were to:

1. ascertain the effect of feeding diets containing 1100 ppm and 2200 ppm *Lactobacillus* to laying pullets and the effect of supplementing these *Lactobacillus* diets with 1% and 3% fat on pullet performance and dietary nitrogen, calcium and phosphorus retention
2. determine the effect of feeding either corn-soybean meal or barley-corn-soybean meal with condensed cane molasses solubles-1100 ppm *Lactobacillus* diets to laying pullets on production performance, nutrient retention, gastrointestinal (GI) feed passage rate and on the anatomical and histological changes of the GI tracts of the laying pullets
3. determine the effect of long-term feeding of *Lactobacillus* to pullets from 7 wk of age (WOA) to 19 WOA (growing phase) and from 20 WOA to 59 WOA (laying phase) on production performance, fat, nitrogen, calcium and phosphorus retentions and the anatomical and histological changes of the gastrointestinal (GI) tract
4. determine the presence of phytase activity in direct-fed microbial source (*Lactobacillus*) and its carrier (condensed cane molasses solubles)
5. determine the effect of feeding condensed cane molasses solubles (CCMS) and *Lactobacillus*-CCMS laying diets containing .45 and .25% available phosphorus on the retentions of phosphorus and calcium, the status of the gastrointestinal pH and the production performance of laying pullets.

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CHAPTER II

REVIEW OF LITERATURE

A. GASTROINTESTINAL TRACT MICROBIOTA

A.1 History

Preservation of food/feed by souring, such as fermented milk and milk products, has been used since ancient times. The popularity of direct-fed microbials; therefore, dates back to ancient times. According to the Persian tradition, the method of fermenting milk was revealed to Abraham by an angel, and to this food he owed his fecundity and longevity. In Deuteronomy 32:14, soured milk and goat milk are named by Moses as being among the foods given to his people by God. It is possible that the longevity of the earth's inhabitants before the flood was attributed to the high consumption of dairy products, which contained *Lactobacillus* species.

Almost every village of ancient Asia, Africa and Europe has their own name for yogurt, most of which embodied the idea of divine food, health of long life (Rossel, 1932). Originally the souring of foods was not well received, but later when man discovered that no harm came from consuming sour food, the bacteria were exploited. Therefore, therapeutic involvement of bacterial cultures has been in existence for a long period of time. However, the Russian scientist, (Metchnikoff, 1903), presented the view that if putrefaction in the large intestine of man could be prevented or decreased, aging and senility would be postponed.

Yogurt was liberally consumed in Bulgaria, where an unusually high proportion of centenarians was found. The conclusion that consuming yogurt would alter the intestinal

microbiota and prolong life was well accepted. Metchnikoff (1908), in his book *Prolongation of Life* cited the possible therapeutic value of *Lactobacilli* for humans. He suggested that many human ills arose from harmful bacteria present in the gastrointestinal (GI) tract and proposed that microorganisms other than *Lactobacilli* produced noxious substances that are absorbed through the intestinal wall into the blood stream and slowly poisoning people and shortening their lives. Since he did not consider *Lactobacilli* to be "noxious organisms", he proposed that *Lactobacilli* in fermented milk displaced the microorganisms of the alimentary tract that produced the noxious substances resulting in improved human health and longevity (Metchnikoff, 1908; Fuller, 1984).

In his later studies, Metchnikoff started inoculating sterilized milk with pure cultures of *Lactobacillus acidophilus* to individuals with typhoid and diarrhea during long distance travels. The advertisement for *Lactobacillus* cultures to prevent diarrhea while travelling was advertised in the 1900's. The idea of Metchnikoff (1903) to replace the putrefactive microbiota with lactic acid producing bacteria, has turned into a realization of the importance of maintaining a well balanced GI microbiota.

The digestive activities of ruminants as well as non-ruminants were described in books prior to 1800. During this century, further investigations were begun (Hungate, 1968). It was the omniscient scientist Pasteur (1885) who was the first to reflect on the importance of these microbes to the host, whether their activities were harmful or beneficial (Jonsson, 1985). Pasteur (1885) was involved in the initiation of this field and is considered to be the godfather of the germ-free animal research. He stated that he did not believe life was possible without bacteria. Dubos *et al.* (1965) started to apply general ecological principles to the GI tract and

its microbiota. The knowledge of the GI microbiota, its relation to and importance for the host has expanded enormously during the last decades but the information is difficult to put into a general overall picture.

The existence of microbes intimately associated with the GI epithelia was noted quite early. Kasai and Kobayashi (1919) described spirochetes from the neck of the fundus glands in the stomach of mice, rabbits, cats and rats. These bacteria have been rediscovered by many investigators but little is known about their role in the GI tract. Another important early discovery was the association of *Lactobacillus* with stomach epithelium of rats (Porter and Rettger, 1940). The evaluation on the importance of the GI microbiota to the host has been founded on the work with germ-free animals. When such animals are associated with one or more microbial species, they are called gnotobiotic.

A.2 Evolution

The GI tract has an adaptation to sequester food, and allowing for motility while feeding or doing other activities (Hungate, 1968, 1984). The indigenous part of GI microbiota is likely to have evolved together with the host (Dubos *et al.*, 1965). It is believed to perform beneficial interactions with the host, such as resistance of the host to infectious diseases by powerful direct bacterial interactions.

A.3 Ecological principles

The GI microbiota form an open ecosystem which is complex but stable. There are interactions between the animal and its microbiota and also between bacteria within the microbiota. In the bacterial ecology, inoculation of foreign species cannot change the number

or composition of an open ecosystem. However, the numbers and composition within the microbiota can be changed by changing the environment (Hungate, 1984). For instance, the rumen microbiota in Hawaiian ruminants can detoxify mimosine of *Leucaena sp* (Jones, 1981) but not that of Australian ruminants. The numbers and composition of the GI microbiota can change with time without any discernible changes in the environment, however; the conditions are not static, but fluctuate. Mutant strains have been reported (Hungate, 1984). These strains appear constantly, some find a niche and replace other strains, then are later replaced in turn. The microbes within the GI tract can be classified either as indigenous (autochthonous), belonging to the GI tract or as non-indigenous (allochthonous), not belonging there (Dubos *et al.*, 1965; Savage, 1977). The indigenous (autochthonous) microorganisms are always found in normal adults, can grow anaerobically, colonize particular areas of the tract, colonize their habitats during succession in infant animals, maintain stable population levels in climax communities in normal adults and may be associated intimately with the mucosal epithelium in the area colonized (Savage, 1977).

According to Alexander (1971) and Savage (1977), an ecosystem is composed of habitats and niches. Habitat is the physical space in the ecosystem and is normally occupied by climax communities of autochthonous organisms. A niche in the ecosystem is defined by the way the organisms make their living in the habitat. Allochthonous organisms are frequently found in any given habitat, passing through the GI tract but contributing little to the economy of the system.

A.4 Establishment in the young animal

Indigenous microbial communities in adults (climax communities) are formed as a result of sequential processes that begin in animals after birth (Savage, 1977). The healthy

fetus (animals) or embryo (poultry) are sterile within the uterus and the bird's egg, respectively. During and after birth or hatch, the young are inoculated by the rich environmental microbiota. The bacteria which develop early in the GI tract has been believed to originate from the vagina and feces of the mother in animals and by contact with the environment and coprophagy in poultry (Smith, 1965b; Bettelheim *et al.*, 1974). Mann (1963) cited evidence that airborne transmittance can occur at least within confined spaces of farm buildings. Although the neonate is met by the complex microbiota of the vagina, feces and environment, the bacterial species which establish first in the GI tract do not belong to any of these dominating species (Ducluzeau, 1983). It seems, therefore, as if there are some mechanisms in the neonate which act to select the special microbiota of the GI tract. The diet of both the neonate and its mother might be of great importance for the establishment of the GI microbiota (Ducluzeau *et al.*, 1981; Lhuillery *et al.*, 1981).

Small numbers of bacteria can be found in the GI tract within a few hours after birth/hatch and the highest populations are reaches within 24 hrs (Smith 1965a; Ducluzeau, 1983). The order in which the different bacterial species appear in the young varies with the animal species. Anaerobic bacteria have been considered incapable of developing in the GI tract of germfree animals without a precedence of facultative anaerobic bacteria (Morishita *et al.*, 1972; Fonty *et al.*, 1983).

The establishment of the gastrointestinal microbiota can be impaired if the animal is born and reared under conditions which prevent normal contact with indigenous microbes (Bryant and Small, 1960). Bare and Wiseman (1964) noted a delay of 3 wks for the establishment of *Lactobacilli* in chicks kept in an environment not previously occupied by chicks. Piglets born and reared in a scrupulously clean environment secreted HCl in the stomach from the second day of life, while piglets born and reared in a conventional

environment had stomach fermentation within 1 wk yielding lactic acid concentrations of up to 250 mM in the stomach contents. When HCl production occurred it was usually accompanied by a reduction in lactic acid production. There is, therefore, an inverse relationship in the concentration of HCl and lactate concentrations in the stomach of young suckling piglets (Barrow *et al.*, 1977). However, if the animals were born in a conventional and later moved to a clean environment, the microbiota developed normally (Gouet *et al.*, 1984). Coates and Fuller, (1977) reported that the microbiota of the unweaned mammal is less stable than that of the adult animal due to the changing composition of the diet and the onset of HCl production and increased enzymatic activity.

The microorganisms in the GI tract can be found either free-living in the lumen, attached to feed particles or to the epithelia. The latter attachment can either be associated with the mucus or real adhesion to the epithelial cells (Savage, 1980). Indigenous microbials may also be found deep in the crypts of lieberkuhn (Savage, 1989). The epithelial communities can differ from luminal communities and both of these can differ from cryptal communities in the microbial genera and species present and in their population levels (Savage, 1989). In these attached locations, the bacterial communities are composed of both gram negative and gram positive genera that cannot multiply in atmospheres containing oxygen. Most of the species are intolerant to oxygen. More than 99.9% of the total microbial population in the GI tract acquire their energy through anaerobic processes.

Association of certain endogenous *Lactobacillus* strains with alimentary epithelial surface has been documented by Fuller (1988). This colonization of beneficial organisms maintain a bacterial balance in the intestine of the animal. Free-living bacteria remain in the

gut by growing at least as fast as the general passage rate, unless the animal has special mechanisms to retain them, such as in the large intestines of equines and rodents (Sperber *et al.*, 1983; Bjornhag *et al.*, 1984).

Lactobacilli, a direct-fed microbial, can survive in the lumen of the small intestine and stomach. These bacteria do not use oxygen in their metabolic processes (Savage, 1977). They are usually present in the hindgut in populations of significant size, but are still outnumbered by as much as 100 to 1 by bacteria of anaerobic classes (Savage, 1977; Hentges, 1983, 1989). Some evidence supports the hypothesis that strains of particular species are indigenous to cecal or colonic habitats in mammals and birds of certain taxonomic classes (Mitsuoka, 1969; Savage, 1977; White, 1982; Jonsson, 1986). In some species of birds and mammals, microorganisms may be derived only from the crop (stomach), and therefore, would be transient in the hindgut. In these cases, they may be found in habitats distal to the stomach, only because they are shed from their epithelial communities in the foregut and pass down the intestine to accumulate in the sluggish environments of the distal small bowel, cecum and colon.

Strains of *Bifidobacterium* and *Streptococcus* species, in addition to *Lactobacilli*, are sometimes found in direct-fed microbials often along with microorganisms of other taxonomic groups, and are indigenous to the hindgut in adult mammals and birds of many species (Mitsuoka and Kaneuchi, 1977). Attachment of bacteria to feed particles occurs often, for example in the case of cellulose digestion, which requires a long time and close contact between the bacteria and its substrate (Hungate, 1968). Attachment of the bacteria to the epithelia is a means of the bacteria to get access to another niche. These bacteria can remain in the gut even at a low multiplication rate (Savage, 1980) and they can obtain energy, carbon

and nitrogen from the mucus (Ross, 1959; Salyers *et al.*, 1977). If they are closely associated with the epithelium, they can obtain oxygen which diffuses from the blood (Cheng *et al.*, 1979; Philip and Lee, 1983).

A.5 Epithelial attachment

The attachment of bacteria to the GI epithelia can be of three types: associated with the mucus, adhesion to stratified squamous epithelium or to columnar epithelium. Bacteria associated with mucus which flow down the tract avoid being washed by multiplying at rates higher than the flow rate. Savage, (1980) observed true adhesion of *Lactobacillus* to squamous epithelium and various other bacteria to columnar epithelium. Adhesion of *Lactobacilli* to squamous epithelium has been shown for several animal species and it seems to include a strong tendency towards species-specificity (Fuller *et al.*, 1978; Lin and Savage, 1984).

A.6 Regulation by microbial metabolites

The GI microbiota can perform a wide range of metabolic activities, using most kinds of substrates such as ingested feed, mucus, digestive secretions and shed cells (Prins, 1977). These metabolic activities encompass biochemical reactions catalyzed by microbial enzymes in the GI canal. The biochemical reactions can be categorized into two groups depending upon whether or not they are essential for the survival of the microbial cells (Savage, 1984 and 1985). Reactions essential for survival are those involved in processes by which the organisms obtain nutrients and transport them into their cells (anabolic and catabolic processes), differentiation and reproductive processes and any mechanisms the cells may have for moving

about in their environment. Non-essential reactions are those where the products are not obviously required for the survival of the microbial population. Examples of these latter processes are reactions in which conjugated xenobiotic compounds entering the tract are deconjugated or transformed by microbial enzymes (Yokoyama and Carlson, 1981; Manning, 1986; Overvik *et al.*, 1990). Most reactions catalyzed by microbial enzymes in the tract can take place only in anaerobic environments and are poised at low oxidation-reduction potential.

Microbial metabolites, such as lactic acid, lowers the pH in the stomach and can be a source of carbon and energy in the small and large intestines of the host. Hydrogen sulfate in the large intestine inhibits growth of microorganisms of certain species. It has been reported that microorganisms can move towards nutrient sources and away from toxic materials and most species may be most rapidly motile in viscous habitats such as mucous gel (Ferrero and Lee, 1988).

Microbial aggregation in all areas of the GI tract inhibits access to adhesion receptors by microbial cells of the same or other species and also facilitates nutritional synergism. The effects of bacteriocins are almost uncertain. However, they are considered potential growth inhibitors of microorganisms of the same or other species (Hudault *et al.*, 1982; Corthier *et al.*, 1985). Antibiotics other than bacteriocins inhibit growth or kill sensitive microbial cells.

Savage (1987) cited evidence of competition for nutrients by microbial cells with animal cells or other microbial cells in all areas of the GI tract. The competition for nutrients can control the microbial populations. Also, synergism among microbial cells may enhance growth and survival of each participant. Association of microbials with the epithelium of the GI tract saves the microbes from being swept downstream in the gut contents by peristaltic action. The microbes attached to the epithelium provide reservoir of inoculants for digesta, facilitates hydrolysis of fibrous materials and growth in lumen (Koopman *et al.*, 1987).

A.7 Regulation by host factors

a. General

The factors that regulate the microbial populations and their biochemical functions are poorly understood (Savage, 1989). Indigenous microorganisms present in various GI habitats can utilize carbon, energy and nitrogen sources-compounds of a wide variety of molecular classes. Countless nutritional and non-nutritional substances are available to them depending upon the animal species of which they are a part of and where they are located in the GI tract. These microbes may derive these nutritional substances from the animal's ingesta, mucinous glycoproteins, urea, antibodies and enzymatic proteins. Mucin and urea may be especially important nutritional substances for indigenous microorganisms, especially for bacteria colonizing habitats in mucus gels on epithelial surfaces (Miller and Hoskins, 1981; Gustafsson *et al.*, 1986; Stanley *et al.*, 1986).

b. Nutritional factors

Even though many indigenous species can utilize compounds produced by an animal's glandular and structural tissues, the hosts ingesta is still a major factor regulating which microbial species can form populations and biochemically function in habitats in the GI tract of a mammal or bird of any species. In the stomach (rumen), dietary components of the host are modified by salivary enzymes, HCl and other animal or microbial enzymes and may be used as sources of carbon, energy, nitrogen and macromolecule precursors (Wilson and Perini, 1988). In the small intestine, dietary components of the host, which are altered in the stomach, are progressively modified by dilution, pancreatic, intestinal and microbial enzymes.

Epithelial absorption may be used as sources of carbon, energy, nitrogen and macromolecule precursors by the host (Wilson and Perini, 1988; Macfarlane *et al.*, 1989). The host's dietary components (principally fibrous material) are neither digested nor absorbed in the small intestine. These dietary components and microbial cells from proximal areas of the small intestine are modified progressively by dehydration (water absorption) and compaction (peristalsis). Microbial enzymes in the GI tract may be used as sources of carbon, energy, nitrogen and macromolecule precursors by the host (Varel *et al.*, 1987; Wedekind *et al.*, 1988).

Gastric, pancreatic, and epithelial enzymatic antibody and mucus proteins in the large and small intestine may serve as sources of carbon, energy, nitrogen and macromolecule precursors for the host animal. Taurine and glycine, resulting from the microbial deconjugation of bile salts may serve as large intestine sources of carbon, energy, nitrogen or macromolecule precursors for the host (Binder *et al.*, 1975; Sung *et al.*, 1990). Sulfates may serve as terminal electron acceptors. Urea may serve as a source of carbon and nitrogen in all areas of the GI tract. Sloughing off of epithelial cells in all parts of the GI tract may be used as sources of carbon, energy, nitrogen, macromolecule precursors and other nutrients by the host animal. Oxygen in all areas of the GI tract serve as terminal electron acceptor (Nugon-Baudon *et al.*, 1985; Nipper *et al.*, 1987).

c. Environmental factors

Oxygen in the stomach of the host may be derived from the host's food, ingested air and from blood by diffusion. Presence of oxygen restricts habitats of anaerobes and selects for facultatives and bacteria that grow at partial pressures of oxygen well below that of air. In the

small and large intestine, oxygen is derived from blood by diffusion, and acts the same as in stomach (Miller *et al.*, 1985).

Temperature in all parts of the GI tract (approximately 37 C) is optimum for growth of the microorganisms (Itoh and Freter, 1989). The acidic medium of the stomach influences growth and survival. The neutral and alkaline medium in the small and large intestines is optimum pH for growth of microorganisms and also influences metabolism (Perman *et al.*, 1981).

Peristalsis is periodic in the stomach and as a consequence, the movement of the stomach contents is slow. In the small intestine, peristalsis is rapid in the upper regions and sluggish in the distal regions. The sluggish movement of lumenal contents and mucous gel to distal area allows microbial multiplication. Movement of digesta in the large intestine is periodic and sluggish, allowing for multiplication of microorganisms (Koopman *et al.*, 1987). Villous contraction in the small intestine adds to the movement of the contents and mucous gel due to peristalsis (Koopman *et al.*, 1987).

Phagocytic cells in the crypts of lieberkuhn, laminal propria and Peyer's patch epithelium are destructive to microbial cells. Membranes of certain epithelial cells over Peyer's patches may serve as microbial habitat epithelium (Wold, 1989).

Conjugated and deconjugated bile acids in the small and large intestine have detergent effects and thus bind to cells and may be lethal. Epithelial turnover in all areas of the GI tract, resulting from sloughing off of cells, necessitates constant replacement of adherent microbial cells. Mucous gel in all areas of the GI tract forms a layer of hydrated gel on all columnar epithelia and serve as microbial habitat. The host's diet provides lumenal habitats, especially surfaces of fibrous materials (Lee-Wickner and Chassey, 1985; Koopman *et al.*, 1987).

A.8 Effect to the host

The activities of the microbiota in the GI tract can be either beneficial, harmful or of no importance to the animal. Beneficial activities include production of volatile fatty acids (VFA's) from carbohydrates, (otherwise undigestible to the host), vitamins and amino acids. Detoxification of toxic compound such as mimosine of leucaena (Jones, 1981) is also a further beneficial influence of microbial metabolites. If substances from microbial fermentation are to be of any value to the host, they must be absorbed. Absorption of VFA's and folate have been confirmed by Miller and Luckey (1963). Ammonia, but not amino acids, is absorbed in the large intestine (Deguchi *et al.*, 1978; Just *et al.*, 1981). The microbiota form *de novo* fatty acids and modify dietary fatty acids, especially unsaturated ones. The microbiota deconjugates bile acids and renders them less absorbable and also lowers the total body pool of cholesterol (Coates, 1984; Eyssen and Van Eldere, 1984).

Some substances produced by the microbes can be detrimental to the host. Also, non-toxic precursors from the feed can be converted into toxic compounds (Jayne-Williams and Hewitt, 1972). The microbiota also compete with the host for nutrients and can modify the nutrients so that they escape utilization by the host. The hydrogenation of essential fatty acids is an example. Substances that might be physiologically harmful, such as ammonia, amines and phenolic compounds have also been attributed to the activities of the GI microbes (Yokoyama *et al.*, 1982; Holland *et al.*,1983).

B. PROBIOTICS

B.1 Definition

Probiotics is derived from the Greek words meaning "for life" and contrasts with the term "antibiotic", which means "against life". Probiotics are bacterial or yeast in origin containing micro-organisms and microbial metabolites, which when fed to animals of commercial interest may result in better health or productivity (Fox, 1988). These microorganisms, according to the definition of Parker (1974), 'contribute to intestinal microbial balance'. However, in order to avoid the inclusion of antibiotics as probiotics, Fuller (1989) defined probiotics as 'live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance'.

The United States Food and Drug Administration (FDA), in 1989, required manufacturers to use the term direct-fed microbials (DFM's) instead of probiotics. The FDA defines DFM's as "a source of live or viable naturally-occurring microorganisms used in an attempt to balance the intestinal microflora and is beneficial in some way to the host animal".

B.2 Classification

Direct-fed microbials are classified as feed additives (Cheeke, 1991) and are non-nutritive components of animal feeds that improve feed acceptance, feed efficiency, health and metabolism of the animal. Their presence in animal feed enhances productive and reproductive performance (Cheeke, 1991). These compounds are also digestion modifiers. They are added into a feed to facilitate the digestion of complex feed ingredients, maintain

acid-base balance, improve metabolic efficiency, increase saliva production and improve intestinal microbial balance. Other examples of digestion modifiers are ionophores and acidifiers.

B.3 Desired characteristics

A good DFM is one that is a normal inhabitant of the intestine (Gordon *et al.*, 1957) and is capable of habituating in the intestinal tract (Harter and Kendall, 1908). It should exert beneficial effect on the host animal and increase growth or disease resistance. According to Gordon *et al.* (1957) and Gilliland (1979), a good DFM is non-pathogenic, non-toxic, is present as viable cells in large numbers and is active in the carrier food before consumption and with maximum efficiency at a given dose. They also suggested that a good DFM is capable of surviving the upper digestive tract to reach the intestines, survive and grow in the intestine. A good DFM is capable of metabolizing in the gut and producing enough metabolites to exert their effects. It is resistant to low pH and remain viable for long storage periods (Gilliland, 1979; Fuller, 1989).

C. FREQUENTLY USED DIRECT-FED MICROBIALS

Table II.1 presents a list of the microorganisms generally recognized as safe (GRAS) for use in animal production (Sogaard and Suhr-Jessen, 1990).

Table II.1. Some direct-fed microorganisms[1]

Lactobacillus acidophilus	*Lactobacillus bulgaricus*
Lactobacillus cellobiosus	*Lactobacillus lactis*
Lactobacillus plantarum	*Lactobacillus fermentum*
Streptococcus lactis	*Streptococcus thermophilus*
Streptococcus faecium	*Streptococcus intermedius*
Bacillus coagulans	*Bacillus subtilis*
Bacteroides ruminocola	*Bacteroides amylophilus*
Bifidobacterium infantis	*Bifidobacterium animalis*
Aspergillus niger[2]	*Aspergillus oryzae*[2]

[1] Sogaard and Suhr-Jessen, 1990

[2] yeast or fungi

C.1 Yeast

a. General

Yeasts are unicellular, eukaryotic organism which are capable of reproducing both sexually and asexually. Yeasts are fungi, which do not contain chlorophyll and are unable to synthesize their organic needs from inorganic components. They, therefore, lead a saprophytic or parasitic life (Phaff *et al.*, 1978) and are considered direct-fed microbials. They are added into feed as "yeast culture" which is a dry product comprising of yeast and the media in

which it is grown. The yeast culture, therefore, contains a lot of metabolites. It is these metabolites, along with some viable yeast cells that are the principal functional components of this type of culture.

Yeasts grow actively at low pH and are less expensive and easier to harvest compared to bacteria. Large scale yeast cultures can be produced and kept without any threat from contaminating microorganisms (Barnett *et al.*, 1990). Baker's and brewer's yeasts are good sources of the water soluble vitamins thiamine, riboflavin, and pantothenic acid (Burden and Eveleigh, 1990).

The three main types of products from which yeast is derived are categorized according to the relationship of the product to the biochemistry of the organism (Lyons, 1986). These products are (1) cell constituents, such as proteins, vitamins and minerals of the whole cell or the contents of the yeast cells combined with their growth medium. This is the major type of yeast product used by the feed industry, (2) excretion products, such as fermented products of grown yeast. These are beer, wine, cider and carbon dioxide, and (3) interactions between enzyme substrates which involves the utilization of whey by *Kluyveromyces fragilis*.

b. Mode of action

In anaerobic conditions, yeast metabolism produces ethanol and carbon dioxide as major products with small amounts of glycerol and succinic, acetic and lactic acids. In aerobic conditions, specific lipids and succinic, acetic and zymonic acids are produced (Phaff et al., 1978). Studies in feeding yeast cultures to ruminants and non-ruminants have been reported.

The effect of the yeast culture has been associated with alteration of the activities of mixed ruminal bacteria. Dawson and Newman (1987) observed a decrease in rumen ammonia

concentrations when feeding a yeast culture. Other alterations in metabolism of ruminants were changes of VFA production (Williams *et al.* 1991), decreased lactic acid concentrations (Williams, 1989), increased ethanol concentrations, stabilized fermentation (Harrison *et al.*, 1988), and increased concentration of anaerobic and cellulolytic bacteria (Harrison *et al.*, 1988; Dawson, 1990).

The exact mode of action of yeast culture in non-ruminants remains known (Glade and Sist, 1987). However, some results have shown significant improvement in performance of animals fed diets containing yeast culture. Yeast has been shown to stimulate microbial activity, which in turn results in reduced pH of the GI tract. The low pH may be essential in the absorption of nutrients such as calcium, phosphorus and other minerals. Yeast has been shown to be responsible for the improved feed efficiency, organic phosphorus utilization, egg quality and litter condition in poultry (Lyons, 1990). Pagan (1989) reported that yeast culture produces certain enzymes (phytase), vitamins (niacin and biotin) and amino acids (glycine, lysine, methionine) in the digestive system of the host animals.

When turkey breeder hens were fed diets containing XP yeast cultures (*Saccharomyces cerevisciae*), a decrease in the incidence of parthenogenesis was observed (Savage *et al.* (1993). In similar studies, early embryonic mortality (0-10 days) of eggs stored 1-7 days prior to incubation was reduced (Bradley *et al.*, 1993) and hatchability was improved (Hayat *et al.*, 1992). Hayat *et al.* (1992) suggested that genotype may influence subsequent incubation performance of eggs from turkey breeder hens fed a yeast culture.

Inclusion of yeast culture into diets of swine and poultry improved phytate phosphorus utilization as a result of increased phytase activity of the microbial population already present in the hindgut of the animals (Thayer *et al.*, 1978 and Pagan, 1989).

C.2 *Lactobacilli*

a. General

Lactobacillus species have been classified into two categories based on their glucose fermentation characteristics (Brown, 1977). The homofermentative *Lactobacillus* species produce more than 85% lactic acid and the heterofermentative species produce approximately 50% lactic acid and small quantities of acetic acid, ethanol and carbon dioxide. The optimum temperature for growth of *Lactobacillus* is 37 C and the optimum pH range from 5.5 to 5.8. *Lactobacillus* are the only known microorganisms that attach to the villi of the GI lining of the host animal.

b. Mode of action

Lactobacilli species are the major component of direct-fed microbials. Therefore, in many discussions when authors mention direct-fed microbials, they refer primarily to *Lactobacilli* (Conway, 1989). The modes of action of *Lactobacilli* to their animal hosts are very controversial. These modes of action of the direct-fed microbials are hypothesized and need further research and confirmation because very little is known about the mechanisms by which such products could possibly function to enhance an animal's health, growth and efficiency in utilizing its foodstuffs. Little is known with certainty about how the microbiota is regulated and influences host function.

One of the most recent theories and most provocative mode of action of DFMs is immunostimulation. Intestinal microflora are known to stimulate the gut associated lymphoid tissues. These microflora maintain lymphoid tissues in a highly responsive state (Naqi *et al.*,

1984). Investigating the distribution of immunoglobulin-bearing cells in the gut associated lymphoid tissues of day-old poults, Naqi *et al.* (1984) concluded that the microflora became established in the gut of the bird soon after hatch and the immunopotentiation of the gut associated lymphoid tissue appeared to be an important and complementary process.

Animals reared in crowded production facilities are very susceptible to diseases caused by microorganisms of various taxonomic groups. Some of the diseases, especially those involving the digestive tract, may be due to a loss of colonization resistance. Such losses could result from changes in the properties of certain animal cells involved in immunological (resistance) mechanisms (Hentges *et al.*, 1984; Berg, 1989) and function in the gastrointestinal microbiota (Savage, 1984) or both of these phenomena.

It has been proposed that components of direct-fed microbials of certain indigenous communities can reestablish in regions of the tract that are targets of microbial pathogens (Freter, 1974; Clements *et al.*, 1981; Lidbeck *et al.*, 1989). The immunological mechanisms of the host are primed to react promptly to antigens of the pathogen (Op Den Camp *et al.*, 1985). *Lactobacillus* antigens such as lipoteichoic acids (Op den Camp *et al.*, 1985) and surface proteins (Conway and Kjelleberg, 1989) or even the bacterial cells themselves could translocate the mucosal epithelium into the areas containing the animal cells that mediate immunological functions.

Leeson and Major (1990) stated that it is only under a situation of stress, when coliforms often increase in numbers, that a direct-fed microbial will be of measurable benefit. During stress, hormonal changes in animal are known to occur. These hormonal changes have been associated with the deterioration of the mucus lining in the gut and the loss of beneficial microflora that are attached to or otherwise associated with this mucus gut covering. The overall effect of a DFM is, therefore, more of a preventive than therapeutic effect.

The indigenous part of the GI microbiota is likely to have evolved together with the host (Dubos *et al.*, 1965). In fact, some of these organisms share antigens with the mucosa and do not induce any immunological response (Foo and Lee, 1974). These microbiota are believed to perform beneficial interactions with the host, such as host resistance to infectious diseases by powerful direct bacterial interactions. This has been termed the barrier effect (Ducluzeau and Raibaud, 1974), colonization resistance (Van der Waaij *et al.*, 1971) or competitive exclusion (Snoeyenbos *et al.*, 1978). The indigenous microbes may also contribute to the utilization of otherwise indigestible carbohydrates (Prins, 1977) and to the vitamin supply (Coates and Fuller, 1977).

Lactic acid and VFA's (acetic acid in particular), carbon dioxide (Holdeman and Moore, 1975; Bailey, 1987), amines (Hill *et al.*, 1970), ammonia, phenolic compounds and hydrogen peroxide (Collins and Aramaki, 1980) produced by *Lactobacilli* species have been claimed to produce specific antibacterial effects, but in some cases, their significance is still obscure. *Lactobacilli* have been reported to produce a variety of antibacterial substances with activity *in vitro* both towards closely and remotely related bacteria as *E. coli*, *S. aureus* and *Salmonella ssp*. Acetic acid has a stronger antibacterial effects than lactic acid. This effect is enhanced by low pH due to higher degree of dissociation of the acid, but is diminished in the presence of organic matter, such as peptones and proteins (Bergeim, 1940; Rubin, 1985). Some *Streptococci* produce nisin and diplococcin (Oxford, 1944). Nisin is used as a food preservative (Hurst and Collins-Thompson, 1978). Diplococcin is a protein-like substance which inhibits the growth of organisms of other strains of the *Streptococci* trains.

Lactobacilli are able to deconjugate bile acids (Gilliland and Speck, 1977; Brown, 1977) and these bile acids can be inhibitory to some fecal bacteria when tested *in vitro* (Floch *et al.*, 1972). Although *Lactobacilli* themselves are inhibited by free bile acids, they are able

to resist these acids better than potential pathogens such as *Clostridium* species and *Enterococci* (Floch *et al.*, 1972; Binder *et al.*, 1975). Bile acids are secreted as conjugated acids, and enter the anterior part of the small intestine where no extensive microbial activity is found in many of the animal species. The greater part of the bile acids are then reabsorbed in the jejunum. Therefore, the effect of bacterial deconjugation of bile acids in the small intestine is probably limited. The small amounts of bile acids entering the large intestine are degraded and this might have some controlling effect on the microbiota but the extent of this is not known.

Lactobacillus are known to inhibit pathogenic bacteria by competing for nutrients or association sites (Morishita and Ogata, 1970). Competition with the microbiota for endogenous nutrients might be harsh, and *Lactobacilli* can probably gain advantage in certain feeding regimes and compete successfully with the pathogenic bacteria. Although competition for adhesion sites between *Lactobacilli* and yeast (Savage, 1969) has been shown to occur on squamous epithelium, *Lactobacilli* do not adhere to columnar epithelium. They are merely associated with the mucus of the intestine. The supposed effect is, therefore, more likely to be a metabolic competition. Gilliland and Kim (1984) reported that there is a possibility of enzyme production by *Lactobacilli* which could improve digestion of lactose.

c. Harmful effects to the host

There may be detrimental effects such as competition between *Lactobacillus* and the host for nutrients, such as starch (Champ *et al.*, 1983). The voluminous microbiota of the anterior GI tract is likely to withdraw some food from the host for its own sustenance. Bacterial overgrowth of the small intestine may result in formation of a variety of protein end

products which are no longer of use by the host. The proteins normally available to the host are changed by the intestinal flora. However, such detrimental effects can in many instances be reversed by appropriate direct-fed microbial activity.

Using rats, Miller (1971) observed that lysine was not degraded to piperidine, and the amino acids arginine and ornithine were not converted to pyrrolidine. However, in the event of detrimental bacterial overgrowth in the intestine, these products were produced by the bacteria and both dietary and microbial protein was wasted. Another detrimental influence of bacteria is the laxative effect observed by Gordon *et al.* (1957). Watkins and Kratzer (1983) also noted that too high doses [9 x 10^{10} cell forming units (cfu) per chick per day] of *Lactobacilli* depressed growth of the broiler chicks. Dosing the broiler chicks with lower numbers of *Lactobacillus* (5 x 10^{10} cfu per chick per day) resulted in the same level of colonizing (populating) of the GI tract in the chicken as the higher doses (9 x 10^{10} cfu per chick per day). Also, the prevalence of bacteria in the GI tract increases the passage rate of the digesta and it is not unlikely that it could be affected if total counts of bacteria in the gut were increased. Vitamin B_{12} deficiency may also occur as a result of destruction of the vitamin by intestinal bacteria under conditions of poor bacterial balance (Dellipiani, 1968).

d. Dietary inhibitors

Several factors, some of which are contributed by *Lactobacilli*, discourage growth and sometimes the survival of the microbes. Brockett and Tannock (1981) reported that fatty acids have influenced bacterial populations in the GI tract. Lhuillery *et al.* (1981) observed that linoleic acid reduced fecal *Lactobacillus* populations in rats and mice receiving a semi-

synthetic diet. High concentrations of linoleic and oleic acids (> 1%) may be inhibitory to *Lactobacilli*, while at lower concentrations (< 0.1%), these fatty acids may be growth factors (Lhuillery et al., 1981).

e. Effect on animal performance

1. Ruminants

The microbiota of the rumen, abomasum and intestine is less well known. However, McBee (1977) stated that the microbiota of the hindgut is not uniquely different from that of the rumen. *Lactobacillus* were reported to be the dominating genus in the anterior parts of the GI tract, although high numbers of anaerobic bacteria have also been found. In later studies, Marshall *et al.* (1982) isolated *Lactobacilli* associated with the epithelium of the esophageal groove, omasum, abomasum and duodenum at levels of 10^4-10^7 per cm^2. Some of these strains could adhere to cells from the forestomach and abomasum and when tested *in vitro* produced hydrogen peroxide. Rumen bacteria, particularly *Lactobacilli*, are adapted to grow and reproduce in anaerobic conditions at a pH range from 5.5 to 7.0 and a temperature range from 30 to 40 C. The steady supply of nutrients from feed ingesta and the continuous removal of fermentative products maintain a relatively constant condition for dense populations of bacteria to develop (Hungate, 1966). The rumen bacteria become adapted competing for nutrients such as carbohydrates, proteins, fats and organic compounds such as vitamins and minerals.

The majority of bacteria in a ruminant receiving predominantly a hay or forage ration are gram-negative. With high grain rations, there is an increased proportion of gram-positive

bacteria. Hungate (1966) suggested that possibly this shift reflects the increased numbers of *Lactobacilli* under the more acidic conditions usually accompanied with high grain rations.

Lactobacillus has been used in diets of cattle for several years. Thomas *et al.* (1973) reported no differences in weight gains for calves fed *L. acidophilus*. Later studies (Bechman *et al.*, 1977) fed *L. acidophilus* to calves from 4 days of age to weaning at 42 days in two trials and observed 7 and 17% increases in weight gains. Fewer calves were observed to be scouring when fed *Lactobacillus* (Thomas *et al.*, 1973; Stern and Storrs, 1975; Bechman *et al.*, 1977). Schwab *et al.*(1980) reported difference in gain in one of three trials when a non-viable *L. bulgaricus* whey fermented product was fed to Holstein bull calves from 2 to 5 days of age.

Feeding a living nonfreeze-dried *L. acidophilus* improved appetite and gain of feedlot cattle (Hutchenson *et al.*, 1980; Anonymous, 1981; Gill *et al.*, 1987). Studies conducted by Hutchenson *et al.* (1980) indicated lower butyrate fermentation levels in cattle. He also stated that consumption of more than 15 g (10^7 cfu) of *L. acidophilus* per head per day may result in over population of the lower tract, which in turn results in reduced nutrient absorption. Speck (1976) suggested that ingestion of excessive numbers ($> 10^8$ to 10^9 viable cells) of *L. acidophilus* cells daily may induce mild gastrointestinal disturbances. Wren (1989) cited evidence that viable lactic acid producing bacteria, primarily *Lactobacillus* and *Streptococci*, have been used in times of stress to restore proper intestinal bacterial balance. Swingle *et al.* (1985) incubated *L. acidophilus* with jojobe meal containing concentrations of simmondsin and simmondsin-2-ferulate toxicants. The *Lactobacillus* addition reduced the toxicants, but the jojobe meal was not as acceptable as cottonseed meal to steers or sheep.

2. Non-ruminants

a. Swine

The GI microbiota of the pig is dominated by gram positive bacteria, especially *Lactobacilli*. In the anterior tract, *Streptococci* are also prevalent, while *Bifidobacterium* are more numerous in the posterior parts. In the stomach, the major bacterial genus is *Lactobacillus* which is found both in the contents and adhering to the non-secreting squamous epithelium and the *pars esophageal* area which is analogous to the chicken crop (Fuller *et al.*, 1978). These investigators suggested that the *Lactobacilli* adhering to the *pars esophageal* area is likely to be the origin of *Lactobacillus* found in the gastric contents.

In the suckling piglet, the major fermentative product is lactate (Friend *et al.*, 1963; Cranwell *et al.*, 1976), while only minor amounts of acetate are formed. In the adult pig, VFA's are produced in equal amounts to lactate (Clemens *et al.*, 1975). The lactate in the stomach of the young piglet helps the animal to maintain a low pH (Cranwell *et al.*, 1976; Barrow *et al.*, 1977). The lactate production and the barrier effect of the microbiota are probably the means by which the pig resists infections.

Muralidhara *et al.* (1977) challenged pigs with an enteropathogenic *E. coli* to determine the possible protective effect of including *Lactobacillus* in the feed. Feeding *Lactobacillus lactis* resulted in a lower number of *E. coli* in the small intestine and these were non-enteropathogenic in contrast to the strains isolated from the control pigs. *Lactobacillus acidophilus* treatment of gnotobiotic pigs also resulted in an increase in the *Lactobacillus* population of tissue and digesta samples although this was not the case with conventionally

reared animals (Pollmann *et al.*, 1980). Following administration of *Bacillus subtilis* to weaned pigs, Ozawa *et al.* (1981) observed stabilization of the indigenous flora of the pigs.

Feeding *L. acidophilus* to newborn pigs resulted in protection against the development of diarrhea (Kohler and Bohl, 1964; Harker, 1989). Feeding *Lactobacillus* to growing pigs enhanced weight gain, feed conversion and improved the digestibility of protein from both cereal grain and proteinaceous sources of both male and female growing pigs (Gombos, 1991; Bourne, 1991).

b. Poultry

1. Chickens

a. General

Lactobacillus ssp are normal inhabitants of the intestinal tract of poultry (Fuller, 1973; Gilliland *et al.* 1975; Sarra *et al.* 1985). Within a few hours after hatching, various bacteria including fecal streptococci, enterobacteria and clostridia may be found randomly scattered through the alimentary tract, and within a few days, *Lactobacilli* become established (Barnes, 1979). An association between *Lactobacilli* and the epithelial lining of the chicken crop is established within a few days after hatching and persists throughout the life of the chicken (Fuller, 1973; Fuller and Brooker, 1974).

b. Broilers

Nurmi and Rantala (1973) reported that colonization of *salmonella* ssp in broiler chickens could be reduced by providing a pathogen-free, adult intestinal flora to newly hatched chicks. These treated chicks were less likely to be colonized by *Salmonella*, and, even if colonized, had reduced level of *Salmonella* excretion in caecal and fecal materials. Barrow and Tucker (1986) prevented caecal colonization by *Salmonella typhimurium* in day-old broiler chicks by a pretreatment with a mixture of three strains of E. coli. In earlier studies, Impey *et al.* (1982) demonstrated the protective effect of indigenous gut flora against *Salmonellas* in young chickens by dosing them with a mixed suspension of 48 different strains of intestinal organisms containing *Lactobacilli*, *Streptococci* and *E. coli*.

Unlike mixed cultures, mono cultures of *Lactobacilli* originating from the ceca of protected broiler chicks were unable to prevent *Salmonella* infection when given in feed or by gavage (Barnes *et al.*, 1980). It has been reported that antagonistic effects of *Lactobacilli* towards *Salmonellas* may occur in the crop. This activity may not occur, or to a limited extent in the ceca of conventionally reared or gnotobiotic chicks (Adler and Da Massa, 1980; Soerjadi *et al.*, 1981; Watkins and Miller, 1983). Fuller (1978) reported that colonizing chickens intestine with an intestinal strain of *Lactobacillus sp.* suppressed the counts of *E. coli* in the chicken crop.

The liver biotin content of broiler chicks dosed with nonhost specific *Lactobacilli* (strain 40) was assayed by Buenrostro and Kratzer (1983). They found that *Lactobacilli* administered in the drinking water of chicks fed a marginal biotin diet sustained decreased liver biotin. A significant increase in plasma free fatty acids and foot dermatitis in broiler chicks were observed when fed diets containing *Lactobacillus* which are indirect indications of

a biotin deficiency. The alterations in the fatty acids were observed only when the plasma samples were collected from fed chickens. The possibility of competition between *Lactobacillus* and the chicks for dietary biotin was suggested. Later studies (Watkins and Kratzer, 1983) indicated a trend for liver biotin to be higher in chicks dosed with 7.0×10^{10} CFU than chicks dosed with either 5.0 or 9.0×10^{10} cfu or no culture. However, these differences were not significant ($P > .05$). Because *Lactobacillus* responds to biotin (Rogosa *et al.*, 1961), it is probable that they compete with the host and biotin-dependent gut microflora for dietary biotin and may also be involved in the development of the Sudden Death Syndrome in which a biotin deficient state has been found (Payne *et al.*, 1974; Buenrostro and Kratzer, 1982). Watkins and Kratzer (1983) further suggested that there is possibly a proper level of *Lactobacilli* required by the broiler chicken that provides the most benefits. Inoculating below or above this level of *Lactobacillus* may result in undesirable effects such as bacterial competition for biotin.

Increased daily gains of chicks on diets supplemented with *L. acidophilus* compared to birds in control groups have been related to the increasing numbers of *Lactobacilli* in the small intestines and the ceca (Tortuero, 1973). Although not statistically significant, differences in fat digestibility and nitrogen retention were reported. The implantation of *Lactobacillus* in the GI tract resulted in lower ceca and excreta weights. The increased numbers of *Lactobacilli* were associated with decreased number of *Enterococci* in the birds receiving the feed supplemented with *L. acidophilus*. In later studies, Dilworth and Day (1978) found that the addition of various levels of *Lactobacillus* to broiler diets improved growth rate and feed efficiency. *Lactobacillus* supplementation of diets containing suboptimal levels of sulfur amino acids and lysine promoted a growth rate equal to broilers fed diets containing adequate amino acid levels. Using gnotobiotic chicks, Watkins *et al.* (1982)

observed a decrease in mortality from 66.7% to zero when *Lactobacillus*-fed chicks were challenged with *E. coli*. Body weights were not affected by feeding the *Lactobacillus*. They also observed that continual dosing with *Lactobacillus* lowered pH of the crop, cecum and rectum of the chicks.

Okumura and Kino (1984) reported that chicks can utilize nonprotein nitrogenous compounds such as urea and diammonium citrate. However, presence of gut microflora was necessary before urea could be used to furnish nitrogen for growth. In other studies, Yokota *et al.* (1989) cited evidence that microorganisms in the gut were not necessary for diammonium citrate to be used as a source of nitrogen for the synthesis of non-essential amino acids.

Data from laboratory trials with broilers raised under commercial conditions indicated that broilers fed diets with supplemental *Lactobacillus* had improved feed efficiency (Burkett *et al.*, 1977; Couch, 1978; Crawford, 1979), increased body weights (Couch, 1978; Crawford, 1979), decreased mortality, and reduced incidences of pasted vents (Couch, 1978). Jones (1994) fed a DFM (Primalac®) to broiler breeders and observed an increase in feed consumption and the movement of feed through the gut. He also reported an improved feed efficiency and reduced heat stress mortality.

Lactobacillus species may influence the bioavailability of minerals either by decreasing the pH of the GI tract of broilers (Bailey, 1987) and the secretion of microbial enzymes, which alter the properties of metal binding substances.

c. Egg-type

Using gnotobiotic chicks, Fuller (1977) compared two strains of *L. acidophilus* and their effectiveness in controlling *E. coli*. *Lactobacillus* exerted inhibitory effect towards the *E. coli*. Miles *et al.* (1981) used a dried preparation of *L. acidophilus* to evaluate the influence on the number of coliform bacteria in the intestine of commercial laying hens. They observed an increase in the number of coliforms with time; however, there were fewer coliforms in the hens receiving the dried preparations of *L. acidophilus*.

Water administration of *Lactobacillus* did not affect wet viscera weights or wet and dry small intestinal weights, although Tortuero (1973) observed decreased cecal weights of 12-d-old Leghorn chicks. Huber *et al.* (1976) observed *Lactobacillus* organisms rapidly proliferated in the intestinal tract when high-energy carbohydrate rations were fed. *Lactobacillus* growth was optimum between pH values of 5.5 to 5.8. The gut pH of chickens and the diets fed to the Leghorn chickens seem to meet the criteria for maximum *Lactobacilli* proliferation and growth. In later studies, Goodling *et al.* (1987) fed a dried, non viable *Lactobacillus* culture to laying hens. No improvement in hen-day egg production nor feed efficiency were observed. The addition of a viable *Lactobacillus* product to rations of differing protein levels did not improve hen-day egg production, livability nor egg size of laying hens. The addition of a non-viable *Lactobacillus* fermentative product to diets of laying hens did not improve feed efficiency, egg weight and hen-day egg production (Cerniglia *et al.*, 1983).

Krueger *et al.* (1977) reported that the addition of a *Lactobacillus* complex to laying hen rations increased egg production and feed efficiency. In similar studies, Hargis and Creger (1978) did not observe any improvement in feed efficiency when feeding *Lactobacillus*

complex to laying hens. However, Crawford (1979) and Miles *et al.* (1981) reported that feeding *Lactobacillus* to commercial egg-type layers improved egg production and feed conversion. Feeding the *Lactobacillus* did not influence egg quality nor egg weight. Later studies (Nakaue and Mirosh, 1991) fed direct-fed microbials to Single Comb White Leghorn laying pullets and observed an increase in feed consumption and improvement in egg production, egg mass, egg weight and egg size.

2. Turkeys

Francis *et al.* (1978) observed an improvement in turkey poult body weight and feed efficiency when *Lactobacillus* product or zinc bacitracin was added to the diet. However, when the product was combined with the zinc bacitracin in the diet, poult performance was not as good as when the *Lactobacillus* product or the zinc bacitracin was fed alone. In later studies, Potter *et al.* (1979) observed that feeding turkeys a dry *L. acidophilus* culture in combination with varying protein levels significantly enhanced body weight up to 12 wks of age. At 16 wks of age, weight gain and feed efficiency were not different for the turkey poults fed the *Lactobacillus* cultures.

A mixed *Lactobacillus* culture incorporated into the diets of broad breasted large white turkey hens (Damron *et al.*, 1981) did not exhibited a beneficial response for any criteria measured. Seuna *et al.* (1985) studied the effect of gentamicin and an anaerobic culture derived from the cecal contents of adult turkeys on salmonella infection in turkey poults and cited evidence that the cecal contents were more effective than gentamicin treatment in preventing the spread of *salmonella*.

Al-Zubaidy and Sullivan (1977) fed large White female turkeys diets containing a live *Lactobacillus* culture and two sources of copper with zinc bacitracin. Turkeys fed *Lactobacillus* had significantly increased gains compared to nontreated turkeys. In a similar experiment, body weights at 4 wks of age were significantly greater in birds fed a combination of the *Lactobacillus* and either zinc bacitracin or penicillin-streptomycin (1:3) than with any of the three supplements alone. Tahir *et al.* (1983) supplemented drinking water of turkey poults from hatch to 20 wk of age with *L. acidophilus* (4.5×10^8 cfu per bird per day). After 12 wk, they observed an increase in weight gain and feed intake and also improved feed efficiency of the poults.

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CHAPTER III

PRODUCTION VARIABLES AND NUTRIENT RETENTION IN SINGLE COMB WHITE LEGHORN LAYING PULLETS FED DIETS SUPPLEMENTED WITH A DIRECT-FED MICROBIAL[1]

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ABSTRACT

Two experiments were carried out for six and seven 28-d periods, respectively, with Dekalb XL Single Comb White Leghorn laying pullets to ascertain the effect of feeding 1,100 mg *Lactobacillus* (Lacto)/kg diet (ppm) and 2,200 ppm Lacto diets and the supplementation of these diets with 1 and 3% fat on layer performance and nitrogen, calcium, and phosphorus retention. Three dietary treatments were corn-soybean meal (C-S) control; C-S plus condensed cane molasses solubles (CCMS)-1,100 ppm Lacto (4.4×10^7 cfu/mg Lacto) and C-S plus CCMS-2,200 ppm Lacto (8.8×10^7 cfu/mg Lacto) for Experiment 1. Seven dietary treatments were C-S control; C-S plus CCMS-1,100 ppm Lacto with 0, 1, and 3% fat; C-S plus CCMS-2,200 ppm Lacto with 0, 1, and 3% fat for Experiment 2. The CCMS served as a carrier for the Lacto and the Lacto premixes were incorporated at 2% of the diets.

In Experiment 1, mean hen-day egg production, daily feed consumption, egg mass, egg weight and egg size of pullets fed 1,100 ppm Lacto diets were increased ($P < .05$) by 1.7, 4, 2.8, 1, and 6%, respectively, and egg mass and internal egg quality of pullets fed 2,200 ppm Lacto were increased ($P < .05$) by 1.5 and 1.7%, respectively, when compared with pullets fed the control diet. Hen-day egg production, feed consumption, feed conversion, egg weight, egg size, internal egg quality, egg specific gravity, body weight gains and mortality of pullets fed the 1,100 and 2,200 ppm Lacto diets were not different. Mean feed conversion of pullets fed both Lacto levels was 2.5% lower than for pullets fed the control diet. In Experiment 2, supplementing the 1,100 and 2,200 ppm Lacto in layer diets increased ($P < .05$) hen-day egg production, daily feed consumption, egg mass, egg weight, egg size, body weight gains, nitrogen, calcium, and phosphorus retention by 1.1, 1.7, 1.7, .4, 2.3, 35, 25, 34 and 76%, and 1.4, 2.6, 2.3, .7, 4.4, 48, 25, 32 and 139%, respectively, when

compared with the diet without Lacto. Supplementing the layer diets with 1% fat improved ($P < .05$) egg size, and nitrogen and phosphorus retention by 2.4, 35 and 62%, respectively, when compared with pullets fed the diet without fat. Supplementing the layer diet with 3% fat decreased ($P < .05$) feed consumption and nitrogen, calcium and phosphorus retentions by 3.4, 15, 14, and 31%, respectively, and improved ($P < .05$) feed conversion and body weight gain by 3 and 19%, respectively, when compared with pullets fed the diet with 1% fat. Positive correlations between Lacto diets and nitrogen retention, calcium retention, daily feed consumption and egg size and between supplemental fat and nitrogen retention, egg mass, egg weight, egg size and feed conversion were found.

According to the experimental conditions, feeding either 1,100 or 2,200 ppm Lacto to laying pullets stimulated appetite, improved egg production, egg mass, egg weight, egg size, internal egg quality, and nitrogen, calcium, and phosphorus retention. Further supplementing the two Lacto diets with 1 and 3% fat reduced daily feed consumption, provided better feed conversion, egg masses, egg sizes, body weight gains and nitrogen, calcium, and phosphorus retentions.

(Key words: direct-fed microbials, *Lactobacillus*, laying pullets, performance, nutrient retention)

INTRODUCTION

Confinement of pullets, to a large extent, can be a predisposing factor to increased susceptibility to disease because of the dense bird population in a given area (Webster, 1984). Probiotics (direct-fed microbials) may be used to reduce this disease susceptibility by improving the health of the gastrointestinal tract and improve production performance of the pullets.

Direct-fed microbials, such as *Lactobacillus,* are living or dead beneficial microorganisms for livestock and poultry, which may be bacterial or yeast in origin. These microorganisms may enhance an animal's health, growth, and efficiency in utilizing its foodstuffs. Watkins and Kratzer (1984) and Goodling *et al.* (1987) reported that microbial cultures typically containing *Lactobacillus* were successfully used as an alternative to antibiotics. Evidence suggests that *Lactobacillus* colonizes the gut wall and is responsible for the suppression of pathogenic *Escherichia coli* in the crop and gut wall of chickens (Fuller, 1973, 1977, 1989; Watkins *et al.*, 1982; Baba *et al.*, 1991). *Lactobacillus* has also been reported to successfully control microbial populations of *Salmonellae* (Smyser and Snoeyenbos, 1979; Vanderwall, 1979; Weinack *et al.*, 1985; Dunham *et al.*, 1993).

There are numerous forms of *Lactobacillus* products that are used in poultry feeds at various concentrations and their effect on layer performance can differ. Feeding liquid, non-viable *Lactobacillus* fermentation product to laying pullets at .236, .473, and .709 L/ton of feed did not improve feed efficiency, egg weight, hen-day egg production, and egg size (Cerniglia *et al.*, 1983). Goodling *et al.* (1987) fed a dried viable and nonviable *Lactobacillus* products each at 227, 454, and 686 g/ton of feed to laying pullets and observed no improvement in hen-day egg production, feed efficiency, livability and egg size. However, the

addition of live cultures of *Lactobacillus acidophilus* at .0125, .0375, and .0625% of the layer diets increased feed consumption and egg size (Miles *et al.*, 1981). Krueger *et al.* (1977) fed 454 and 2,270 g of *Lactobacillus* complex/ton of feed each with and without gentian violet to laying hens and reported an improvement in feed efficiency and hen day egg production for hens fed only *Lactobacillus*. When laying hens were fed a viable *Lactobacillus* fermentation product, egg size was increased (Hargis and Creger, 1978).

Because the influence of feeding live cultures of *Lactobacillus* on the productive performance of laying pullets and the effective level of supplementation in layer diets are controversial and not well documented, this report describes two experiments conducted to ascertain the effect of feeding diets containing 1,100 and 2,200 ppm *Lactobacillus* to laying pullets and the effect of supplementing these *Lactobacillus* diets with 1, and 3% fat on pullet performance and dietary nitrogen, calcium, and phosphorus retention.

MATERIALS AND METHODS

Two experiments were carried out with Dekalb XL Single Comb White Leghorn laying pullets starting at 28 wk of age (WOA) in Experiment 1 and 34 WOA in Experiment 2. The experiments were carried out for six (Experiment 1) or seven (Experiment 2) 28-d periods. Five hundred and seventy-six laying pullets were used in Experiment 1 and 1,344 laying pullets were used in Experiment 2. Experiment 1 was completely randomized and Experiment 2 was completely randomized in a 2 by 3 factorial arrangement with 2 levels of *Lactobacillus* (Lacto) and 3 levels of fat, plus a control. The pullets were raised according to standard methods as outlined by North and Bell (1990). The dietary compositions and treatments for Experiments 1 and 2 are presented in Tables III.1 and III.2, respectively. In Experiment 1, the dietary treatments consisted of corn-soybean meal (C-S, control), C-S plus condensed cane molasses solubles (CCMS)-1,100 ppm Lacto [4.4 x 10^7 cfu/mg Lacto] and C-S plus CCMS-2,200 ppm Lacto (8.8 x 10^7 cfu/mg Lacto). In Experiment 2, the dietary treatments were C-S (control), C-S plus CCMS-1,100 ppm Lacto with 0, 1, and 3% fat and C-S plus CCMS-2,200 ppm Lacto with 0, 1, and 3% fat. Condensed cane molasses solubles served as a carrier for the Lacto, and the Lacto premixes were incorporated at 2% of the diet. The diets were formulated according to NRC (1984) recommendations and fed in mash form.

The dietary treatments were randomly assigned to rows of 24 individual cages (21 cm wide x 46 cm deep x 46 cm high) with sloping wire floors in a stair-step arrangement per bank. Each cage housed two pullets (483 cm^2 per pullet). Each row of 24 cages served as a replicate and each dietary treatment was replicated four times. The caged pullets were housed in a windowless, positive pressure, mechanically ventilated house. Fourteen hours (0400 to 1800 h) of artificial lighting with a light intensity of 5.4 lx was provided

daily. Feed was provided for *ad libitum* consumption. Water was provided for 15-min intervals every 2 h in continuous flow water troughs during the daily light period.

Five pullets from each replicate were identified and weighed individually prior to and at the end of the study. Body weight gain was determined for the entire experimentation period. Egg production was recorded daily. Mortality was recorded as it occurred and percent mortality determined at the end of each 28-d period. Bulk egg weights for each row were obtained for 3 consecutive d at the end of every 28-d period. The same eggs were used to determine egg size (jumbo, extra large and large) at the end of Periods 1, 2, 3, 4 and 6 in Experiment 1 and at the end of each 28-d period in Experiment 2. Egg sizes were determined by the USDA grading system (Anonymous, 1983) using a Modermatic model D5-V egg grader.[2] Egg mass was calculated by multiplying percentage hen-day egg production by the average egg weight in grams as described by North and Bell (1990).

Internal egg quality and egg specific gravity were measured at the end of Periods 1, 3, and 6 in Experiment 1, and at the end of Periods 1, 3, 5, and 7 in Experiment 2. Two eggs from each replicate were selected, weighed individually, and broken on glass break-out stand with a reflective mirror to detect blood spots on the under side of the egg. Albumen heights were measured using a micrometer,[3] the incidence of blood spots recorded, and Haugh units calculated using the formula described by Roush (1981). Egg specific gravity, which estimates shell strength was determined using the procedure outlined by Arscott and Bernier (1961). The procedure is based on the reports of Olsson (1934) and Hamilton (1982) that the internal contents of the egg have a specific gravity (SG) of 1, which is equal to the SG of

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[2] Modern Poultry Supplies Inc., Lancaster, PA 17601.

[3] B.C. Ames Co., Waltham, MA 02154

water, while the egg shell has a SG of 2. Thus, factors that influence the percentage of shell will influence the SG of eggs, and as specific gravity increases, there is a concomitant increase in shell thickness and strength. A floatation technique was used whereby eggs from each replicate were immersed sequentially, by means of plastic egg baskets with weights to hold them down, in buckets with series of saline solutions of ascending SG that ranged from 1.056 to 1.104, in increments of .004. The SG of the saline solution when an egg floated first was the approximate SG of that egg. The number of eggs that floated at each increment was recorded and the average egg specific gravity of each replicate of each treatment group was determined. The SG of the saline solutions was adjusted using a hydrometer and non-iodine salt at a temperature of 15.5 C.

During the fifth period of Experiment 2, two pullets from each replicate of each treatment were randomly selected and fed the experimental diets containing .3% chromium oxide marker to determine the percentage of nitrogen, calcium, and phosphorus retention. The marked feeds were fed for 7 d prior to 3 d of excreta collection. After collection, excreta samples were homogenized and dried in an oven at 27 C for 24 h. Excreta samples from each replicate of each treatment group were ground separately in a Wiley mill[4] through a 60-mesh screen. Chromium oxide levels in feed and excreta were determined by acid digestion and spectrophotometric methods described by Czarnocki *et al.* (1961) and Edwards and Gillis (1959). The samples were ground according to the methods of Association of Official Analytical Chemists (AOAC, 1980). Feed and excreta nitrogen levels were determined by the Kjeldahl analysis (AOAC, 1980). Feed and excreta samples were dry ashed at 550 C for 8 h prior to analysis for calcium, and phosphorus levels. Calcium was determined by the atomic

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[4] Arthur H. Thomas Co., Scientific Apparatus, Philadelphia, PA 19131

absorption spectrophotometry (AOAC, 1980), and phosphorus by the phosphomolybdic acid method (Fiske and SubbaRow, 1925). Percentage nutrient retentions were calculated using the formula described by Edwards and Gillis (1959).

Statistical Analysis

Percentage data (egg production, egg size, and nutrient retentions) were transformed into arc sine coefficients prior to analysis. Data were subjected to analysis of variance using the General Linear Models (GLM) procedure of SAS® (SAS Institute, 1988) with Lacto as treatment effect in Experiment 1 and Lacto and fat as treatment effects in Experiment 2. All variables were analyzed using repeated measurements with an exception of nutrient retentions. Correlation analyses among treatment effects, performance variables, and nitrogen, calcium, and phosphorus retentions were also computed using the GLM procedure.

The statistical model used for egg production, feed consumption, feed conversion, egg mass, egg weight, egg size, internal egg quality, egg specific gravity, body weight gain, and mortality in Experiment 1 was: $X_{ijk} = \mu + L_i + P_j + R_{ijk} + (LP)_{ik} + \varepsilon_{ijk}$; where μ = the overall mean; L_i = effect of Lacto, i= 1, 2, 3; P_j = effect of periods, j= 1...6; R_{ijk} = replications, k= 1...4; $(LP)_{ij}$ = interaction between Lacto and periods; and ε_{ijk} = error term.

The statistical model used for egg production, feed consumption, feed conversion, egg weight, egg mass, internal egg quality, egg specific gravity, body weight gain, and mortality in Experiment 2 was: $Y_{ijkl} = \mu + L_i + F_j + P_k + R_{ijkl} + (LF)_{ij} + (LP)_{ik} + (FP)_{jk} + (LFP)_{ijk} + \varepsilon_{ijkl}$ where μ = overall mean; L_i = effect of Lacto, i= 1, 2, 3; F_j = effect of fat, j = 1, 2, 3; P_k = effect of periods, k = 1...7; R_{ijkl} = replications, l = 1...4; $(LF)_{ij}$ = interaction of Lacto and fat; $(LP)_{ik}$ = interaction of Lacto and periods; $(FP)_{jk}$ = interaction of fat and periods, $(LFP)_{ijk}$ = interaction of Lacto, fat and periods and ε_{ijkl} = error term.

The statistical model used for nitrogen, calcium, and phosphorus retentions was: $Y_{ijk} = \mu + L_i + F_j + R_{ijk} + (LF)_{ij} + \varepsilon_{ijk}$ where μ = overall mean; L_i = effect of Lacto, $i = 1, 2, 3$; F_j = effect of fat, $j = 1, 2, 3$; R_{ijk} = replications, $k = 1...4$; $(LF)_{ij}$ = interaction of Lacto and fat, and ε_{ijk} = error term.

If significant differences ($P \leq .05$) were observed, least significant difference (LSD) comparisons were used between treatment means for the main effects (Steele and Torrie, 1980). Significance implies $P \leq .05$, unless stated otherwise.

RESULTS AND DISCUSSION

No significant treatment by period interactions were observed for Experiment 1; therefore, the performance data were pooled over periods and analyzed for treatment effects. Mean performance data of Experiment 1 from laying pullets fed Lacto diets are presented in Table III.3. Hen-day egg production was significantly higher by 1.7% for pullets fed the 1,100 ppm Lacto diet than the control diet. This improvement in egg production was consistent with the findings of Krueger *et al.* (1977), and Miles *et al.* (1981). Hen-day egg production of pullets fed the 2,200 ppm Lacto diet was not different from those fed 1,100 ppm Lacto and the control diet.

Daily feed consumptions were significantly increased by 4 and 3.4% for pullets fed the 1,100 ppm and 2,200 ppm Lacto diets, respectively, when compared with the pullets fed the control diet without Lacto. Feed consumptions for pullets fed the 1,100 and 2,200 ppm Lacto were not different. Contrary to our observation on feed consumption, Cerniglia *et al.* (1983) and Goodling *et al.* (1987) did not observe significant effects on feed consumption when low doses of Lacto-fermented products (either 0, .26, .52, and .78 L/ton of feed or .236, .473 and .709 L/ton of feed, respectively) were fed to laying hens. This difference may be attributed to the sources of Lacto and the levels of Lacto fed by the researchers. Viable Lacto with CCMS carrier was used in this experiment whereas the latter groups used Lacto-fermented products.

Feed conversion was significantly decreased by 2.5% when pullets were fed the diets containing 1,100 and 2,200 ppm Lacto diets when compared with the control. This finding was contrary to the findings of Krueger *et al.* (1977). The better feed conversion of the control diets may be partly associated with less feed consumption by the laying pullets.

Egg mass, egg weight, and egg size were significantly increased by 2.8, 1, and 6% for pullets fed the 1,100 ppm Lacto diets than the pullets fed the control diet. Furthermore, egg mass of pullets fed the 1,100 ppm Lacto diet was better by 1.3% than for pullets fed the 2,200 ppm Lacto diet. The effects on egg size were in agreement with the reports of Hargis and Creger (1978), who fed a *Lactobacillus* fermentation product to laying hens and observed an increase in egg size. No differences in egg weight and egg size were observed between pullets fed the 1,100 and 2,200 ppm Lacto diets.

Internal egg quality (Haugh units) was significantly improved by 1.7% in pullets fed the 2,200 ppm Lacto diet compared with the control. No differences in the internal egg quality were observed between either the 1,100 and 2,200 ppm Lacto diets or the control and 1,100 ppm Lacto diets. There were no blood spots detected in any of the treatment groups. Egg specific gravity, body weight gain, and mortality did not differ among the dietary treatments.

No significant Lacto by fat x period interactions were observed for Experiment 2; therefore, data were pooled over periods and analyzed for main effects. Mean performance data of Experiment 2 for pullets fed Lacto diets with 0, 1, and 3% fat are presented in Table III.4. Fat was added to the Lacto diets in an attempt to lower the effect of the increased feed consumption observed in Experiment 1. Hen-day egg production of all pullets fed the 1,100 and 2,200 ppm Lacto diets were significantly improved by 1.1 and 1.4%, respectively, when compared with the pullets fed no Lacto (control) diet. No differences in hen-day egg production were observed between pullets fed the 1,100 and 2,200 ppm Lacto diets and between pullets fed diets without and with 1 and 3% fat.

Pullets fed the 1,100 and 2,200 ppm Lacto diets consumed 1.7 and 2.6% more feed daily per layer, respectively, than the pullets fed the diet without Lacto (control). Daily feed

consumption of pullets fed the diets containing the 1,100 and the 2,200 ppm Lacto were not different. This was also observed in Experiment 1. Daily feed consumptions of pullets fed diets with 3% supplemental fat were significantly reduced by 5 and 3.4% when compared with pullets fed the diets without and with 1% fat, respectively. No differences in daily feed consumption were observed between pullets fed the diets without and with 1% fat.

Feed conversions for pullets fed no Lacto, 1,100 and 2,200 ppm Lacto diets were not different. These results were not consistent with our findings in Experiment 1 and those of Krueger *et al.* (1977); however, these findings were consistent with the report of Cerniglia *et al.* (1983) and Goodling *et al.* (1987). Feed conversions of pullets fed diets with 3% fat were significantly improved by 4 and 3% when compared with those of pullets fed diets without and with 1% fat, respectively. The improvements in feed conversions were the result of decreased daily feed consumptions and better egg production.

Egg masses were improved significantly by 1.7 and 2.3% for all pullets fed the 1,100 and 2,200 ppm Lacto diets, respectively, when compared with pullets fed diets without Lacto; however, no differences in egg masses were observed between pullets fed the 1,100 and 2,200 ppm Lacto diets. Egg masses of pullets fed diets without and with 1 and 3% fat were not different. Mean egg weights were .4 and .7% heavier for pullets fed 1,100 and 2,200 ppm Lacto diets, respectively than pullets fed the diet without Lacto. Egg weights of pullets fed diets with 1 and 3% fat were 1% heavier than those of pullets fed the diet without fat. The increase in egg weight of pullets fed the diets with 1 and 3% fat when compared with pullets fed the diet without fat may be associated with the levels of linoleic acid in the diets with supplemental fat. The diets containing 0, 1, and 3% supplemental fat were calculated to have 1.3, 1.5 and 1.8% linoleic acid, respectively. Several investigators (Jensen *et al.*, 1958;

Shutze *et al.*, 1962; Hoyle and Garlich, 1987; Sell *et al.*, 1987; Whitehead *et al.*, 1991; Mannion *et al.*, 1992) observed improvement in egg weight with increasing levels of dietary linoleic acid.

Egg sizes were significantly improved by 4.4 and 2% for pullets fed the 2,200 ppm Lacto diet than those fed diets without and with 1,100 ppm Lacto, respectively. Egg sizes were increased by 2.3% for pullets fed the 1,100 ppm Lacto diet when compared with pullets fed the diet without Lacto. The pullets fed diets containing 1% fat had a 2.4 and 1.8% increase in egg size when compared with pullets fed the diets without and with 3% fat, respectively. The 1.8% decrease in egg size of pullets fed the diet with 3% fat when compared with pullets fed the diet containing 1% fat may be associated with a decrease in feed consumption of the pullets fed the 3% fat diet. These increases in egg size may be associated with the stimulation of appetite with Lacto-fed pullets and the supplemental fat contributing linoleic acid in the diet.

Internal egg quality (Haugh units) was improved by 1.2% when pullets were fed the 2,200 ppm Lacto diet than pullets fed diets without and with 1,100 ppm Lacto. No differences in internal egg quality were observed between the pullets fed diets without and with 1,100 ppm Lacto. The improvement in internal egg quality may be partly associated with the higher nitrogen retention of pullets fed diets containing Lacto. Egg Specific gravity and the incidence of blood spots were not different among Lacto and fat levels.

Body weight gain of pullets fed the 2,200 ppm Lacto diet was 48 and 9% greater than pullets fed diets without and with 1,100 ppm Lacto, respectively. Body weight gain of pullets fed 1,100 ppm Lacto diet was 36% greater than that of pullets fed the diet without Lacto. The increase in body weight gain may be due to an increase in feed consumption which may have resulted in fat deposition in the adipose tissue. Body weight gain of pullets fed 3% fat diet

was 20 and 19% greater than that of pullets fed diets without and with 1% fat, respectively. No differences in body weight gain were observed between the pullets fed the diet with 1% fat and those fed the diet without fat. The greater body weight gains observed in pullets fed the diets with 1 and 3% fat may be the result of the efficiency of the pullets to utilize fat. Possibly, the excess dietary fat was deposited in the adipose tissue. High fat diets may slow down the passage rate of digesta through the GI tract (Golian and Maurice, 1992), hence allowing more time for utilization of nutrients such as nitrogen, which may be associated with the better body weight gains. Mortality was not different among the Lacto and the fat levels.

No significant Lacto by fat interactions were observed for the percentage nutrient retentions; therefore, the data were analyzed for main effects. Mean nitrogen, calcium, and phosphorus retention data calculated from feed and excreta samples of pullets for Experiment 2 are presented in Table III.5. Nitrogen retentions of pullets fed either the 1,100 or the 2,200 ppm Lacto diets were increased by 25% when compared with the pullets fed the diet without Lacto. No differences in nitrogen retention were observed between pullets fed the 1,100 and those fed the 2,200 ppm Lacto diets. Nitrogen retentions of pullets fed 1% fat diet were 35% higher than those of pullets fed the diets without fat. Nitrogen retention was decreased by 15% for pullets fed 3% fat diets when compared with 1% fat diet. Nitrogen retention was better for pullets fed the 3% fat diet than pullets fed the diet without fat. The low retention of nitrogen by pullets fed the diet without fat may be due to an increase in feed consumption. Excess nitrogen cannot be stored, therefore, increased amounts of nitrogen might have been excreted when the pullets met their nitrogen requirement. The retention of nitrogen increased partly because of the decrease in feed consumption when the diets were supplemented with 1% fat. The low nitrogen consumption, most of which was retained, may have also contributed to the increase in percentage nitrogen retention. The decrease in nitrogen retention

by pullets fed diets containing 3% fat compared to pullets fed diets with 1% fat may be due to a decrease in feed consumption of the pullets fed the 3% fat diet. The retentions of calcium were 34 and 32% higher for pullets fed the 1,100 and 2,200 ppm Lacto diets, respectively, than pullets fed the diet without Lacto. No differences in calcium retention were observed between pullets fed the 1,100 and the 2,200 ppm Lacto diets. Although not significant, a 6% increase in calcium retention was observed when a layer diet was supplemented with 1% fat. However, supplementing the layer diet with 3% fat significantly reduced the retention of calcium by 14% when compared with 1% fat diet. The decrease in calcium retentions in the diet with 3% fat may be the result of complexing calcium with fatty acids and thus increasing the proportion of fecal calcium soaps (Hakansson, 1975; Sibbald and Price, 1977; Atteh and Leeson, 1983, 1984, 1985; Rising *et al.* 1990).

Phosphorus retentions of pullets fed 1,100 and 2,200 ppm Lacto diets were 76 and 139% higher, respectively, when compared with the pullets fed the diet without Lacto. The retention of phosphorus was better for pullets fed the 2,200 ppm Lacto diets when compared with pullets fed the 1,100 ppm Lacto diet. Phosphorus retention was 62% higher for pullets fed the diet with 1% fat than the diets without fat. Supplementing a layer diet with 3% fat decreased phosphorus retention by 31% when compared with a 1% fat diet. No differences in phosphorus retention were observed between pullets fed the diets without and with 3% fat. The higher fat levels may have interfered with the phosphorus retention.

Correlation coefficients between Lacto diets, fat, nitrogen, calcium and phosphorus retentions and performance variables of laying pullets for Experiment 2 are presented in Table III.6. Positive correlations between Lacto diets and nitrogen retention, calcium retention, feed consumption, and egg size; between fat and calcium retention, nitrogen retention, egg mass,

and egg weights, between nitrogen retention and egg weights, egg mass, and egg size were observed. The positive correlation between nitrogen and calcium retentions indicates that proteins may affect mineral nutrition. Wapnir (1989) suggested that proteins may affect mineral nutrition either by altering the integrity of the intestinal mucosa, or the synthesis of hydrolytic enzymes and absorption mediators or binding and making available certain minerals. Negative correlation was observed between dietary fat and feed consumption; as expected, because dietary fat decreased feed consumption. Although most of these correlations are low, they present the interrelationships that may exist between nutrients and their contribution to the performance of the pullets. The low and significant correlations may be a result of the large sample size used in this study.

According to the experimental conditions, feeding either 1,100 or 2,200 ppm *Lactobacillus* to laying pullets stimulated appetite, improved egg production, egg mass, egg weight, egg size, internal egg quality, and nitrogen, calcium, and phosphorus retentions. Further supplementation of the Lacto diets with 1 and 3% fat reduced feed consumption, provided better feed conversion, egg production, egg masses, egg sizes, body weight gains, and nitrogen, calcium, and phosphorus retentions.

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Table III.1. Composition of diets for Experiment 1

Ingredients and analyses	Dietary Lacto[1,2] levels		
	0	1100	2200
	(%)		
Corn (yellow)	66.45	64.45	64.45
Soybean ml (47.5% CP)	18.50	18.50	18.50
Barley (8.7% CP)	5.00	5.00	5.00
CCMS[3]-Lacto premix (55 g/kg)	---	2.00	---
CCMS-Lacto premix (110 g/kg)	---	---	2.00
Limestone flour (37% Ca)	4.00	4.00	4.00
Oyster shell (38% Ca)	3.80	3.80	3.80
Dicalcium phosphate (21% Ca, 18% P)	1.70	1.70	1.70
Salt	.25	.25	.25
Trace mineral premix[4]	.05	.05	.05
Vitamin premix[5]	.20	.20	.20
D,L methionine (98%)	.05	.05	.05
Calculated analyses			
CP, %	15.3	15.3	15.3
ME, Kcal/kg	2855	2819	2819
Ca, %	3.60	3.60	3.60
Avail. P, %	.40	.40	.40
Total P, %	.69	.69	.69
Met., %	.30	.30	.30
Met. + Cys., %	.60	.60	.60
Linoleic acid, %	1.30	1.30	1.30
Analyzed levels			
CP, %	15.3	15.1	15.2
Ca, %	3.58	3.60	3.59
Total P, %	.71	.65	.70

[1] *Lactobacillus.* [2] Milligrams per kilogram.

[3] Condensed cane molasses solubles.

[4] Provided per kilogram of diet: manganese, 60 mg; iodine, 1.2 mg; iron, 20 mg; copper, 2 mg; zinc, 20 mg; and cobalt, .2 mg.

[5] Provided per kilogram of diet: vitamin A (retinyl acetate), 3,300 IU; vitamin D_3, 1,100 ICU; dl-α-tocopheryl acetate, 1.10 IU; menadione bisulfite complex, .55 mg; vitamin B_{12}, 5.5 μg; riboflavin, 3.3 mg; pantothenic acid, 5.5 mg; niacin, 22 mg; choline chloride, 220 mg; folic acid, 220 μg; and ethoxyquin, 62.4 mg.

Table III.2. Composition of diets for Experiment 2

Ingredients and analyses	Dietary Lacto[1,2] and fat[3] levels						
	No Lacto No fat	1100 No fat	1100 1	1100 3	2200 No fat	2200 1	2200 3
	(%)						
Corn (yellow)	66.45	64.45	62.75	60.75	64.45	62.75	60.75
Soybean ml (47.5% CP)	18.50	18.50	19.20	19.20	18.50	19.20	19.20
Barley (8.7% CP)	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Poultry blended fat	---	---	1.00	3.00	---	1.00	3.00
CCMS[4]-Lacto premix (55 g/kg)	---	2.00	2.00	2.00	---	---	---
CCMS-Lacto premix (110 g/kg)	---	---	---	---	2.00	2.00	2.00
Limestone flour (37% Ca)	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Oyster shell (38% Ca)	3.80	3.80	3.80	3.80	3.80	3.80	3.80
Dicalcium phosphate (21% Ca, 18% P)	1.70	1.70	1.70	1.70	1.70	1.70	1.70
Salt	.25	.25	.25	.25	.25	.25	.25
Trace mineral premix[5]	.05	.05	.05	.05	.05	.05	.05
Vitamin premix[6]	.20	.20	.20	.20	.20	.20	.20
D,L methionine (98%)	.05	.05	.05	.05	.05	.05	.05
Calculated analyses							
CP, %	15.3	15.3	15.3	15.3	15.3	15.3	15.3
ME, Kcal/kg	2855	2819	2861	2947	2819	2861	2947
Ca, %	3.60	3.60	3.60	3.60	3.60	3.60	3.60
Avail. P, %	.40	.40	.40	.40	.40	.40	.40
Total P, %	.69	.69	.69	.69	.69	.69	.69
Met., %	.30	.30	.30	.30	.30	.30	.30
Met. + Cys., %	.60	.60	.60	.60	.60	.60	.60
Linoleic acid, %	1.30	1.30	1.50	1.80	1.30	1.50	1.80
Analyzed levels							
CP, %	15.2	15.1	15.2	15.3	15.0	15.7	15.1
Ca, %	3.58	3.60	3.59	3.61	3.58	3.60	3.59
Total P, %	.71	.65	.70	.70	.63	.64	.67

[1] *Lactobacillus.* [2] Milligrams per kilogram. [3] Percent of diet

[4] Condensed cane molasses solubles.

[5] Provided per kilogram of diet: manganese, 60 mg; iodine, 1.2 mg; iron, 20 mg; copper, 2 mg; zinc, 20 mg; and cobalt, .2 mg.

[6] Provided per kilogram of diet: vitamin A (retinyl acetate), 3,300 IU; vitamin D_3, 1,100 ICU; dl-α-tocopheryl acetate, 1.10 IU; menadione bisulfite complex, .55 mg; vitamin B_{12}, 5.5 μg; riboflavin, 3.3 mg; pantothenic acid, 5.5 mg; niacin, 22 mg; choline chloride, 220 mg; folic acid, 220 μg; and ethoxyquin, 62.4 mg.

Table III.3. Performance variables of Single Comb White Leghorn laying pullets fed corn-soybean meal (C-S) diets containing 1,100 and 2,200 ppm *Lactobacillus* (Lacto) for six 28-d periods, Experiment 1

Dietary Lacto levels	Hen-day egg production	Daily feed consumption	Feed conversion	Egg mass	Egg weight	Egg Size ≥large	Internal egg quality	Egg specific gravity	Body[1] weight gain	Cumulative mortality
Lacto (ppm)	(%)	(g/hen)	(kg/doz eggs)	(g/hen/day)	(g/egg)	(%)	(HU)[2]	(1.07--)	(g/hen)	(%)
0	88.9[b]	117[b]	1.58[b]	52.7[c]	59.4[b]	80.3[b]	83.1[b]	89[a]	210[a]	.27[a]
1100	90.4[a]	122[a]	1.62[a]	54.2[a]	60.0[a]	85.1[a]	83.8[ab]	82[a]	190[a]	.08[a]
2200	89.5[ab]	121[a]	1.62[a]	53.5[b]	59.8[a]	84.1[a]	84.5[a]	83[a]	170[a]	.17[a]
Pooled SEM	.5	.6	.01	.3	.2	1.0	.5	.0003	10	.004
Source of variation	----------Probabilities----------									
Lacto	.02	.001	.001	.001	.002	.001	.04	NS	NS	NS
Period	.001	.001	.001	.001	.001	.001	.001	.001	.001	NS
Lacto x Period	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a,b] Mean values within columns with no common superscript differ significantly ($P \leq .05$).

[1] Cumulative for six 28-day periods

[2] HU:Haugh units.

Table III.4. Performance variables of Single Comb White Leghorn laying pullets fed corn-soybean meal (C-S) diets containing 1,100 and 2,200 ppm *Lactobacillus* (Lacto) with 1 and 3% supplemental fat for seven 28-d periods, Experiment 2

Main effects	Hen-day egg production	Daily Feed consumption	Feed conversion	Egg mass	Egg weight	Egg size ≥large	Internal egg quality	Egg specific gravity	Body[1] weight gain	Cumulative mortality
Lacto levels (ppm)	(%)	(g/hen)	(kg/doz eggs)	(g/hen/day)	(g/egg)	(%)	(HU)[2]	(1.07--)	(g/hen)	(%)
No Lacto	85.9[b]	115[b]	1.61[a]	52.6[b]	61.3[b]	90.5[c]	80.4[b]	72[a]	225[c]	.38[a]
1,100	87.0[a]	117[a]	1.62[a]	53.5[a]	61.6[a]	92.6[b]	80.3[b]	66[a]	307[b]	.30[a]
2,200	87.1[a]	118[a]	1.63[a]	53.8[a]	61.7[a]	94.5[a]	81.4[a]	70[a]	334[a]	.25[a]
Pooled SEM	.2	.4	.01	.2	.1	.4	.2	.0002	16	.10
Fat levels (%)										
No fat	87.0[a]	120[a]	1.65[a]	53.5[a]	61.1[b]	92.6[b]	81.3[a]	68[a]	299[b]	.34[a]
1	87.0[a]	118[a]	1.63[a]	53.7[a]	61.7[a]	94.9[a]	80.8[a]	68[a]	303[b]	.27[a]
3	87.2[a]	114[b]	1.58[b]	53.8[a]	61.7[a]	93.1[b]	80.5[a]	68[a]	360[a]	.23[a]
Pooled SEM	.3	.3	.01	.3	.1	.4	.5	.0004	14	.01
Source of variation	----------	----------	----------	----------	Probabilities	----------	----------	----------	----------	----------
Lacto	.03	.002	NS	.04	.05	.001	.04	NS	.001	NS
Fat	NS	.001	.001	NS	.04	.001	NS	NS	.001	NS
Period	.001	.001	.001	.001	.001	.001	.04	.03	.05	NS

[a,b] Mean values within columns with no common superscript differ significantly ($P \leq .05$).

[1] Cumulative for seven 28-d periods. [2] HU:Haugh units.

Table III.5. Retention of nitrogen, calcium, and phosphorus by Single Comb White Leghorn laying pullets fed corn-soybean meal (C-S) diets containing 1,100 and 2,200 ppm *Lactobacillus* (Lacto) with 1 and 3% fat for seven 28-d periods, Experiment 2

Main effects	N	Ca	P
Lacto levels (ppm)	----------(%)----------		
No Lacto	38.3[b]	44.1[b]	14.7[c]
1,100	47.9[a]	59.0[a]	25.8[b]
2,200	47.8[a]	58.0[a]	35.2[a]
Pooled SEM	1.0	1.8	1.3
Fat levels (%)			
No fat	41.0[c]	58.9[ab]	24.4[b]
1	55.4[a]	62.6[a]	39.6[a]
3	47.0[b]	54.0[b]	27.5[b]
Pooled SEM	1.3	2.2	1.1
Source of variation	----------Probabilities----------		
Lacto	.01	.001	.008
Fat	.001	.001	.001
Lacto x Fat	NS	NS	NS

[a,b] Mean values within columns with no common superscript differ significantly ($P \leq .05$).

Table III.6. Correlation coefficients among *Lactobacillus* (Lacto) diets, supplemental fat, nitrogen, calcium, phosphorus retentions, and performance variables of Single Comb White Leghorn laying pullets fed corn-soybean meal (C-S) diets containing *Lactobacillus* with 1 and 3% fat, Experiment 2

	Nutrients retained			Performance parameters								
Treatments	N	Ca	P	Hen-day egg production	Feed conversion	Feed consumption	Egg mass	Egg weight	Egg size	Internal egg quality	Shell thickness	Body weight gains
Lacto	.85**	.54**	.27	.01	-.13	.56**	.23	.40	.44*	.22	.16	.25
Fat	.52*	.53**	.37	.15	.03	-.73**	.49*	.52*	.54	.30	.23	.45*
Nutrients retained												
N		.76**	.20	.10	-.03	-.02	.70**	.71**	.59**	.06	.06	.10
Ca			.82**	.22	.22	.34*	.41*	.40*	.30	.02	.24	.20
P				.10	.48*	.41*	-.20	.26	-.08	-.09	.26	.10

* $P \leq .05$.

** $P \leq .01$.

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CHAPTER IV

PERFORMANCE OF SINGLE COMB WHITE LEGHORN LAYERS FED CORN-SOYBEAN MEAL AND BARLEY-CORN-SOYBEAN MEAL DIETS SUPPLEMENTED WITH A DIRECT-FED MICROBIAL[1]

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ABSTRACT

An experiment was conducted with Single Comb White Leghorn (SCWL) layers to determine the effect of feeding either corn-soybean meal (C-S) or barley-corn-soybean meal (B-C-S) diets with condensed cane molasses solubles (CCMS)-1,100 mg *Lactobacillus* (Lacto)/kg diet (ppm) on performance, nutrient retentions, gastrointestinal (GI) passage rate and anatomical and histological changes of the GI tracts of the layers. Six dietary treatments were fed for eight 28-d periods and consisted of C-S (control), C-S + CCMS, C-S + CCMS-1,100 ppm Lacto, B-C-S (control), B-C-S + CCMS and B-C-S + CCMS-1,100 ppm Lacto. The CCMS were used as the carrier for the Lacto, and the CCMS-Lacto premix was incorporated at 2% of the diet.

Lactobacillus supplementation in C-S diets improved ($P < .05$) egg weight, egg mass, egg size and body weight gains, and in B-C-S diets improved body weight gains. There were no differences in feed consumption, feed conversion, internal egg quality and mortality among dietary treatments. Passage rates of digesta were increased ($P < .05$) when B-C-S layer diets were supplemented with Lacto. *Lactobacillus* supplementation of the C-S and B-C-S diets increased ($P < .05$) fat and calcium, and fat, phosphorus and manganese retentions, respectively and increased cellularity of Peyer's patches in the ileum, which are part of the mucosal immune system which respond to antigenic stimuli by secreting immunoglobulin (IgA).

Feeding Lacto in C-S and B-C-S diets improved egg weight, egg mass, egg size, body weight gains, fat and calcium retention, and body weight gains, fat, phosphorus and manganese retention and rate of passage of digesta in layers, respectively. Feeding Lacto increased cellularity of Peyer's patches of the ileums.

(Key words: Direct-fed microbials, layers, nutrient retention and passage rate, peyer's patch)

INTRODUCTION

Cereal grains, such as corn and barley, are provided as a source of energy in poultry feeds. Barley, in particular, is an important cereal grain because of its wide ecological range and the diverse adaptation of the many varietal types. It is an outstanding grain crop from the standpoint of being able to mature in a shorter growing season grain, and therefore, a very important crop in many areas of the world where the growing season may be cut short either by lack of sufficient rainfall or by low temperatures. Barley, however, has high fiber and low energy contents. The presence of β-D-glucans, the growth depressing, highly viscous, water-soluble mixed-linked (1-3), (1-4) carbohydrates (White *et al.*, 1981; Hesselman and Aman, 1986) lower the digestibility of barley. The β-D-glucans are found in the cell wall, and are resistant to digestive enzymes of poultry and animals, and therefore, protect starch and protein from digestion in the small intestine (Hesselman and Aman, 1986). In most animals, endogenous enzymes cannot cleave β(1-4) linkages in carbohydrates (Lehninger, 1975), although these linkages may be broken down by the microflora of the gastrointestinal (GI) tract. Microbials have been employed to improve the digestibility of barley in monogastric animals. These microorganisms may rupture the endosperm cell walls surrounding the starch granules in the grain, making available starch for digestive degradation in the GI tract (Hesselman and Aman, 1986).

In previous studies, Graham *et al.* (1986) reported that mixed-linked β-glucans were degraded in the stomach of the pig where *Lactobacilli* were present; however, there was doubt whether any direct benefit was obtained through the release of glucose residues from β-glucans. Other studies conducted to determine the effect of *Lactobacillus* supplementation in C-S layer diets have shown a lot of variability in results partly because of the different forms and concentrations of *Lactobacillus* added to the diets. Feeding either dried viable or dried

non-viable *Lactobacillus* products at .25, .5, and .75 g/kg of feed in laying hen diets did not improve hen-day egg production, feed efficiency, livability and egg size (Goodling *et al.*, 1987). However, Krueger *et al.* (1977) reported improvement in feed efficiency and hen-day egg production when laying hen diets were supplemented with .5, and 2.5 g of *Lactobacillus* complex/kg of feed. Miles *et al.* (1981) added live cultures of *Lactobacillus acidophilus* at .0125, .0375, and .0625% of the diets and observed an increase in feed consumption and egg size. Nahashon *et al.*(1992, 1993) fed 1,100 and 2,200 mg *Lactobacillus*/kg of layer diets in condensed cane molasses solubles carrier and observed appetite stimulation, increased egg size, egg mass, egg weight and nitrogen, calcium and phosphorus retention in layers.

Since no information is available on the possible involvement of *Lactobacillus* cultures in the utilization of barley in layer diets, the objective of this study was to determine the effect of feeding either corn-soybean meal (C-S) or barley-corn-soybean meal (B-C-S) with condensed cane molasses solubles containing *Lactobacillus* (Lacto) on layer performance, fat, nitrogen, calcium, phosphorus, zinc, manganese, copper, iron and magnesium retentions, gastrointestinal (GI) passage rate and on the anatomical and histological changes of the GI tracts of layers.

MATERIALS AND METHODS

Five hundred seventy six Dekalb XL Single Comb White Leghorn (SCWL) layers were raised according to standard methods as outlined by North and Bell (1990). The hens were fed one of 6 dietary treatments for eight 28-d periods beginning at 30.5 wk of age. The study was completely randomized in a 2 x 2 x 2 factorial arrangement with 2 grain sources, 2 levels of CCMS and 2 levels of Lacto. The diets were corn-soybean meal (C-S, control), C-S + CCMS, C-S + CCMS-1,100 ppm (4.4×10^7 cfu/mg Lacto) diets, barley-corn-soybean meal (B-C-S, control), B-C-S + CCMS and B-C-S + CCMS-1,100 ppm Lacto diets and are presented in Table IV.1. Equal quantities of barley and corn were incorporated in the grain portion of the diets. In order to introduce the Lacto into the feeds, condensed cane molasses solubles served as the carrier for the Lacto, and the Lacto premix was incorporated at 2% of the diet. The diets were formulated according to NRC (1984) recommendations.

Dietary treatments were randomly assigned to rows of 24 individual cages (21 cm wide x 46 cm deep x 46 cm high) with sloping wire floors in a stair-step arrangement per bank. Each cage housed one layer (966 cm^2 per layer). Each row of 24 cages served as a replicate and each dietary treatment was replicated four times. The housing, lighting, feeding and watering conditions were previously described in Chapter III.

The same five layers from each replicate were randomly selected, identified and weighed individually prior to and at the end of the study period. Egg production, weight, mass and sizes (jumbo, extra large and large) were measured at the end of each period using the previously described procedures (Chapter III). Internal egg quality (Haugh units) and egg specific gravity were measured at the end of periods 1, 3, 5 and 8 as described in Chapter III. Mortality was recorded as it occurred. Yolk color was compared to the Roche yolk color fan with the color score ranging from 1 being light yellow to 15 being orange color.

During the 6th period, 2 individual layers from each replicate of each treatment group were randomly selected and fed quantities of each of the experimental diets containing .3% chromium oxide marker to determine crude fat, nitrogen, calcium, phosphorus, zinc, manganese, copper, iron and magnesium retentions. The marker feeds were fed for 7 days prior to 3 continuous days of excreta collection. Digesta passage time was measured by collecting excreta samples hourly from 0 to 4 h; every 2 h from 4 to 12 h; and every 4 h from 12 to 32 h. After collection, excreta samples were homogenized and dried in an oven at 27 C for 24 h. Fecal samples from each individual layer were ground separately in a Wiley mill[2] through a 60 mesh screen. Fat was extracted using the Soxhlet apparatus (AOAC, 1980). Chromium oxide, nitrogen, calcium and phosphorus levels in feed and excreta were determined using the procedures described in Chapter III. Zinc , manganese, copper, iron and magnesium in feed and excreta were determined by atomic absorption spectrophotometry (AOAC, 1980). Percent nutrient retentions were calculated using the formula described by Edwards and Gillis (1959).

At the end of the 8th period, 5 layers from each treatment group were randomly selected and euthanatized for subsequent tissue histological examinations. Sections of the duodenum, jejunum and ileum from each layer were excised and flushed with .08% normal saline prior to fixation in 10% neutral bufferred formalin solution for histological examinations. Samples of the jejunum and ileum were obtained from the proximal and distal ends of the Meckel's diverticulum, respectively. The tissues were processed through a

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[2] Arthur H. Thomas Co., Scientific Apparatus, Philadelphia, PA 19131.

standard alcohol dehydration-toluene series, embedded in paraffin wax, sectioned and stained with hematoxylin + eosin dyes for microscopic observation. Photomicrographs of the tissues were taken where gross changes were observed.

Statistical Analysis

Percentage data (egg production, egg size and nutrient retentions) were transformed into arc sine coefficients prior to analysis. Data were subjected to analysis of variance using the General Linear Model (GLM) of Statistical Analysis Systems (SAS, 1988) with dietary compositions, CCMS and Lacto as treatment effects. All variables were analyzed using repeated measurements with an exception of nutrient retentions.

The statistical model used for egg production, feed consumption, feed conversion, egg mass, egg weight, egg size, internal egg quality, egg specific gravity, yolk color, body weight gains and mortality was: $X_{ijklm} = \mu + C_i + L_j + R_{ijk} + G_l + P_m + (CG)_{il} + (LG)_{jl} + (GP)_{lm} + (CP)_{im} + (LP)_{jm} + (CGP)_{ilm} + (LGP)_{jlm} + \varepsilon_{ijklm}$; where X_{ijklm} = individual observation; μ = the overall mean; C_i = the effect of CCMS, i= 1, 2; L_j = the effect of Lacto, j= 1, 2 ; R_{ijk} = the inter experimental unit (rows of cages) error term, k= 1,..,4; G_l= the effect of grain source, l=1, 2; P_m = the effect of period, m = 1,..,8; CG_{il} = the interaction between CCMS and grain source; $(LG)_{jl}$ = the interaction between Lacto and grain source; $(CP)_{im}$ = the interaction between CCMS and periods; $(LP)_{jm}$ = the interaction between Lacto and periods; $(CGP)_{ilm}$= the interaction between CCMS, grain source and periods, $(LGP)_{jlm}$ = the interaction between Lacto, grain source and periods; and ε_{ijklm} = the intra experimental unit error term.

The statistical model used for the retention of fat (ether extract), nitrogen, calcium, phosphorus, zinc, manganese, copper, iron and magnesium was: $X_{ijkl} = \mu + C_i + L_j + R_{ijk}$

$+ G_l + (CG)_{il} + (LG)_{jl} + \varepsilon_{ijkl}$; where X_{ijkl} = individual observation; μ = the overall mean; C_i = the effect of CCMS, i= 1, 2; L_j = the effect of Lacto, j= 1, 2 ; R_{ijk} = the inter experimental unit (layers) error term, k= 1,..,5; G_l= the effect of grain source, l=1, 2; CG_{il} = the interaction between CCMS and grain source; $(LG)_{jl}$ = the interaction between Lacto and grain source; and ε_{ijkl} = the intra experimental error term.

The mean cumulative excreta chromium oxide concentration for each dietary treatment was regressed with time after introducing the chromium oxide feed to layers.

When significant ($P \leq .05$) F-values were observed, least significant difference (LSD) comparisons were used between treatment means for main effects (Steele and Torrie, 1980). Significant implies $P \leq .05$, unless stated otherwise.

RESULTS AND DISCUSSION

Significant grain source x CCMS x Lacto interactions were observed on performance parameters; however, no significant grain source x period, CCMS x period and Lacto x period interactions; therefore, the data were pooled over periods and analyzed for treatment effects. Mean performance data of Single Comb White Leghorn layers fed diets containing corn-soybean meal (C-S) and barley-corn-soybean meal (B-C-S) with CCMS and CCMS-Lacto premix are presented in Table IV.2. Hen-day egg production was significantly higher for layers fed C-S + CCMS, C-S + CCMS-Lacto and B-C-S + CCMS diets than C-S and B-C-S (controls) diets. These findings were contrary to previous reported findings (Krueger *et al.*, 1977; Hargis and Creger, 1978; Nahashon *et al.*, 1992), where *Lactobacillus* supplementation increased hen-day egg production of Single Comb White Leghorn Layers. The lack of effect of *Lactobacillus* on hen-day egg production may be explained by the fact that *Lactobacillus* become established in the gut of most species of animals soon after birth or hatch (Savage *et al.*, 1968; Timms, 1968), and only under stressful conditions, coliforms increase in number, and direct-fed microbials have measurable benefit (Leeson and Major (1990) and R. B. Parker (RBPi, 16398 S. W. 72nd, Portland, Oregon 97224, personal communication). Unlike previous studies (Nahashon *et al.*, 1992), where two layers were housed in a single cage (483 cm^2 per layer), layers in this study were raised under relatively ideal conditions because each layer was housed in an individual cage (966 cm^2 per layer) where *Lactobacillus* supplementation to layer feeds may not show beneficial effects.

Lacto supplementation did not improve daily feed consumptions of layers fed either C-S or B-C-S diets with and without Lacto. These results were not consistent with previous findings (Nahashon *et al.*, 1992, 1993). The failure of Lacto to stimulate appetite may be due

to the fact that layers in this study were raised under relatively ideal conditions where *Lactobacillus* supplementation to layer feeds may not show beneficial effects.

Feed conversions were not different among treatment groups. These findings were consistent with those of Goodling *et al.* (1987). However, these findings were contrary to the reports of Krueger *et al.* (1977) who observed an improvement in feed conversion when supplementing layer diets with Lacto.

Egg weights were better for layers fed the diets containing C-S + CCMS-Lacto than diets containing C-S with and without CCMS. These findings were consistent with our previous findings (Nahashon *et al.*, 1992). Layers fed the B-C-S diets with and without CCMS and CCMS-Lacto premix produced heavier eggs than layers fed the C-S diets with and without CCMS and CCMS-Lacto premix.

Egg mass from layers fed the C-S + CCMS-Lacto diet was significantly higher than the egg mass from layers fed C-S (control) and C-S + CCMS diets. Egg masses were also higher for layers fed the diets containing B-C-S + CCMS and B-C-S + CCMS- Lacto than either B-C-S or C-S diets with and without CCMS or CCMS-Lacto.

Egg size of layers fed the C-S + CCMS-Lacto diet were larger than layers fed the C-S control and C-S + CCMS diets. The increases in egg mass, egg weight and egg size with Lacto supplementation of the C-S diet were consistent with our previous finding (Nahashon *et al.*, 1992). Egg sizes were larger for layers fed all these B-C-S diets than C-S diets. The supplementation of 6% fat in the B-C-S diets may have further influenced the egg size due to the linoleic acid levels in the C-S and B-C-S diets which were 1.8 and 2.1%, respectively (Sell *et al.*, 1987; Whitehead *et al.*, 1991; Mannion *et al.*, 1992).

Internal egg quality (Haugh units), egg specific gravity and mortality were not different among dietary treatments.

Body weight gains of layers fed diets containing B-C-S + CCMS-Lacto were more than layers fed the B-C-S and C-S diets either with or without CCMS. Layers fed the C-S + CCMS-Lacto diet gained more body weights than layers fed the C-S diet with and without CCMS. Since the B-C-S diets contained more supplemental fat than the C-S diets, the larger body weight gains observed from layers fed B-C-S diets may be partly due to the efficiency of the layers to utilize fat. Golian and Maurice (1992) reported that high fat diets may slow down the rate of passage of digesta through the GI tract allowing more time for utilization of nutrients, however, excessive fat is deposited in the adipose tissue.

Yolk colors of layers fed B-C-S diets were significantly lighter than the yolk color from layers fed the C-S diets. The decrease in yolk color intensity of layers fed the B-C-S diets was a result of the low levels of xanthophylls in these diets as opposed to C-S diets. The calculated levels of xanthophylls in B-C-S diets and the C-S diets were 7.6 and 14.7 mg/kg, respectively.

The chromium oxide concentration in excreta of layers fed C-S (control) and C-S with either CCMS or CCMS-Lacto diets are presented in Figure IV.1. The cumulative chromium oxide concentration in excreta for layers fed the three dietary treatments rose linearly with time to 12 h post feeding and was constant from 12 to 32 h post feeding. The chromium oxide concentration in excreta of layers fed B-C-S (control) and B-C-S with either CCMS or CCMS-Lacto diets are presented in Figure IV.2. The cumulative chromium oxide concentration in excreta of layers fed the three dietary treatments rose linearly with time to 15 h post feeding and was constant from 15 to 32 h post feeding. Two linear regression lines of Y on X with Y-intercepts at 0 and 12 h for each C-S diet and at 0 and 15 h for each B-C-S diet were constructed. The regressions of Y on X were calculated using mean values for 0 to 12 h and 12 to 32 h for C-S diets and 0 to 15 h and 15 to 32 h for B-C-S diets. The slopes of

regression lines for 12 to 32 h and for 15 to 32 h for C-S and B-C-S diets, respectively, were not different and therefore, they were not presented.

The equations of mean cumulative excreta chromium oxide concentration for each dietary treatment (Y) regressed with time after introduction of the chromium oxide marker feed to layers (X) are presented in Table IV.3. These regression equations represent excreta chromium oxide concentrations between 0 and 12 h and 0 and 15 h for C-S and B-C-S diets, respectively. The variations in intercepts of the regression lines are due to marked differences in the amounts and/or types of feeds consumed by the layers. The individual regressions were calculated for each replicate and the resulting coefficients subjected to analysis of variance and separation of means by LSD when there was a significant F-value. The slopes of the regression lines for layers fed either C-S + CCMS or C-S + CCMS-Lacto diets were significantly greater than the slope of the regression line of layers fed the C-S (control) diet. The slopes of the regression lines for layers fed C-S + CCMS, C-S + CCMS-Lacto and B-C-S + CCMS-Lacto diets were not different. However, the slopes of the regression lines for layers fed B-C-S + CCMS-Lacto diet were greater than slopes of the regression lines of layers fed either B-C-S (control) or B-C-S + CCMS diets. The slope of the regression line for layers fed C-S + CCMS-Lacto diet was the same as layers fed either C-S (control) or C-S + CCMS diets. These findings are supported by the suggestion of Dellipiani (1968) that the prevalence of bacteria in the GI tract increases the passage rate of digesta. The slope of the regression line for layers fed C-S (control) diet was significantly greater than that of layers fed B-C-S (control) diet. These differences in slopes imply that the layers fed the C-S diets were voiding excreta at a higher rate than layers fed the B-C-S diets. The difference in rate of passage may be attributed to the B-C-S diets having more supplemental fat (6%) than the C-S

diets (3%). Golian and Maurice (1992) cited evidence that high fat diets slow down the rate of passage of digesta through the GI tract.

Significant grain source x CCMS x Lacto interactions on nutrient retentions were observed; therefore, the data were analyzed for treatment effects. Mean fat, nitrogen, calcium and phosphorus retentions are presented in Table IV.4. Fat retention was higher for layers fed diets containing C-S + CCMS-Lacto and B-C-S + CCMS-Lacto than for layers fed C-S and B-C-S diets with and without CCMS. These findings were consistent with our previous findings (Nahashon *et al.*, 1992, 1993). *Lactobacillus* species have been cited to deconjugate bile acids (Brown, 1977) which may enhance fat absorption.

Nitrogen retentions were higher for either the C-S or the B-C-S diets with CCMS and CCMS-Lacto than respective C-S and B-C-S control diets.

Calcium retention was higher with the C-S + CCMS-Lacto diet than either the C-S diets with and without CCMS or the B-C-S diets. The increase in calcium retention observed with the supplementation of the C-S diet with Lacto was consistent with our previous findings (Nahashon *et al.*, 1992). Calcium retention in layers fed either the C-S + CCMS or C-S + CCMS-Lacto diets was higher than in layers fed B-C-S diets. Since the B-C-S diets had higher level of supplemental fat than the C-S diets, the low calcium retention in layers fed B-C-S diets may be caused partly by the complexing of calcium with the fatty acids and result with increasing the proportion of fecal Ca soaps (Atteh and Leeson, 1983, 1984, 1985; Rising *et al.*, 1990). The fiber content of C-S and B-C-S diets was 2.1 and 3.0%, respectively. The higher level of fiber in B-C-S diets may have also influenced the low calcium retention. Ashmead *et al.* (1985) reported that fiber is responsible for preventing the absorption of minerals such as calcium. Kalsay (1981) hypothesized that fiber may inhibit mineral absorption by forming stable and unabsorbable mineral-fiber complexes, and by directly

altering luminal-to-serosal transport mechanisms in the mucosa. Phosphorus retention was better with B-C-S + CCMS-Lacto diet than either B-C-S or C-S diets with and without CCMS.

There were significant grain source x CCMS x Lacto interactions on nutrient retentions; therefore, the mean comparisons for C-S and B-C-S dietary treatments were made separately. Mean zinc, manganese, copper, iron and magnesium retentions are presented in Table IV.5. The zinc, manganese, copper and magnesium retentions of layers fed C-S (control), C-S + CCMS and C-S + CCMS-Lacto diets were not different. However, iron retention was better for layers fed C-S + CCMS diet compared with the C-S (control) and C-S + CCMS-Lacto diets. Zinc and iron retentions were better for layers fed the B-C-S (control) and B-C-S + CCMS-Lacto diets than B-C-S + CCMS diet. The low retention of zinc and iron by layers fed the CCMS diet may be due to an increase in dietary levels of these elements, much of which was excreted. However, the supplementation of layer diets with CCMS-Lacto increased the utilization of these elements. Manganese retention was better for layers fed B-C-S + CCMS-Lacto diets compared with B-C-S (control) and B-C-S + CCMS diets. The retention of copper by layers was improved when feeding B-C-S + CCMS diets. *Lactobacillus* supplementation to the C-S and B-C-S diets had no effect on copper and magnesium retention by layers. However, supplementation of B-C-S layer diets with CCMS improved copper retention in the layers.

The retentions of zinc, iron and magnesium were significantly better for pullets fed the B-C-S control diet than layers fed the C-S control diet. The improvement in the retention of these elements may be associated with the reduction in the rate of passage of digesta in layers fed the B-C-S diet. Supplementation of CCMS in C-S diet improved zinc and iron retention and in B-C-S diet decreased the retention of these elements. The decrease in

percentage retention of these elements in the B-C-S diet supplemented with CCMS may be due to an increase in dietary levels of the elements, much of which was excreted.

Histological examinations indicated an increased cellularity of Peyer's patches in the ileac sections of the GI tract of layers fed either C-S or B-C-S diets with CCMS-Lacto (Figure IV.3.) than layers fed either C-S + CCMS and B-C-S + CCMS (Figure IV.4.) or the control (Figure IV.5.) diets. The presence of Peyer's patches caused a substantial increase in tissue mass at the base of the villi in layers fed the Lacto diets. Peyer's patches are organized cellular aggregates in lamina propria, generally known as gut associated lymphoid tissue. They are widely distributed tissues and are part of the mucosal immune system that respond to antigenic stimuli primarily by secreting immunoglobulin (IgA) (Bondy and Pestka, 1991). This finding on immunostimulation is supported by the observation of Op den Camp *et al.*, (1985) where they observed that in the presence of a pathogen, the immunological mechanisms of the host were primed to react promptly to the antigens of the pathogen. The improvement in performance of the layers in this experiment may be linked to the immunopotentiation of the gut lining of the layers (Motyka *et al.*, 1993). Naqi *et al.*, (1984) concluded that the intestinal microflora became established in the gut of the bird soon after hatch and the immunopotentiation of the gut associated lymphoid tissue appeared to be an important and complementary process for local resistance in the gut.

Feeding C-S + CCMS-Lacto diet to layers improved egg weight, egg mass, egg size, body weight gains, fat and calcium retention, and feeding B-C-S + CCMS-Lacto diet improved body weight gains, fat, phosphorus and manganese retention and the rate of passage of the digesta in the layers. Feeding B-C-S diets increased egg weight, egg mass, egg size and body weight gains of layers compared to feeding C-S diets. Layers fed C-S and B-C-S diets did not differ in hen-day egg production, feed conversion and daily feed consumption.

However, yolk color was lighter for layers fed B-C-S diets than C-S diets. Feeding Lacto stimulated the mucosal immune system by inducing cellularity of Peyer's patches of the ileums.

Table IV.1. Composition of experimental diets

Ingredients and analyses	Diets					
	C-S[1]	C-S	C-S	B-C-S[2]	B-C-S	B-C-S
	(%)					
Corn (yellow)	58.94	56.94	56.94	30.56	29.76	29.76
Soybean ml (47.5% CP)	22.20	22.20	22.20	22.00	21.60	21.60
Barley (8.7% CP)	5.00	5.00	5.00	30.56	29.76	29.76
Poultry blended fat	3.00	3.00	3.00	6.00	6.00	6.00
Oyster shell (38% Ca)	4.60	4.60	4.60	4.60	4.60	4.60
Limestone flour (37% Ca)	4.00	4.00	4.00	4.00	4.00	4.00
Dicalcium phosphate(16% Ca, 21% P)	1.65	1.65	1.65	1.65	1.65	1.65
Salt	.30	.30	.30	.30	.30	.30
CCMS[3]	----	2.00	----	----	2.00	----
CCMS-Lacto[4] premix (55 g/kg)	----	----	2.00	----	----	2.00
Trace mineral premix[5]	.05	.05	.05	.05	.05	.05
Vitamin premix[6]	.20	.20	.20	.20	.20	.20
D,L methionine (98%)	.06	.06	.06	.08	.08	.08
Calculated analyses						
CP, %	15.3	15.3	15.3	15.3	15.3	15.3
ME, Kcal/kg	2938	2901	2901	2884	2858	2858
Ca, %	3.60	3.60	3.60	3.60	3.60	3.60
Avail. P, %	.45	.45	.45	.45	.45	.45
Total P, %	.65	.65	.65	.65	.65	.65
Met., %	.33	.32	.32	.33	.32	.32
Met. + Cys., %	.61	.61	.61	.60	.59	.59
Linoleic acid, %	1.83	1.79	1.79	2.16	2.13	2.13
Fiber, %	2.12	2.11	2.11	3.09	3.04	3.04
Analyzed levels						
CP, %	15.5	15.4	15.6	15.7	15.3	15.1
Ca, %	3.60	3.60	3.58	3.60	3.56	3.55
Total P, %	.62	.61	.61	.61	.63	.64

[1] Corn-soy. [2] Barley-corn-soy. [3] Condensed cane molasses solubles.

[4] *Lactobacillus*.

[5] Provided per kilogram of diet: manganese, 60 mg; iodine, 1.2 mg; iron, 20 mg; copper, 2 mg; zinc, 20 mg; and cobalt, .2 mg.

[6] Provided per kilogram of diet: vitamin A (retinyl acetate), 3,300 IU; vitamin D_3, 1,100 ICU; dl-α-tocopheryl acetate, 1.10 IU; menadione bisulfite complex, .55 mg; Vitamin B_{12}, 5.5 μg; riboflavin, 3.3 mg; pantothenic acid, 5.5 mg; niacin, 22 mg; choline chloride, 220 mg; folic acid, 220 μg; and ethoxyquin, 62.4 mg.

Table IV.2. Performance variables of Single Comb White Leghorn laying pullets fed corn-soybean meal (C-S) and barley-corn-soybean meal (B-C-S) diets containing condensed cane molasses solubles (CCMS) and *Lactobacillus* (Lacto)-CCMS premix for eight 28-d periods

Dietary grain source, CCMS and Lacto levels			Hen-day egg production	Daily feed consumption	Feed conversion	Egg weight	Egg mass	Egg size ≥Large	Internal egg quality	Egg specific gravity	Body[1] weight gains	Yolk color
Grain source	CCMS (%)	Lacto (ppm)	(%)	(g/hen)	(kg/doz eggs)	(g/egg)	(g/hen/day)	(%)	(HU)[2]	(1.07--)	(g/hen)	
C-S	0	0	88.5[b]	110[a]	1.49[a]	59.0[c]	52.4[c]	86.7[c]	80.3[a]	77[a]	195[c]	9.8[a]
C-S	2	0	89.3[a]	112[a]	1.49[a]	59.3[c]	53.0[c]	85.7[c]	79.8[a]	65[a]	199[c]	9.8[a]
C-S	2	1,100	89.4[a]	112[a]	1.49[a]	60.3[b]	53.9[b]	88.9[b]	79.4[a]	67[a]	247[b]	9.8[a]
B-C-S	0	0	88.7[b]	110[a]	1.50[a]	61.5[a]	54.5[b]	94.8[a]	80.5[a]	65[a]	248[b]	7.5[b]
B-C-S	2	0	90.0[a]	112[a]	1.49[a]	61.7[a]	55.6[a]	93.8[a]	79.2[a]	66[a]	258[b]	7.7[b]
B-C-S	2	1,100	88.9[ab]	111[a]	1.49[a]	61.9[a]	55.0[a]	94.8[a]	79.2[a]	60[a]	312[a]	7.8[b]
Pooled SEM			.7	1.2	.01	.2	.3	.8	.8	.0004	21	.01
Source of variation			----------	----------	----------	Probabilities	----------	----------	----------	----------	----------	----------
Grain source			NS	NS	NS	.001	.001	.001	NS	NS	.03	0.1
CCMS			.03	NS	NS	.001	.001	NS	NS	NS	NS	NS
Lacto			NS	NS	NS	.001	.01	.01	NS	NS	.04	NS
Period			.001	.001	.001	.001	.001	.001	.001	.001	.001	.001

[a,b] Means within columns with no common superscript differ significantly ($P \leq .05$).

[1] Cumulative for eight 28-d periods.

[2] Haugh units.

Table IV.3. Regression equations for rate of passage of digesta through the gastrointestinal (GI) tract of Single Comb White Leghorn laying pullets fed corn-soybean meal (C-S) and barley-corn-soybean meal (B-C-S) diets containing condensed cane molasses solubles (CCMS) and *Lactobacillus* (Lacto)-CCMS premix

Dietary grain source, CCMS and Lacto levels			Regression equations	$(R^2)^1$
Grain source	CCMS %)	Lacto (ppm)		
C-S	0	0	$Y = .053 + .088^{b} X$	.966
C-S	2	0	$Y = .169 + .092^{a} X$	.847
C-S	2	1100	$Y = .152 + .095^{a} X$	.899
B-C-S	0	0	$Y = .019 + .082^{c} X$	.920
B-C-S	2	0	$Y = .050 + .086^{bc} X$	.918
B-C-S	2	1100	$Y = .063 + .097^{a} X$	.957

[1] Coefficients of determination were calculated using 8 degrees of freedom.

[a,b] Means within columns with no common superscript differ significantly ($P \leq .05$).

Table IV.4. Retention of fat (ether extract), nitrogen, calcium, and phosphorus by Single Comb White Leghorn laying pullets fed corn-soybean meal (C-S) and barley-corn-soybean meal (B-C-S) diets containing condensed cane molasses solubles (CCMS) and *Lactobacillus* (Lacto)-CCMS premix for eight 28-d periods

Dietary grain source, CCMS and Lacto levels			Fat	N	Ca	P
Grain source	CCMS (%)	Lacto (ppm)	----------(%)----------			
C-S	0	0	86.1^{bc}	30.0^{b}	29.1^{bc}	19.1^{b}
C-S	2	0	84.3^{c}	41.1^{a}	38.3^{b}	19.7^{b}
C-S	2	1,100	90.1^{a}	42.4^{a}	40.8^{a}	20.4^{b}
B-C-S	0	0	86.7^{bc}	29.8^{b}	23.3^{c}	19.1^{b}
B-C-S	2	0	87.5^{b}	41.3^{a}	28.2^{c}	21.2^{b}
B-C-S	2	1,100	92.3^{a}	44.5^{a}	32.2^{c}	29.4^{a}
Pooled SEM			1.0	3.4	3.6	2.1
Source of variation			----------Probabilities----------			
Grain source			.05	NS	.001	NS
CCMS			NS	.001	NS	NS
Lacto			.001	NS	.05	.05
Grain source x CCMS			.02	.01	.001	.03
Grain source x Lacto			.01	.02	.05	.05

[a,b] Means within columns with no common superscript differ significantly ($P \leq .05$).

Table IV.5. Retention of zinc, manganese, copper, iron, and magnesium by Single Comb White Leghorn laying pullets fed corn-soybean meal (C-S) and barley-corn-soybean meal (B-C-S) diets containing condensed cane molasses solubles (CCMS) and *Lactobacillus* (Lacto)-CCMS premix for eight 28-d periods

Dietary grain source, CCMS and Lacto levels			Zn	Mn	Cu	Fe	Mg
Grain source	CCMS (%)	Lacto (ppm)	(%)				
C-S	0	0	14.7^{c}	7.4^{b}	31.8^{ab}	16.5^{c}	25.8^{c}
C-S	2	0	15.9^{bc}	8.5^{b}	26.5^{ab}	35.6^{a}	28.9^{bc}
C-S	2	1,100	13.8^{c}	8.4^{b}	27.3^{ab}	29.3^{c}	31.9^{abc}
B-C-S	0	0	23.6^{a}	8.2^{b}	24.4^{b}	30.7^{bc}	34.5^{ab}
B-C-S	2	0	7.5^{d}	8.7^{b}	33.5^{a}	24.2^{d}	32.6^{ab}
B-C-S	2	1,100	20.6^{ab}	15.7^{a}	34.1^{a}	34.7^{ab}	35.2^{a}
Pooled SEM			1.4	.7	1.2	1.7	1.1
Source of variation			Probabilities				
Grain source			.02	.001	NS	.01	.01
CCMS			.05	.NS	.05	.03	NS
Lacto			NS	.04	NS	NS	NS
Grain source x CCMS			.001	.001	.01	.001	.05
Grain source x Lacto			.001	.001	.01	.001	.05

[a,b] Means within columns with no common superscript differ significantly ($P \leq .05$).

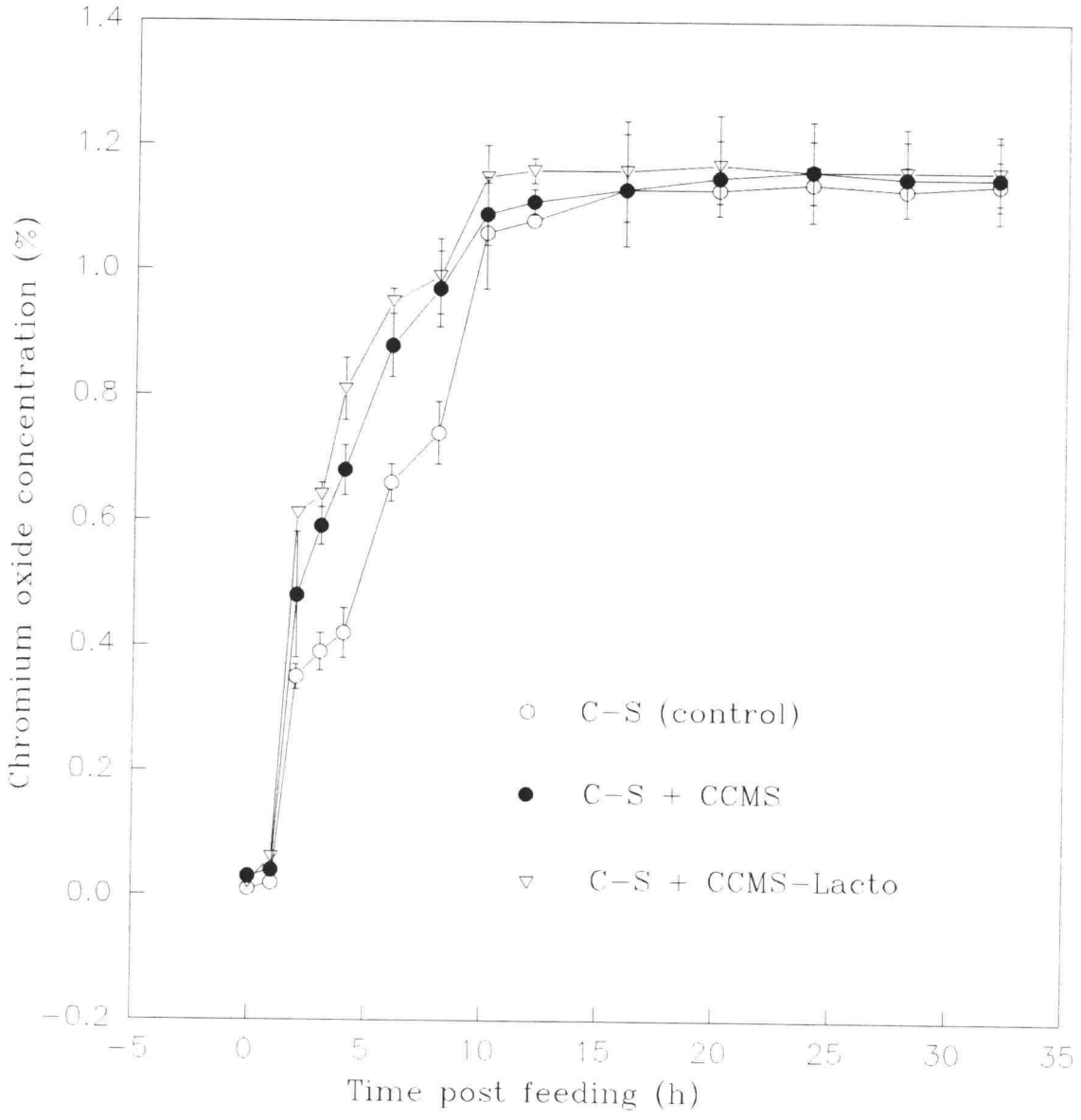

FIGURE IV.1. Chromium oxide concentration in excreta of Single Comb White Leghorn layers fed corn-soybean meal (C-S) diets containing condensed cane molasses solubles (CCMS) and CCMS-*Lactobacillus* premix

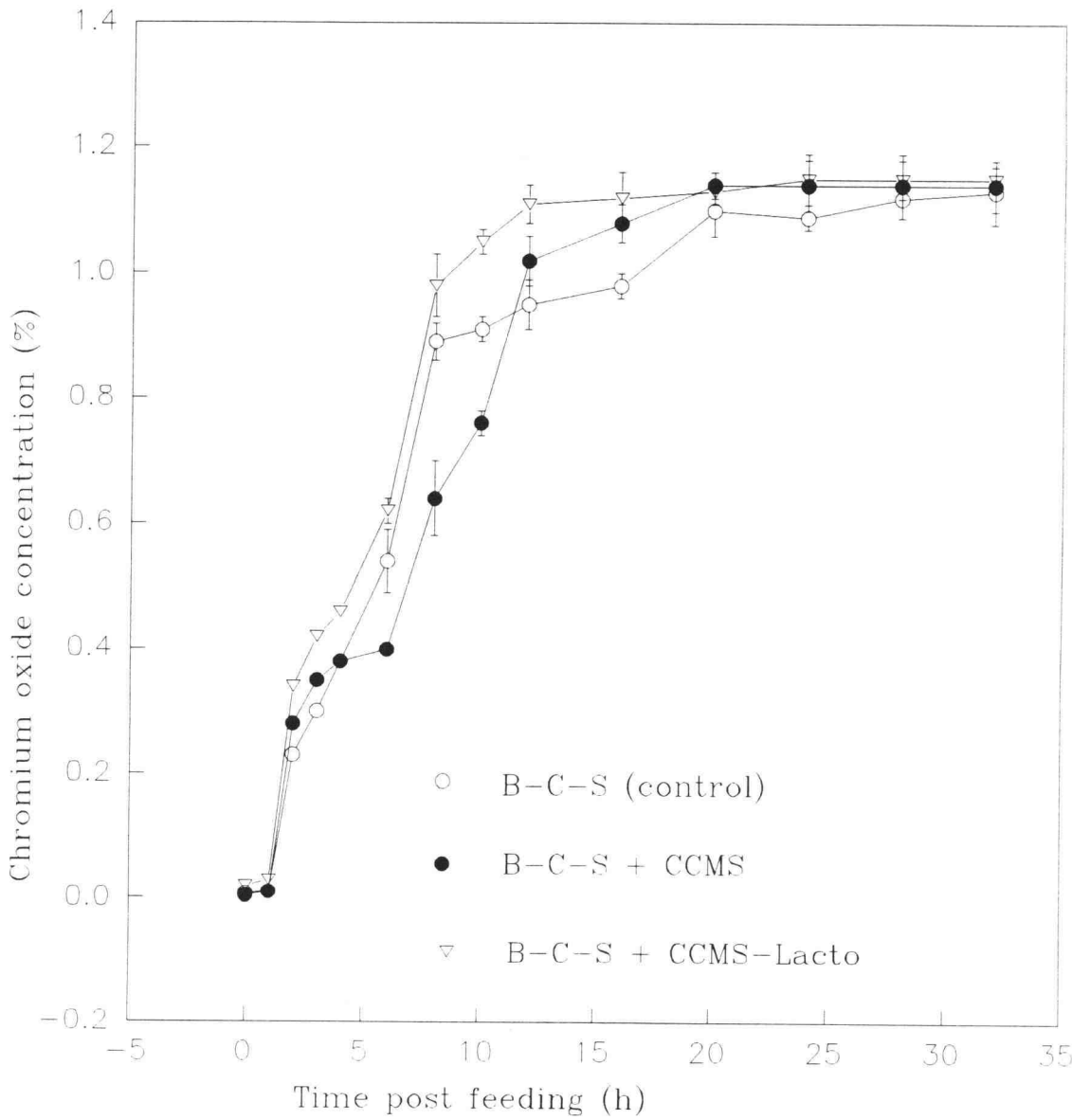

FIGURE IV.2. Chromium oxide concentration in excreta of Single Comb White Leghorn layers fed barley-corn-soybean meal (B-C-S) diets containing condensed cane molasses solubles (CCMS) and CCMS-*Lactobacillus* premix

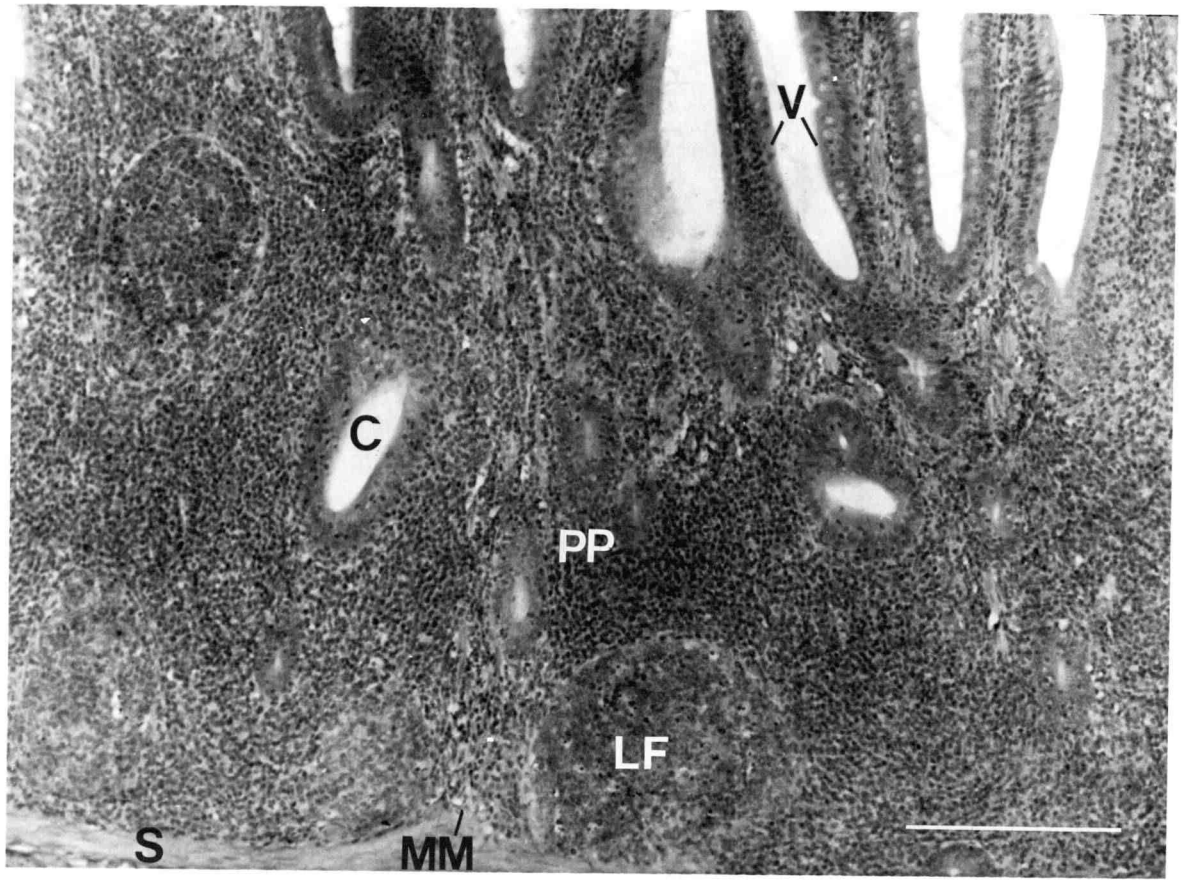

FIGURE IV.3. Photomicrograph of the ileum of Single Comb White Leghorn layers fed corn-soybean meal (C-S) and barley-corn-soybean meal (B-C-S) diets containing condensed cane molasses solubles (CCMS) and CCMS-*Lactobacillus* premix. They illustrate the distribution of lymphoid cells and follicles (LF) which constitute the Peyer's patches (PP) overlying the submucosa (S) and the muscularis mucosae (MM). The villi (V) are reduced and crypts (C) increased in size due to enlargement of the lymphoid tissue. H & E (100x). Bar = .2μ.

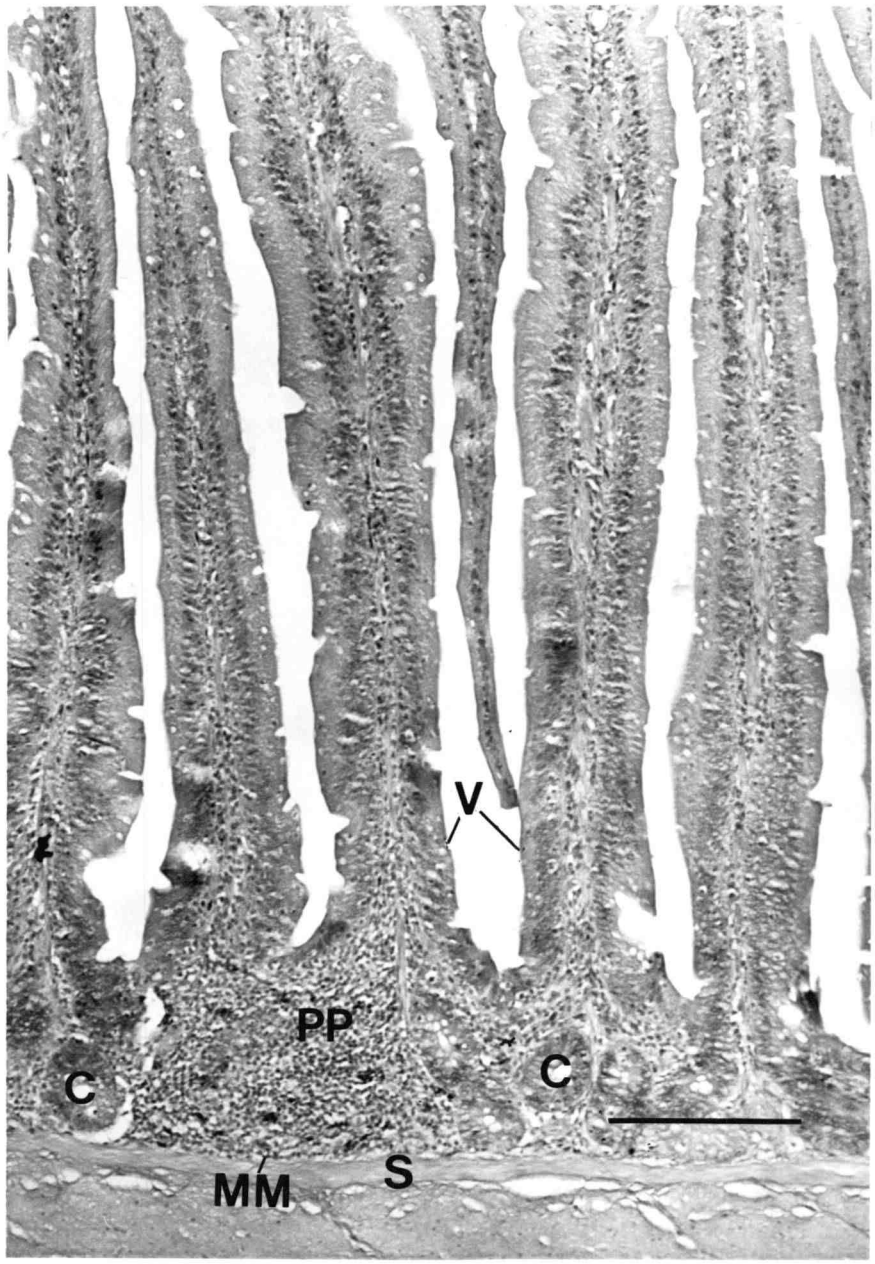

FIGURE IV.4. Photomicrograph of the ileum of Single Comb White Leghorn layers fed corn-soybean meal (C-S) and barley-corn-soybean meal (B-C-S) diets containing condensed cane molasses solubles (CCMS). They illustrate few lymphoid cells constituting a Peyer's patch (PP) overlying the submucosa (S) and the muscularis mucosae (MM). The villi (V) and crypts (C) are not different from the control. H & E (100x). Bar = .2μ.

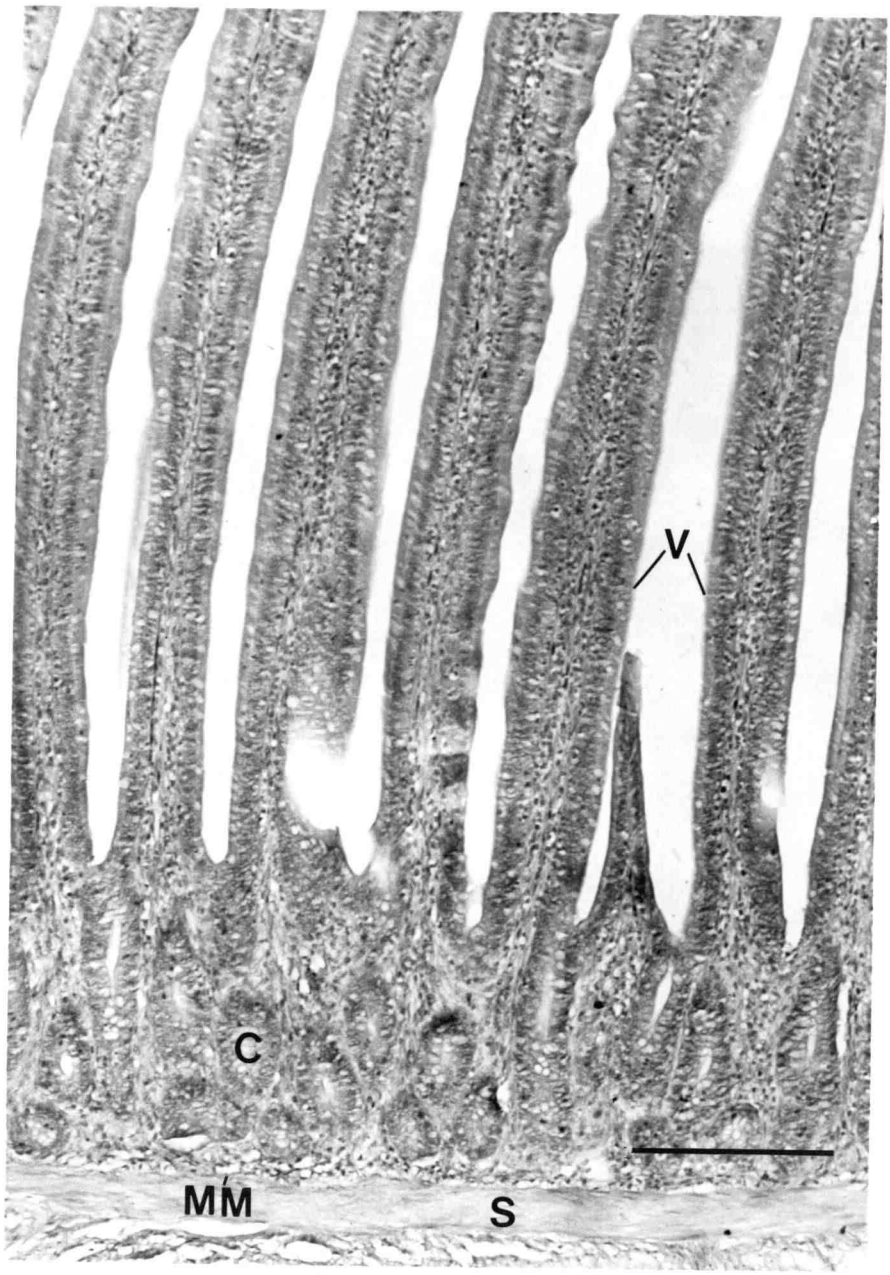

FIGURE IV.5. Photomicrograph of the ileum of Single Comb White Leghorn layers fed corn-soybean meal (C-S) and barley-corn-soybean meal (B-C-S) control diets. They illustrate the distribution of crypts (C) and the villi (V) and the muscularis mucosae (MM) and submucosa (S). H & E (100x). Bar = .2μ.

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CHAPTER V

NUTRIENT RETENTION AND PERFORMANCE OF SINGLE COMB WHITE LEGHORN LAYERS FED A DIRECT-FED MICROBIAL DURING GROWTH AND EGG LAYING PHASES[1]

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ABSTRACT

Dekalb XL Single Comb White Leghorn pullets were fed corn-soybean meal (C-S) diets containing condensed cane molasses solubles (CCMS) or CCMS-1,100 mg *Lactobacillus* (Lacto)/kg diet (ppm) from 7 wk of age (WOA) to 59 WOA to determine the long term effect of feeding Lacto diets on the retention of fat (ether extract), nitrogen, calcium, and phosphorus, layer performance, and anatomical and histological changes of the gastrointestinal (GI) tracts. The pullets were fed three dietary treatments consisting of C-S (control), C-S + CCMS and C-S + CCMS-1,100 ppm Lacto (4.4×10^7 cfu/mg Lacto). Condensed cane molasses served as the carrier for the Lacto, and the CCMS and CCMS-Lacto premix were incorporated at 2% of the diet.

Between 7 and 19 WOA, pullets fed the C-S + CCMS-Lacto diets had better ($P < .05$) daily feed consumption and body weight gains than pullets fed either C-S (control) or C-S + CCMS diets. No differences in shank length were observed among dietary treatments.

During the laying phase (20-59 WOA), nitrogen and Ca retentions were better ($P < .05$) for layers fed the C-S + CCMS-Lacto diet than pullets fed either C-S (control) or C-S + CCMS diets. Fat retentions were not different among dietary treatments. Phosphorus retention was better for pullets fed either C-S + CCMS-Lacto or C-S + CCMS diets than pullets fed C-S (control) diet. Pullets fed the C-S + CCMS-Lacto diets had increased ($P < .05$) daily feed consumption, egg mass, and egg size compared to pullets fed either C-S (control) or C-S + CCMS diets. *Lactobacillus* supplementation to pullet diets increased cellularity of Peyer's patches in the ileum and decreased intestinal weight and length. Positive and significant ($P < .05$) correlations between C-S + CCMS-Lacto diet and nitrogen, retention, calcium retention, daily feed consumption and egg mass were observed.

Feeding a *Lactobacillus* diet to replacement pullets from 7 to 19 WOA improved daily feed consumption and body weight gain. Increased daily feed consumption, egg size, nitrogen and calcium retentions and decreased length and weight of intestines were observed in laying pullets fed *Lactobacillus* diet from 7 to 59 WOA.

(Key words: *Lactobacillus*, direct-fed microbials, pullet performance, nutrient retention, mucosal histology)

INTRODUCTION

Direct-fed microbials (*Lactobacillus species*) benefit the host animal by stimulating appetite (Nahashon *et al.*, 1992, 1993), improve intestinal microbial balance (Fuller, 1989), synthesize vitamins (Coates and Fuller, 1977), and stimulate immune system (Naqi *et al.*, 1984; Op den Camp *et al.*, 1985; Conway and Kjelleberg, 1989). *Lactobacillus species* also produce the digestive enzyme, lactase (Gilliland and Kim, 1984), utilize undigestible carbohydrates (Prins, 1977), stimulate lactic and volatile fatty acids production (Bailey, 1987), produce specific antibacterial compounds such as hydrogen peroxide (Collins and Aramaki, 1980) and compete with other microbes for adhesive site in the gastrointestinal tract of the host (Snoeyenbos et al., 1978; Baba *et al.*, 1991; Dunham *et al.*, 1993).

Developing a profitable pullet is one of most important items in an egg laying operation. The quality and profitability of a laying pullet is determined by its performance beginning from day-old to onset of sexual maturity (18-20 wk of age). Chickens are subjected to a number of stresses such as beak trimming, vaccination, extreme environmental temperatures and/or disease during the growing and laying periods. Most of these stresses reduce feed consumption which may result in poor bird performance. If these stresses could be reduced or minimized, the laying pullet will perform better and become profitable.

Possibly, the stresses may be reduced by supplementing diets of pullets with direct-fed microbials such as *Lactobacillus*. Variability in the results can occur when different forms and concentrations of *Lactobacillus* are supplemented in layer diets. Feeding a liquid, non-viable *Lactobacillus* fermentation product to laying pullets at .26, .52 and .78 ml/kg of feed did not improve feed efficiency, egg weight, hen-day egg production and egg size (Cerniglia *et al.*, 1983). Goodling *et al.* (1987) observed no improvement in hen-day egg production, feed

efficiency, livability and egg size when laying pullets were fed a dried, non-viable *Lactobacillus* product at .25, .5, and .75 g/kg of feed. While the addition of *Lactobacillus acidophilus* at .0125, .0375, and .0625% of the layer diets increased feed consumption and egg size (Miles *et al.*, 1981). Supplementing layer diets with live cultures of *Lactobacillus* at 1,100 mg and 2,200 mg *Lactobacillus*/kg of diet increase feed consumption, egg mass, egg weight and fat, nitrogen, calcium, and phosphorus retentions of the layers (Nahashon *et al.*, 1992).

Since there is no report on the involvement of live cultures of *Lactobacillus* on performance of Single Comb White Leghorn pullets, the objective of this study was to determine the effect of long-term feeding of *Lactobacillus* to pullets from 7 WOA to 19 WOA (growing phase) and 20 WOA to 59 WOA (laying phase) on fat, nitrogen, calcium, and phosphorus retentions, bird performance, and anatomical and histological changes of the gastrointestinal (GI) tracts.

MATERIALS AND METHODS

Three hundred and sixty Dekalb XL Single Comb White Leghorn (SCWL) pullets were raised on commercial starter diets to 6 WOA using standard brooding and rearing methods outlined by North and Bell (1990). At 7 WOA, all pullets were wingbanded for identification and placed on 3 dietary treatments for three 28-d periods.

The dietary compositions and treatments fed from 7 to 19 WOA and 20 to 59 WOA are presented in Tables V.1, and V.2, respectively. The diets were corn-soybean meal (C-S, control), C-S plus CCMS and C-S plus CCMS-1,100 mg *Lactobacillus* per kg feed (ppm) [4.4 x 10^7 cfu/mg Lacto]. The diets were formulated according to NRC (1984) recommendations and fed in mash form. Condensed cane molasses solubles served as the carrier for Lacto and the CCMS and CCMS-Lacto premix were incorporated at 2% of the diet.

During the growing period (7-19 WOA), the dietary treatments were randomly assigned to concrete floor pens which were covered with pine wood shavings litter to a depth of 10 cm. Each pen (240 cm x 150 cm) housed 30 pullets and served as a replicate. Each dietary treatment was replicated four times. The pullets received 8 h of artificial lighting daily while feed and water were supplied for *ad libitum* consumption. Feed was provided in trough feeders, and water was available in bell water fountains (30 cm diameter). Two trough feeders and one water fountain were provided in each pen. Feed consumptions from 7 to 19 WOA were measured at 11, 15 and 19 WOA and body weights and shank lengths were measured at the beginning of the experiment (7 WOA) and at 11, 15 and 19 WOA. Mortality was recorded as it occurred.

At 19 WOA, the pullets were moved to the laying house and placed in laying cages where they were continued on the same dietary treatments using layer feeds as shown in Table

V.2. The laying experiment consisted of ten 28-d periods between 20 WOA and 59 WOA. The diets were randomly assigned to rows each containing 24 individual cages (21 cm wide x 46 cm deep x 46 cm high) with sloping wire floors in a stair-step arrangement per bank. Each cage housed one pullet (966 cm^2 per pullet). Each row of 24 cages served as a dietary replicate and was replicated four times.

The housing, lighting, feeding and watering conditions were similar to those described in Chapter III. Five pullets from each replicate were identified and weighed individually prior to and at the end of the study. Egg production, feed conversion, daily feed consumption, egg weights, egg mass and egg size were determined at the end of each 28-d period using the procedures described in Chapter III. Mortality was recorded as it occurred. Internal egg quality (Haugh units) and egg specific gravity were measured at the end of periods 3, 5, 7 and 9 according to the procedures described in Chapter III.

During the 5th period, ten pullets from each treatment group were fed quantities of the experimental diets with the addition of .3% chromium oxide as a marker. The pullets were fed the marker feed for 7 d followed by 3 d of fecal collection. Fecal samples from each pullet for the 3 d collection were homogenized and dried in an oven at 27 C for 24 h. Feed and excreta samples were ground in a Wiley Mill using a 60 mesh screen. Chromium oxide in feed and excreta were determined by acid digestion and spectrophotometric methods described by Czarnocki *et al.* (1961) and Edwards and Gillis (1959). Feed and excreta nitrogen, fat, calcium and phosphorus levels were determined by the AOAC (1980) procedures described in Chapter III. Percent nutrient retentions were calculated using the formula described by Edwards and Gillis (1959).

At the end of the laying phase, 5 pullets from each dietary treatment were randomly selected and euthanatized for histological examinations. Ten additional pullets from each

dietary treatment were randomly selected and euthanatized for anatomical examinations of the digestive tract. The intestinal samples were excised between the distal end of the gizzard and cloaca and their lengths measured. The intestinal samples were washed with distilled water, blotted with paper towel and weighed. These samples were dried in an oven at 27 C for 48 h after which their dry weights were recorded. Sections of the duodenum, jejunum and ileum were excised and flushed with .08% normal saline prior to fixation in formalin solution for later histological examinations. The jejunum and ileum tissues were obtained from the immediate proximal and distal ends of the Meckel's diverticulum, respectively. The tissues were processed through a standard alcohol dehydration-toluene series, embedded in paraffin wax, sectioned and stained with hematoxylin + eosin dyes, for microscopic examinations.

Statistical Analysis

Percent data (egg production, egg size and nutrient retentions) were transformed to arc sine coefficients prior to analyses. Data were subjected to analysis of variance using the General Linear Model (GLM) procedure of Statistical Analyses Systems (SAS, 1988) with CCMS and Lacto as main effect. All variables were analyzed using repeated measurements with an exception of nutrient retentions. Correlation analyses among treatment effects, performance parameters and the retention of fat (ether extract), nitrogen, calcium, and phosphorus were computed using the GLM procedure.

The mathematical model used for egg production, feed conversion, feed consumption, egg weight, egg mass, egg size, internal egg quality, egg specific gravity, body weight gains and mortality was: $Y_{ijkl} = \mu + C_i + L_j + R_{ijk} + P_l + (CP)_{il} + (LP)_{jl} + \varepsilon_{ijkl}$ where Y_{ijkl}=individual observation; μ= the overall mean, C_i= effect of CCMS, i=1, 2; L_j= the effect of Lacto, j=1, 2; R_{ijk}= the inter experimental unit (rows of cages) error term,

$k=1,..,4$; P_l= the effect of periods, $l=1,..,10$; $(CP)_{il}$ = the interaction between CCMS and periods, $(LP)_{jl}$ = the interaction between Lacto and periods and ε_{ijkl} = the intra experimental unit error term.

The mathematical model used for fat (ether extract), nitrogen, calcium, and phosphorus retentions was: $Y_{ijk} = \mu + C_i + L_j + R_{ijk} + \varepsilon_{ijk}$ where Y_{ijk}=individual observation; μ = the overall mean, C_i= the effect of CCMS, $i=1, 2$; L_j= the effect of Lacto, $j=1, 2$; R_{ijk}= the inter experimental unit (pullets) error term, $k=1,..,5$; and ε_{ijk} = the intra experimental unit error term. When significant ($P \leq .05$) F-values were observed, least significant difference (LSD) comparisons were used between treatment means for the main effects (Steele and Torrie, 1980).

RESULTS AND DISCUSSION

Growing phase

No significant treatments x period interactions were observed for the performance data; therefore, the data were pooled by treatments and periods and analyzed for treatment effects. Mean performance data of SCWL replacement pullets fed C-S diets containing CCMS and CCMS-Lacto are presented in Table V.3. Daily feed consumption of pullets fed the C-S + CCMS-Lacto diet was better than pullets fed either the control or C-S + CCMS diets.

Body weight gains were higher for pullets fed the C-S + CCMS-Lacto diet than the pullets fed either the control or C-S + CCMS diets. Pullets fed the C-S + CCMS diet had depressed body weights compared to pullets fed either the control or C-S + CCMS-Lacto diets. The lower body weight gains of the pullets fed C-S + CCMS diet may be due to the level of CCMS in the diets. The incorporation of 2% CCMS in the diet might be excessive for the young pullets (7-19 WOA). This response was not expected since Scott *et al.* (1982) suggested that molasses may be used at levels up to 4-5% in poultry feeds.

Feed conversions were improved for pullets fed either the control or C-S + CCMS-Lacto diet than pullets fed C-S + CCMS diet; however, feeding C-S + CCMS diet lowered the feed conversion because of decreased body weight gain of the pullets. No differences in shank length gains nor mortality were observed among treatments throughout the growing period.

Laying phase

No significant CCMS x Lacto x period interactions were observed for the performance data; therefore, the data were pooled and analyzed for treatment effects. The mean performance data for SCWL laying pullets fed C-S diets containing CCMS and CCMS-Lacto premix are presented in Table V.4. Hen-day egg production was higher for pullets fed either C-S + CCMS or C-S + CCMS-Lacto diets than pullets fed the C-S (control) diet. Feed conversion was better for pullets fed the C-S + CCMS diet than either the C-S + CCMS-Lacto or the control diets. These data are contrary to the findings of Hargis and Creger (1978) and Krueger *et al.* (1977) and consistent with the findings of Cerniglia *et al.* (1983) and Goodling *et al.* (1987). The failure of *Lactobacillus* to show a significant effect on egg production and feed conversion may be attributed to the ideal conditions in which these pullets were reared. Since *Lactobacilli* become established in the gut of most species of animals soon after birth (Naqi *et al.*, 1984; Savage *et al.*, 1968 and Timms, 1968), *Lactobacillus* supplementation under relatively ideal conditions may not be beneficial (Leeson and Major, 1990).

Daily feed consumption was higher for pullets fed C-S + CCMS-Lacto diet compared to pullets fed either control or C-S + CCMS diets. These data were consistent with our previous findings (Nahashon *et al.*, 1992). However, Goodling *et al.* (1987) and Cerniglia *et al.* (1983) reported contrary results. This difference in findings on feed consumption may be due to the different source (dried viable and non-viable *Lactobacillus* products, and liquid non-viable *Lactobacillus* fermentation products, respectively) and the levels (.25, .5, and .75 g/kg of diet, and .26, .52, and .75 L/kg of diet, respectively) of Lacto used in the latter two reports.

Egg masses were improved for laying pullets fed C-S + CCMS and C-S + CCMS-Lacto diets than for the pullets fed the control diet. However, no difference in egg mass was observed between laying pullets fed either C-S + CCMS or C-S + CCMS-Lacto diets.

Eggs were heavier for pullets fed either the control or the C-S + CCMS-Lacto diets than laying pullets fed the C-S + CCMS diet. No differences in egg weights was observed between the C-S + CCMS-Lacto and the control diets.

Egg sizes were better in pullets fed C-S + CCMS-Lacto diet than in pullets fed either C-S + CCMS or the control diets. These increases in egg size and weight may be associated with the stimulation of appetite in CCMS-Lacto fed pullets. No differences were observed for internal egg quality, egg specific gravity and body weight gains among the dietary treatments. Mortality was not different among the treatments, therefore, data were not presented.

No significant CCMS x Lacto interactions were observed for nutrient retentions; therefore data were pooled and analyzed for treatment effects. The mean fat (ether extract), nitrogen, calcium and phosphorus retention are presented in Table V.5. No differences in fat retentions were observed among the dietary treatments. Nitrogen and calcium retention of pullets fed C-S + CCMS-Lacto diet were higher than the pullets fed either the C-S + CCMS or the control diets. These improvements in nutrient retentions are in agreement with our previous findings and may be associated with the increased appetite of the Lacto-fed pullets (Nahashon *et al.*, 1992). Phosphorus retention was higher for pullets fed the C-S + CCMS diet than pullets fed the control diet. Phosphorus retention for pullets fed the C-S + CCMS-Lacto diet were not different from pullets fed the control diet.

Correlation coefficients between C-S + CCMS-Lacto diet, C-S + CCMS diet and fat (ether extract), nitrogen, calcium and phosphorus retentions and the performance parameters are presented in Table V.6. Positive correlation between the C-S + CCMS-Lacto diet and

nitrogen retention, calcium retention, daily feed consumption and egg mass; between CCMS and egg size, egg specific gravity and body weight gains were observed.

Mean intestinal dry weights and lengths of laying pullets fed the C-S + CCMS and C-S + CCMS-Lacto diets are presented in Table V.7. The intestinal dry weights of pullets fed either C-S + CCMS or C-S + CCMS-Lacto diets were lower than pullets fed the control diet. These findings were not consistent with a previous report (Nahashon *et al.*, 1993). This difference is due to the pullets in this study being fed the Lacto containing diets for a longer period of time than pullets in previous study (Nahashon *et al.*, 1993). No difference in intestinal dry weights were observed between the pullets fed either C-S + CCMS or C-S + CCMS-Lacto diets.

The intestinal length in pullets fed the C-S + CCMS-Lacto diet were shorter than those of pullets fed either C-S + CCMS or the control diets. The shortened intestine and lowered intestinal dry weights of C-S + CCMS-Lacto fed pullets may possibly be due to the interference of Lacto metabolites with growth receptors in the intestines. However, the shortness of the intestines did not interfere with performance of the pullets probably because the length of intestines might not be a factor in nutrient uptake provided there is sufficient absorptive surface area. The health of the gut of the pullets fed diets containing Lacto might have played a major role on performance of these pullets than the length of the intestines.

Histological examinations of the ileum sections of the intestinal wall indicated an increase in cellularity of Peyer's patches in pullets fed C-S plus CCMS-Lacto diet than either C-S + CCMS or the control diet. This finding was consistent with previous finding (Nahashon *et al.*, 1993). The Peyer's patches are part of the mucosal immune system that responds to antigenic stimuli by producing secretory immunoglobulin (IgA). They are organized cellular aggregates in lamina propria and referred to as gut associated lymphoid

tissue. This finding is also supported by the finding of Op den Camp *et al.* (1985) where they observed that in the presence of a pathogen, the immunological mechanisms of the host were primed to react promptly to antigens of the pathogen. The improvement in the performance of the laying pullets in this study may be linked to the immunopotentiation of the gut lining of the pullets. Naqi *et al.* (1984) concluded that the microflora became established in the gut of the bird soon after hatch and the immunopotentiation of the gut associated lymphoid tissue appeared to be an important and complementary process for local resistance in the gut.

Feeding *Lactobacillus* to replacement pullets from 7 to 19 WOA improved daily feed consumption and body weight gains. Increased feed consumption, egg size, nitrogen and calcium retentions, stimulation of the mucosal immune system and decreased length and weight of intestines were observed in laying pullets fed *Lactobacillus* diet from 7 to 59 WOA.

Table V.1. Composition of grower and developer diets fed between 7 and 19 WOA

Ingredients and analyses	Grower (7-14 WOA)			Developer (15-19 WOA)		
	Dietary CCMS[1,2] and Lacto[3,4] levels					
	No CCMS No Lacto	2 No Lacto	2 1100	No CCMS No Lacto	2 No Lacto	2 1100
	(%)					
Corn (yellow)	62.10	61.60	61.60	63.325	61.625	61.625
Soybean ml (47.5 % CP)	18.90	17.40	17.40	15.00	14.70	14.70
Barley (8.7% CP)	12.30	12.30	12.30	15.00	15.00	15.00
Meat & bone meal	6.10	6.10	6.10	6.10	6.10	6.10
CCMS	----	2.00	----	----	2.00	----
CCMS-Lacto premix (55 g/kg)	----	----	2.00	----	----	2.00
Salt	.25	.25	.25	.25	.25	.25
Trace mineral premix[5]	.05	.05	.05	.05	.05	.05
Vitamin premix[6]	.25	.25	.25	.25	.25	.25
Amprolium premix (25%)	.05	.05	.05	.025	.025	.025
Calculated analyses						
CP, %	17.0	17.0	17.0	16.0	16.0	16.0
ME, Kcal/kg	2982	2981	2981	2987	2986	2986
Ca, %	1.00	1.00	1.00	1.00	1.00	1.00
Avail. P, %	.45	.45	.45	.45	.45	.45
Total P, %	.65	.65	.65	.65	.65	.65
Met., %	.29	.30	.30	.35	.35	.35
Met. + Cys., %	.60	.60	.60	.64	.62	.63
Analyzed levels						
CP, %	16.7	16.9	16.8	15.8	16.0	15.9
Ca, %	1.11	.98	.98	1.15	1.12	1.11
Total P, %	.65	.61	.64	.66	.64	.66

[1] Condensed cane molasses solubles. [2] Percent [3] *Lactobacillus*. [4] milligrams per kilogram

[5] Provided per kilogram of diet: manganese, 60 mg; iodine, 1.2 mg; iron, 20 mg; copper, 2 mg; zinc, 20 mg; and cobalt, .2 mg.

[6] Provided per kilogram of diet: vitamin A (retinyl acetate), 3,300 IU; vitamin D_3, 1,100 ICU; dl-α-tocopheryl acetate, 1.10 IU; menadione bisulfite complex, .55 mg; vitamin B_{12}, 5.5 μg; riboflavin, 3.3 mg; pantothenic acid, 5.5 mg; niacin, 22 mg; choline chloride, 220 mg; folic acid, 220 μg; and ethoxyquin, 62.4 mg.

Table V.2. Composition of layer diets fed between 20 and 59 WOA

Ingredients and analyses	Dietary CCMS[1,2] and Lacto[3,4]		
	No CCMS No Lacto	2 No Lacto	2 1100
Corn (yellow)	63.36	62.01	62.01
Soybean ml (47.5% CP)	21.93	21.27	21.27
Barley (8.7% CP)	3.00	3.00	3.00
Poultry blended fat	1.00	1.00	1.00
Oyster shell (38% Ca)	4.48	4.48	4.48
Limestone flour (37% Ca)	4.00	4.00	4.00
Dicalcium phosphate (16% Ca, 21% P)	1.63	1.64	1.64
Salt	0.30	0.30	0.30
CCMS	----	2.00	----
CCMS-Lacto premix (55 g/kg)	----	----	2.00
Trace mineral premix[5]	0.05	0.05	0.05
Vitamin premix[6]	0.20	0.20	0.20
D.L methionine (98%)	0.05	0.05	0.05
Calculated analyses			
CP, %	15.3	15.3	15.3
ME, Kcal/kg	2861	2833	2833
Ca, %	3.60	3.60	3.60
Avail. P, %	0.45	0.45	0.45
Total P,	0.65	0.65	0.65
Met., %	0.32	0.32	0.32
Met. + Cys., %	0.69	0.69	0.69
Analyzed levels			
CP, %	15.5	15.2	15.4
Ca, %	3.55	3.51	3.53
Total P, %	0.62	0.65	0.61

[1] Condensed cane molasses solubles. [2] Percent [3] *Lactobacillus*. [4] Milligrams per kilogram.

[5] Provided per kilogram of diet: manganese, 60 mg; iodine, 1.2 mg; iron, 20 mg; copper, 2 mg; zinc, 20 mg; and cobalt, .2 mg.

[6] Provided per kilogram of diet: vitamin A (retinyl acetate), 3,300 IU; vitamin D_3, 1,100 ICU; dl-α-tocopheryl acetate, 1.10 IU; menadione bisulfite complex, .55 mg; vitamin B_{12}, 5.5 μg; riboflavin, 3.3 mg; pantothenic acid, 5.5 mg; niacin, 22 mg; choline chloride, 220 mg; folic acid, 220 μg; and ethoxyquin, 62.4 mg.

Table V.3. Performance variables of Single Comb White Leghorn pullets fed corn-soybean meal (C-S) diets containing condensed cane molasses solubles (CCMS) and *Lactobacillus* (Lacto)-CCMS premix from 7 to 19 WOA

Dietary CCMS and Lacto levels		Daily feed consumption	Body weight gains	Feed conversion	Shank length gain
CCMS (%)	Lacto (ppm)	(g/pullet)	(g/pullet)	(feed/gain)	(mm/shank)
0	0	57[b]	261[b]	6.96[b]	9.3[a]
2	0	57[b]	234[c]	8.66[a]	9.0[a]
2	1,100	59[a]	272[a]	6.88[b]	9.4[a]
Pooled SEM		.6	3	.14	.2
Source of variation		----------Probabilities----------			
CCMS		NS	.04	.01	NS
Lacto		.03	.001	NS	NS
Periods		.001	.001	.001	.001
CCMS x Periods		NS	NS	NS	NS
Lacto x Periods		NS	NS	NS	NS

[a,b] Means within columns with no common superscript differ significantly ($P \leq .05$).

Table V.4. Performance variables of Single Comb White Leghorn laying pullets fed corn-soybean meal (C-S) diets containing condensed cane molasses solubles (CCMS) and *Lactobacillus* (Lacto)-CCMS premix from 20 to 59 WOA

Dietary CCMS and Lacto levels		Hen-day egg production	Daily feed consumption	Feed conversion	Egg mass	Egg weight	Egg size ≥large	Internal egg quality	Egg specific gravity	Body[1] weight gain
CCMS (%)	Lacto (ppm)	(%)	(g/hen)	(kg/doz eggs)	(g/hen/day)	(g/egg)	(%)	(HU)[2]	(1.0---)	(Kg/hen)
0	0	88.4[b]	118[b]	1.60[a]	53.1[b]	60.2[a]	85.4[b]	85.7[a]	810[a]	1.42[a]
2	0	90.8[a]	119[b]	1.58[b]	54.2[a]	59.8[b]	84.6[b]	85.5[a]	784[a]	1.34[a]
2	1,100	90.5[a]	121[a]	1.63[a]	54.3[a]	60.1[a]	86.4[a]	86.2[a]	804[a]	1.40[a]
Pooled SEM		.3	1	.01	.2	.1	.8	.5	.001	.04
Source of variation		Probabilities								
CCMS		.01	NS	NS	.01	.05	NS	NS	NS	NS
Lacto		NS	.01	.01	NS	NS	.04	NS	NS	NS
Periods		.001	.001	.001	.001	.001	.001	.001	.001	.04
CCMS x Periods		NS	NS	NS	NS	NS	NS	NS	NS	NS
Lacto x Periods		NS	NS	NS	NS	NS	NS	NS	NS	NS

[a,b] Means within columns with no common superscripts differ significantly ($P \leq .05$).

[1] Cumulative for ten 28-d periods (20 to 59 WOA).

[2] Haugh units.

Table V.5. Percent retention of fat (ether extract), nitrogen, calcium, and phosphorus by Single Comb White Leghorn laying pullets fed corn-soybean meal (C-S) diets containing condensed cane molasses solubles (CCMS) and *Lactobacillus* (Lacto)-CCMS premix from 20 to 59 WOA

Dietary CCMS and Lacto levels		Fat	N	Ca	P
CCMS (%)	Lacto (ppm)	----------(%)----------			
0	0	87.9[a]	33.9[b]	45.6[b]	18.2[b]
2	0	87.3[a]	36.6[b]	47.1[b]	21.4[a]
2	1,100	87.6[a]	44.8[a]	54.9[a]	20.3[ab]
Pooled SEM		.8	1.9	1.3	.8
Source of variation		----------Probabilities----------			
CCMS		NS	.01	.03	.05
Lacto		NS	.01	.01	NS

[a,b] Means within columns with no common superscript differ significantly ($P \leq .05$).

Table V.6. Correlation coefficients among condensed cane molasses solubles (CCMS), *Lactobacillus* (Lacto), fat (ether extract), nitrogen, calcium, and phosphorus retentions and performance variables of Single Comb White Leghorn laying pullets fed corn-soybean meal (C-S) diets containing CCMS and CCMS-Lacto premix from 20 to 59 WOA

	Nutrients retained			performance parameters								
Treatments	N	Ca	P	Hen-day egg prod.	Feed/doz eggs	Daily feed cons.	Egg mass	Egg weight	Egg size	Internal egg quality	Egg specific gravity	Body weight gain
Lacto	.64**	.68**	.13	.10	.12	.48*	.59*	-.03	-.32	.26	-.08	.26
CCMS	.46	.43	.39	.23	-.21	.20	.26	.21	.52*	-.11	-.46*	.59*

* $P \leq .05$.

** $P \leq .01$.

Table V.7. Dry weights and lengths of the intestine from Single Comb White Leghorn laying pullets (59 WOA) fed corn-soybean meal (C-S) diets containing condensed cane molasses solubles (CCMS) and *Lactobacillus* (Lacto)-CCMS premix for ten 28-d periods

Dietary CCMS and Lacto levels		Dry weight	Length
CCMS (%)	Lacto (ppm)	(g/kg body weight)	(cm)
0	0	30[a]	139[a]
2	0	14[b]	135[a]
2	1,100	15[b]	129[b]
Pooled SEM		1	1
Probabilities		.01	.01

[a,b] Means within columns with no common superscript differ significantly ($P \leq .05$).

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CHAPTER VI

PHYTASE ACTIVITY, PHOSPHORUS AND CALCIUM RETENTION AND PERFORMANCE OF SINGLE COMB WHITE LEGHORN LAYERS FED DIETS CONTAINING TWO LEVELS OF AVAILABLE PHOSPHORUS AND SUPPLEMENTED WITH A DIRECT-FED MICROBIAL[1]

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ABSTRACT

The presence of phytase activities in condensed cane molasses solubles (CCMS) and CCMS-*Lactobacillus* (Lacto) were determined. With these two sources, an experiment was carried out for nine 28-d periods to determine the effects of feeding Single Comb White Leghorn layers .45% and .25% available phosphorus (AP) diets supplemented with CCMS and CCMS-Lacto on phytase activities of the gastrointestinal (GI) tract contents and intestine, liver and pancreatic tissues, on the GI tract pH, on the phosphorus, and calcium retention and on layer performance. Six dietary treatments were corn-soybean meal (C-S) control, C-S + CCMS and C-S + CCMS-1,100 mg Lacto/kg diet (ppm) [4.4 x 10^7 cfu/mg Lacto] each with .45% and .25% AP. Condensed cane molasses solubles were used as a carrier for the Lacto, and the CCMS-Lacto premix was incorporated at 2% of the diets.

The presence of phytase activity was much higher in the *Lactobacillus* source (CCMS-Lacto premix) than its carrier (CCMS). Phytase activities of the crop contents were higher with the layers fed the CCMS-Lacto diets regardless of the AP level. Intestinal phytase activity was higher in layers fed the .45% AP CCMS-Lacto diet than the unsupplemented .45% AP diets. *Lactobacillus* supplementation did not stimulate phytase activities in the intestinal contents, and liver and pancreatic tissues.

The pH levels of the crop and intestinal contents were much lower for the Lacto-fed layers than the layers fed unsupplemented diets regardless of dietary AP levels. No differences in calcium retentions were observed with Lacto supplementation regardless of the dietary AP levels. However, higher phosphorus retentions were observed with the Lacto supplementation in the .25% AP diet.

Layers fed .45% and .25% AP Lacto-supplemented diets had lower hen-day egg production, lower feed conversion, consumed slightly more feed, had smaller egg mass, and

laid more larger eggs than the layers fed .45% and .25% AP unsupplemented diets. Lacto supplementation to .25% AP diet produced much thicker egg shells than the unsupplemented .45% AP diet, but not different from unsupplemented .25% AP diet. Layers fed the .25% AP diets had lower body weight gains than layers fed the .45% AP diets regardless of Lacto supplementation.

Under the conditions of this experiment, phytase activity was present in the Lacto source and the presence of phytase and Lacto supplementation to a .25% AP diet improved phosphorus retention and layer performance.

(Key words: direct-fed microbials, *Lactobacillus*, layer performance, phosphorus and calcium retention, phytase activities).

INTRODUCTION

Excessive phosphorus levels in lakes, ponds and slow moving waterways can cause "algae bloom" and water pollution. These excessive phosphorus levels could be derived from poultry manure application on the land which is the most common method of disposing poultry solid waste. Because poultry can utilize only one third of the phosphorus contained in feedstuffs of plant origin (Cromwell, 1989a, 1989b), an improvement in the utilization of phytin phosphorus will reduce the cost of adding inorganic phosphorus sources in the feeds, lower the phosphorus excretion in the manure and subsequently reduce the pollution problems. Therefore, any feed supplement that can improve the utilization of phytin phosphorus in poultry will improve the environment and reduce feed costs.

Ullah (1988), and Harland and Frolich (1989) isolated phytase from microbial (*Pseudomonas* and *Bacillus subtilis*) and fungal (*Saccharomyces cerevisiae*) sources, respectively. Extremely low levels of phytase enzyme have been isolated from the brush border region of mammalian small intestine (Cooper and Gowing, 1983). Patterson (1993) and Ravindran *et al.* (1993) observed that the utilization of phytate phosphorus from poultry feeds was increased by 10% when phytase enzyme at a level of 1,000 units/kg of diet was incorporated into the feed. Simons *et al.* (1990) reported an improvement in phosphorus utilization and feed conversion ratio when broilers were fed 1,000 and 1,500 units phytase/kg diet. Nelson *et al.* (1971) observed a significant increase in phosphorus availability in chicks when their diets were fortified with 3-8 g phytase/kg diet. Nahashon *et al.* (1993a, 1993b) reported that phosphorus retention was improved in layers when the diet was supplemented with *Lactobacillus*.

Therefore, the objectives of this study were to determine the phytase activity in a direct-fed microbial source (*Lactobacillus*) and its carrier (condensed cane molasses solubles) and to determine the effect of feeding *Lactobacillus*-condensed cane molasses solubles in laying diets containing .45% and .25% available phosphorus on the phytase activities of gastrointestinal tract contents and intestine, liver and pancreatic tissues; on the status of the gastrointestinal pH; on the phosphorus and calcium retention, and on the production performance of the layers.

MATERIALS AND METHODS

Phytase Activity in CCMS, CCMS-Lacto Premix and Intestinal Contents and Tissues

Phytase activities in CCMS, CCMS-Lacto premix, intestinal contents and tissues were assayed by the method of Simons *et al.* (1990) using a time course of 1 h and expressed as μmol phosphate liberated from phytic acid in 1 min at pH 5.5 and 40 C. The CCMS and CCMS-Lacto premix samples were centrifuged for 10 min at 1,800 g using a refrigerated Beckman TJ-6 centrifuge[2] prior to analysis for free phosphate in the supernatant, which was determined spectrophotometrically by the phosphomolybdic acid method (Fiske and SubbaRow, 1925).

Phytase activity was measured in the crop and intestine of layers at 59 wk of age. Ten layers from each treatment group were randomly selected, individually weighed and euthanatized. The crop and intestinal contents were collected in 100 ml beakers and diluted with 10 ml ice-cold distilled water prior to pH determinations. The crop, intestinal contents, and 2 g each of small intestine, liver and pancreatic tissues were each homogenized in 4 ml ice-cold water using a Polytron® tissue homogenizer[3]. These tissue samples were centrifuged for 10 min at 1,800 g (3,000 rpm) using a refrigerated Beckman TJ-6 centrifuge and the supernatant was assayed for phytase activity immediately or stored at -70 C until assay.

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[2] Palo Alto, California 94304

[3] Brickman Instruments, Inc., Cantiague Road, Westbury, NY 11590

Feeding trial

Dekalb XL Single Comb White Leghorn (SCWL) layers were fed for nine 28-d periods beginning at 22.5 WOA corn-soybean meal (C-S, control), C-S + condensed cane molasses solubles (CCMS) and C-S + CCMS-1,100 mg *Lactobacillus* (Lacto)/kg diet (ppm) [4.4 x 10^7 cfu/mg Lacto] diets each with .45% and .25% available phosphorus (AP). Condensed cane molasses solubles served as a carrier for the Lacto, and the Lacto premix was incorporated at 2% of the diets. The diets were formulated according to NRC (1984) recommendations and fed in mash form. The dietary compositions and treatments are presented in Table VI.1.

Dietary treatments were randomly assigned to rows of 24 individual cages (21 cm wide x 46 cm deep x 46 cm high) with sloping wire floors in a stair-step arrangement per bank. Each cage housed one layer (966 cm^2 per layer). Each row of 24 cages served as a replicate, and each dietary treatments was replicated four times.

The housing, lighting, feeding and watering conditions were similar to those described in Chapter III. Five layers from each replicate were randomly selected, identified and weighed individually prior to and at the end of the study. Mortality was recorded as it occurred. Egg production, egg weights, egg size (jumbo, extra large and large) and egg mass were measured at the end of each study period as previously described in Chapter III. Egg specific gravity and internal egg quality were measured at the end of periods 1, 4, 7 and 9 as described in Chapter III.

During the 4th period, 2 layers were randomly selected from each replicate of each treatment group and fed their respective diets with the addition of .3% chromium oxide marker to permit the determination of phosphorus and calcium retentions. These feeds were fed for 7 d prior to 3 d of excreta collections. After collection, excreta samples were

homogenized and dried at 27 C for 24 h. The excreta samples from each dietary replicate were ground separately in a Wiley mill using a 60 mesh screen. Chromium oxide in feed and excreta were determined by acid digestion and the spectrophotometric methods described by Czarnocki *et al.* (1961) and Edwards and Gillis (1959). Feed and fecal phosphorus and calcium levels were determined using the AOAC (1980) procedures described in Chapter III. Percent nutrient retentions were calculated using the formula described by Edwards and Gillis (1959).

Statistical Analysis

The phytase activities of condensed cane molasses (CCMS) and CCMS-Lacto premix (Y) were regressed with time post incubation (X). The regressions of Y on X were calculated for the CCMS and CCMS-Lacto premix using mean values for 0 to 60 min. When there was a linear relationship, the individual regressions were calculated for each dietary replicate and the resulting coefficients subjected to analysis of variance and means separated by least significant difference (LSD) when there was a significant F-value.

Percentage data (egg production, egg size and phosphorus and calcium retentions) were transformed to arc sine coefficients and units of phytase activities were $\log_{10}$ transformed prior to analysis. Data were subjected to analysis of variance using the General Linear Model (GLM) procedure of Statistical Analyses Systems (SAS, 1988) with CCMS, CCMS-Lacto and available phosphorus (AP) as main effects. All variables were analyzed using repeated measurements with an exception of nutrient retentions and phytase activities.

The statistical model used for feed consumption, feed conversion, egg production, egg weight, egg mass, body weight gains, internal egg quality, egg specific gravity and body weight gains was: $X_{ijklm} = \mu + C_i + L_j + P_k + R_{ijkl} + T_m + (CT)_{im} + (LT)_{jm} + (PT)_{km} +$

$(CP)_{ik}$ + $(LP)_{jk}$ + $(CPT)_{ikm}$ + $(LPT)_{jkm}$ + ε_{ijklm} where X_{ijklm}=individual observation; μ = the overall mean; C_i = the effect of CCMS, i= 1, 2; L_j = the effect of Lacto, j = 1, 2; P_k = the effect of AP, k=1, 2; R_{ijkl}= the inter experimental unit (rows of cages) error term, l= 1,..,4; T_m = the effect of periods, m=1,..,9; $(CT)_{im}$ = the interaction between CCMS and periods; $(LT)_{jm}$ = the interaction between Lacto and periods; $(PT)_{km}$ = the interaction between AP and periods; $(CP)_{ik}$ = the interaction between CCMS and AP; $(LP)_{jk}$ = the interaction between Lacto and AP; $(CPT)_{ikm}$ = the interaction between CCMS, AP and periods; $(LPT)_{jkm}$ = the interaction between Lacto, AP and periods and ε_{ijklm} = the intra experimental unit error term.

The statistical model used for phosphorus and calcium retentions and units of phytase activities was: $X_{ijkl} = \mu + C_i + L_j + P_k + R_{ijkl} + (CP)_{ik} + (LP)_{jk} + \varepsilon_{ijkl}$ where X_{ijkl}=individual observation; μ = the overall mean; C_i = the effect of CCMS, i= 1, 2; L_j = the effect of Lacto, j= 1, 2; P_k = the effect of AP, k=1, 2; R_{ijkl}= the inter experimental unit (layers) error term, l= 1,..,4; $(CP)_{ik}$ = the interaction between CCMS and AP; $(LP)_{jk}$ = the interaction between Lacto and AP; and ε_{ijkl} = the intra experimental unit error term.

When significant ($P \leq .05$) F-values were observed, LSD comparisons were used between treatment means for main effects (Steele and Torrie, 1980).

RESULTS AND DISCUSSION

The phytase activities in the CCMS and CCMS-Lacto premixes determined over 1 h are presented in Figure VI.1. The phytase activities in both CCMS and CCMS-Lacto premix increased linearly with incubation time. The regression coefficients of phytase activities in the CCMS and CCMS-Lacto premix are presented in Table VI.2. The slopes of regression lines for phytase activities in the CCMS-Lacto premix were significantly greater compared to CCMS.

No significant CCMS x Lacto x AP interactions were observed; therefore, the phytase activity, pH, and calcium and phosphorus retention data were analyzed for treatment effects. Mean phytase activities of the crop and intestinal contents, and the intestinal, liver and pancreatic tissues of layers are presented in Table VI.3. The phytase activities of the crop contents for the layers fed .45% AP CCMS-Lacto and .25% AP CCMS-Lacto diets were higher than for layers fed either the .45% AP control and .25% AP control diets with and without CCMS. The phytase activities of the intestinal contents of layers fed .45% AP diets without and with CCMS and CCMS-Lacto were not different. *Lactobacillus* supplementation to both .45% and .25% AP diets did not improve the phytase activity of the intestinal contents. However, layers fed the .25% AP diets had higher intestinal phytase activities than layers fed .45% AP diets. Phytase activities of the intestinal tissues were higher for layers fed .45% AP CCMS-Lacto diets than .45% AP CCMS and .45% AP control diets. Possibly, Lacto supplementation in the layer diets hydrolyzed phytate phosphorus which met the phosphorus requirements of the layers. The phytase activity of the Lacto in the layer diets may have decreased the tissue phytase activities. The phytase activities of the liver from the layers fed either the .45% AP CCMS-Lacto or the .25% AP CCMS-Lacto diets were lower than in layers fed the CCMS and control diets containing either .45% or .25% AP. The liver

and pancreatic phytase activities of the layers fed the .25% AP control diet were higher than the phytase activity of the other treatment groups. These high activities of phytase were expected because layers will compensate for the low dietary AP levels by increasing their tissue phytase activities.

The pH of the crop and intestinal contents of the layers fed the CCMS and CCMS-Lacto with either .45% or .25% AP diets are presented in Figures VI.2 and VI.3, respectively. The pH of the crop contents of the layers fed the .25% AP CCMS-Lacto diet was lower than that of the layers fed the .25% AP CCMS and control diets. The pH of the crop contents for layers fed the .45% AP CCMS-Lacto diet was lower than for layers fed either the .45% AP diets with or without CCMS. The pH of intestinal contents from the layers fed either .45% AP CCMS-Lacto or .25% AP CCMS-Lacto diets were lower than of layers fed either .25% AP CCMS and the .25% AP control or the .45% AP CCMS and .45% AP control diets. These findings are consistent with the report of Bailey (1987) in which *Lactobacillus* species decreased the pH in the GI tract of broilers.

Mean phosphorus and calcium retentions are presented in Table VI.4. Layers fed the .25% AP CCMS-Lacto diet retained more phosphorus than those fed either the .25% AP CCMS or the .45% AP or the .25% AP control diets. The improvement in phosphorus retention by the layers fed either the .45% AP CCMS-Lacto or .25% AP CCMS-Lacto diets may, in part, be due to the phytase activity of Lacto in the layer diets and the decrease in pH of the GI tract of the layers. Ashmead *et al.* (1985) cited evidence that minerals such as phosphorus and calcium salts require very low pH to solubilize. The acidic environment facilitates the ionization of minerals which is essential for absorption, whereas the basic environment complexes the minerals with the OH^- ions and prevent ionization with a resulting hinderance of the absorption of these minerals. The Lacto supplementation in the .25% AP

diets appear to stimulate phosphorus retention than in the .45% AP diets. The addition of less mono-dicalcium phosphate to the .25% AP diet than in the .45% AP diet created an economic advantage of 18.25 cents per 100 kg of diet with Lacto supplementation.

Calcium retentions were higher for layers fed the .25% AP control, the .45% AP CCMS, and the .45% AP CCMS-Lacto diets than the .45% AP control diet. No differences on calcium retention were observed among the .25% AP diets.

No significant CCMS x Lacto x AP x period interactions were observed; therefore, the performance data were pooled over periods and analyzed for treatment effects. Mean performance data from laying layers fed Lacto diets with .45% and .25% AP are presented in Table VI.5. Hen-day egg production of layers fed the .25% AP CCMS diet was higher than of layers fed either the .45% AP CCMS-Lacto or the .25% AP CCMS-Lacto or the .25% AP control diets. Hen-day egg production of layers fed the .45% AP control diet was not different from the layers fed either the .45% AP CCMS or the .25% AP control diets.

Daily feed consumptions were higher for layers fed either the .25% AP CCMS or .25% AP CCMS-Lacto diets than the .25% AP control diet. However, daily feed consumption of layers fed the .45% AP control, the .45% AP CCMS and the .45% AP CCMS-Lacto diets were not different.

Feed conversions were better for layers fed either the .45% or .25% AP control diets than either the .45% AP CCMS or the .45% AP CCMS-Lacto diets. Layers fed the .25% AP CCMS-Lacto diet had better feed conversion than the layers fed the .45% AP CCMS-Lacto diet. The more efficiency in feed conversions of layers fed either the .45% AP CCMS or the .45% AP CCMS-Lacto diets, may be, a result of appetite stimulation, which was consistent with our previous findings (Nahashon *et al.*, 1992, 1993a).

Egg mass and egg weight were better for layers fed the .25% AP CCMS diet than layers fed either the .25% AP CCMS-Lacto or the .45% AP CCMS-Lacto or the .25% AP control diets. Percentage of large eggs laid by layers fed either the .25% AP or the .45% AP diet with and without CCMS and CCMS-Lacto were higher than the .25% AP and .45% AP control diets.

Internal egg quality expressed as Haugh units was better for layers fed either the .45% AP CCMS or the .45% AP CCMS-Lacto diets than either the .25% AP CCMS or the .25% AP CCMS-Lacto diets. No differences were observed in egg quality between the .45% AP control and the .25% AP control diets; between .45% AP CCMS, .45% AP CCMS-Lacto diets and .45% AP control diets; and between .25% AP CCMS, .25% AP CCMS-Lacto and .25% AP control diets.

Egg specific gravity was better for the layers fed the .25% AP control, .25% AP CCMS and .25% AP CCMS-Lacto diets than either the .45% AP control or the .45% AP CCMS diets. No differences in egg specific gravity were observed between layers fed the .45% AP CCMS-Lacto diet and the .25% AP CCMS-Lacto diet. However, egg specific gravity of layers fed the .25% AP control diet was better than that of layers fed .45% AP control diet. No differences were observed among the dietary treatments with .25% AP. The better egg specific gravity of layers fed the .25% AP diets compared to the .45% AP diets may be explained by the increase in phytase activities of the crop and intestinal contents and the intestine, liver and pancreatic tissues of layers fed diets containing .25% AP compared to diets containing .45% AP. Even though the phytase activity in the diets containing .45% AP is higher than .25% AP (see Figures VI.2 and VI.3), the phosphorus available in the .25% AP with or without supplemental Lacto seem to be sufficient for better egg specific gravity than the .45% AP diets.

Body weight gains of layers fed the .45% AP diets with and without CCMS or CCMS-Lacto were not different. Similar observation was noted between the .25% AP diets; however, body weight gains were superior for layers fed the .45% AP diets than for layers fed the .25% AP diets. Possibly, diets containing .25% AP either may not have met the phosphorus required by the layers for bone formation or growth, which caused the low body weight gains. However, bone condition such as cage layer fatigue was not observed during the experimental period. No data were presented for mortality since no differences were observed among treatment groups.

Even though supplementing layer diets with Lacto improved phosphorus retention and the phytase activities of layers, there was no significant improvement in production performance with the exception of egg specific gravity. The lack of effect of Lacto on selected layer production performance in this study may be due to the relatively ideal conditions (1 layer per cage with an area of 966 cm^2 per layer) where layers were housed. In previous studies (Nahashon *et al.*, 1992, 1993b) laying layers were housed in a stressed conditions (2 layers per cage with an area of 483 cm^2 per layer). Leeson and Major (1990) suggested that only under stressful conditions, coliforms increase in number, and direct-fed microbials have measurable benefit.

In this study, layers fed corn-soybean meal (control) diets containing either .45% AP or .25% AP did not differ in hen-day egg production, feed conversion, egg mass, egg weight, egg size and internal egg quality. However, the addition of Lacto into CCMS increased the phytase activity in the CCMS-Lacto premix. Supplementation of the layer diets with the CCMS-Lacto decreased the pH of the GI tracts, increased phytase activity in crop and intestinal contents and in intestinal tissues, and improved egg specific gravity and phosphorus retention in the layers. The phosphorus retention was better for layers fed the diet containing

.25% AP with CCMS-Lacto than .45% AP control diet. Therefore, reducing the level of AP from .45% to .25% of the diet with less mono-dicalcium phosphate addition and supplementing the diets with Lacto lowered the feed cost by 18.25 cents for every 100 kg of feed.

Table VI.1. Composition of experimental diets

Ingredients and analyses	Dietary CCMS[1], Lacto[2,3] and AP[4] levels					
CCMS	0	2	2	0	2	2
Lacto	0	0	1100	0	0	1100
AP	.45	.45	.45	.25	.25	.25
	(%)					
Corn (yellow)	63.30	62.00	62.00	63.80	62.20	62.20
Soybean ml (47.5% CP)	21.93	21.28	21.28	22.00	21.60	21.60
Barley (8.7% CP)	3.00	3.00	3.00	3.00	3.00	3.00
Poultry blended fat	1.00	1.00	1.00	1.00	1.00	1.00
Mono-dicalcium phosphate (16% Ca, 21% P)	1.63	1.64	1.64	.69	.69	.69
Oyster shell (38% Ca)	4.48	4.48	4.48	4.90	4.90	4.90
Limestone flour (37% Ca)	4.06	4.00	4.00	4.00	4.00	4.00
Salt	.30	.30	.30	.30	.30	.30
CCMS	----	2.00	----	----	2.00	----
CCMS-Lacto premix (55g/kg)	----	----	2.00	----	----	2.00
Trace mineral premix[5]	.05	.05	.05	.05	.05	.05
Vitamin premix[6]	.20	.20	.20	.20	.20	.20
D.L methionine (98%)	.05	.05	.05	.06	.06	.06
Calculated analyses						
CP, %	15.3	15.3	15.3	15.3	15.3	15.3
ME, Kcal/kg	2861	2833	2833	2880	2848	2848
Ca, %	3.60	3.60	3.60	3.60	3.60	3.60
Avail. P, %	.45	.45	.45	.25	.25	.25
Total P, %	.69	.69	.69	.69	.69	.69
Met., %	.32	.32	.32	.32	.32	.32
Met. + Cys., %	.69	.69	.69	.69	.69	.69
Analyzed levels						
CP, %	15.5	15.2	15.4	15.2	15.3	15.3
Ca, %	3.55	3.51	3.53	3.55	3.58	3.56
Total P, %	.62	.65	.61	.51	.48	.50

[1] Percent condensed cane molasses solubles. [2] *Lactobacillus*. [3] Milligrams per kilogram.

[4] Percent available phosphorus.

[5] provided per kilogram of diet: manganese, 60 mg; iodine, 1.2 mg; iron, 20 mg; copper, 2 mg; zinc, 20 mg; and cobalt, .2 mg.

[5] provided per kilogram of diet: vitamin A (retinyl acetate), 3,300 IU; vitamin D_3, 1,100 ICU; dl-α-tocopheryl acetate, 1.10 IU; menadione bisulfite complex, .55 mg; vitamin B_{12}, 5.5 μg; riboflavin, 3.3 mg; pantothenic acid, 5.5 mg; niacin, 22 mg; choline chloride, 220 mg; folic acid, 220 μg; and ethoxyquin, 62.4 mg.

Table VI.2. Coefficients of regression lines for phytase activities of condensed cane molasses solubles (CCMS) and CCMS-*Lactobacillus* (Lacto) premix fed in corn-soybean meal (C-S) diets of Single Comb White Leghorn layers for nine 28-d periods

CCMS and Lacto levels		Slope	R^2	df
CCMS (%)	Lacto (ppm)			
2	0	.24[b]	.92	4
2	1,100	.39[a]	.94	4

[a,b] Means within columns with no common superscript differ significantly ($P \leq .05$).

Table VI.3. Phytase activities of crop and intestinal contents, and intestinal, pancreatic and liver tissues of Single Comb White Leghorn layers fed corn-soybean meal (C-S) diets containing .45 and .25% available phosphorus (AP) with condensed cane molasses solubles (CCMS) and *Lactobacillus* (Lacto)-CCMS premix for nine 28-d periods

Dietary CCMS, Lacto and AP levels			contents		Tissues		
			Crop	Intestine	Intestine	Liver	pancreas
CCMS (%)	Lacto (ppm)	AP (%)	phytase activity (μmol pi/mmol phytic acid/h/kg body weight)				
0	0	.45	13[d]	12[c]	372[c]	208[c]	424[b]
2	0	.45	11[d]	13[c]	339[d]	230[b]	187[d]
2	1,100	.45	52[a]	13[c]	428[b]	91[e]	429[b]
0	0	.25	17[c]	26[a]	604[a]	442[a]	446[a]
2	0	.25	12[d]	18[b]	412[b]	229[b]	312[c]
2	1,100	.25	20[b]	22[ab]	601[a]	151[d]	412[b]
Pooled SEM			1	1	2	2	3
Source of variation			Probabilities				
CCMS			NS	NS	NS	.4	.04
Lacto			.03	NS	.02	NS	NS
AP			.05	.05	.01	.3	.03

[a,b] Means within columns with no common superscript differ significantly ($P \leq .05$).

Table VI.4. Percent phosphorus, and calcium retentions by Single Comb White Leghorn layers fed corn-soybean meal (C-S) diets containing .45 and .25% available phosphorus (AP) with condensed cane molasses solubles (CCMS) and *Lactobacillus* (Lacto)-CCMS premix for nine 28-d periods

Dietary CCMS, Lacto and AP levels			P	Ca
CCMS (%)	Lacto (ppm)	AP (%)	----------%----------	
0	0	.45	18.2[c]	46.0[b]
2	0	.45	31.2[a]	54.1[a]
2	1,100	.45	31.8[a]	53.2[a]
0	0	.25	17.8[c]	54.6[a]
2	0	.25	24.3[b]	51.5[ab]
2	1,100	.25	32.4[a]	51.2[ab]
Pooled SEM			1.5	2.3
Source of variation			----------Probabilities---------	
CCMS			.001	.05
Lacto			.009	NS
AP			NS	NS

[a,b] Means within columns with no common superscript differ significantly ($P \leq .05$).

Table VI.5. Performance variables of Single Comb White Leghorn layers fed corn-soybean meal (C-S) diets containing .45 and .25% available phosphorus (AP) with condensed cane molasses solubles (CCMS) and *Lactobacillus* (Lacto)-CCMS premix for nine 28-d periods

Dietary CCMS, Lacto and AP levels			Hen-day egg production	Daily feed consumption	Feed conversion	Egg mass	Egg weight	Egg size ≥large	Internal egg quality	Egg specific gravity	Body[1] weight gains
CCMS (%)	Lacto (ppm)	Av. P (%)	(%)	(g/hen)	(kg/doz eggs)	(g/layer/day)	(g/egg)	(%)	(HU)[2]	(1.0---)	(g/hen)
0	0	.45	88.7^{ab}	119^{a}	1.62^{c}	53.7^{ab}	60.6^{b}	85.4^{c}	86.6^{ab}	810^{d}	493^{a}
2	0	.45	88.0^{abc}	121^{a}	1.65^{ab}	53.7^{ab}	60.9^{ab}	88.2^{ab}	86.8^{a}	815^{cd}	402^{a}
2	1,100	.45	86.9^{c}	120^{a}	1.67^{a}	52.6^{c}	60.5^{b}	88.4^{ab}	86.9^{a}	821^{bc}	449^{a}
0	0	.25	87.1^{bc}	117^{b}	1.61^{c}	52.9^{bc}	60.7^{b}	86.6^{bc}	85.8^{abc}	829^{a}	380^{b}
2	0	.25	88.8^{a}	120^{a}	1.62^{bc}	54.4^{a}	61.2^{a}	89.8^{a}	85.1^{bc}	824^{ab}	386^{b}
2	1,100	.25	86.8^{c}	119^{a}	1.63^{bc}	52.5^{c}	60.5^{b}	89.4^{a}	84.6^{c}	824^{ab}	383^{b}
Pooled SEM			.5	1	.01	.3	.1	.7	.6	.0002	18
Source of variation			Probabilities								
CCMS			NS	.001	.001	NS	NS	.001	NS	NS	NS
Lacto			.01	NS	.01	.001	.01	.05	NS	NS	NS
AP			NS	.001	.003	NS	NS	.04	.002	.001	.001
Period			.001	.001	.001	.001	.001	.001	.001	.001	.001

[a,b] Means within columns with no common superscript differ significantly ($P \leq .05$).

[1] Cumulative for nine 28-d periods.

[2] Haugh units.

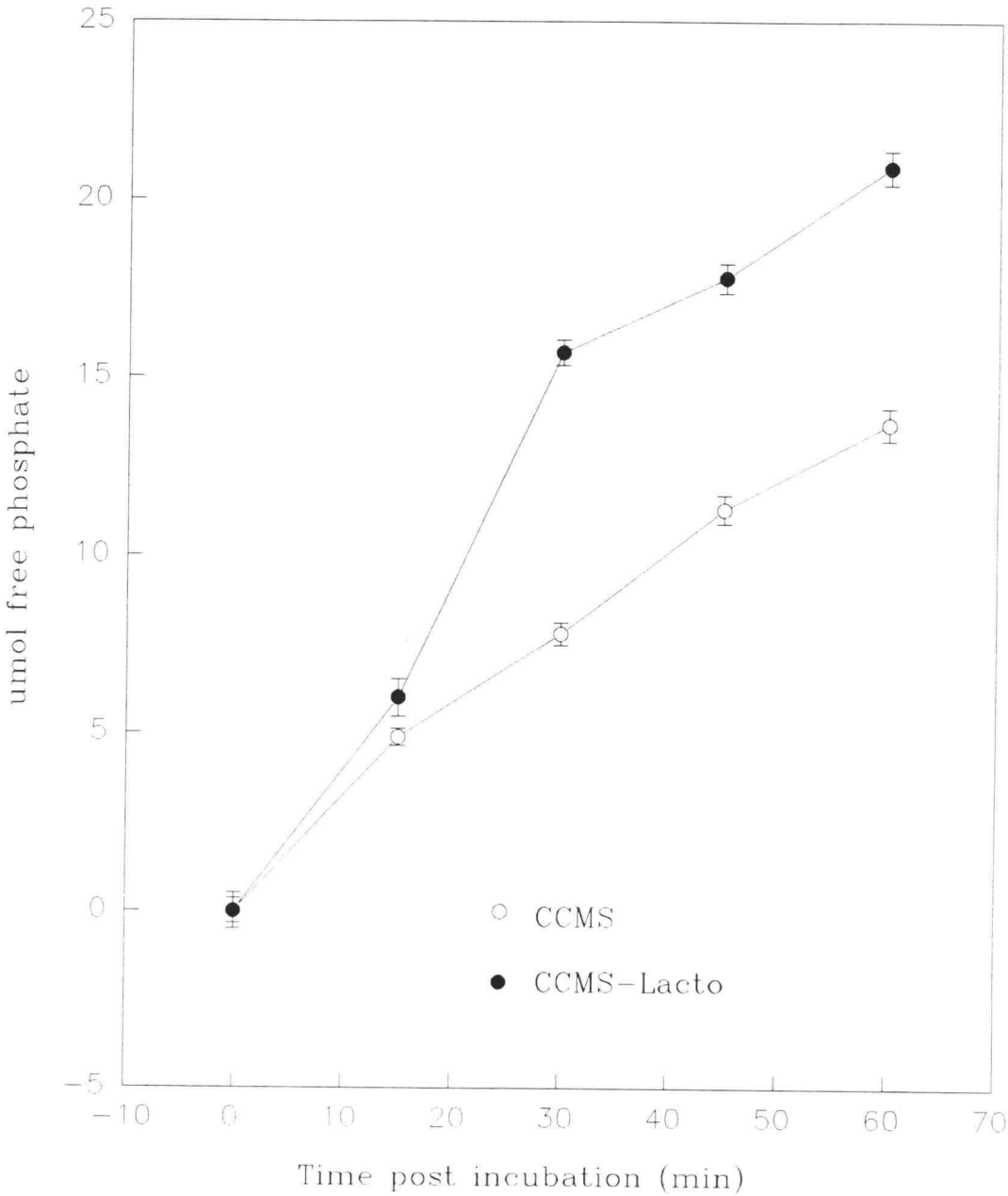

FIGURE VI.1. Phytase activities in condensed cane molasses solubles (CCMS) and CCMS-*Lactobacillus* (Lacto) premix

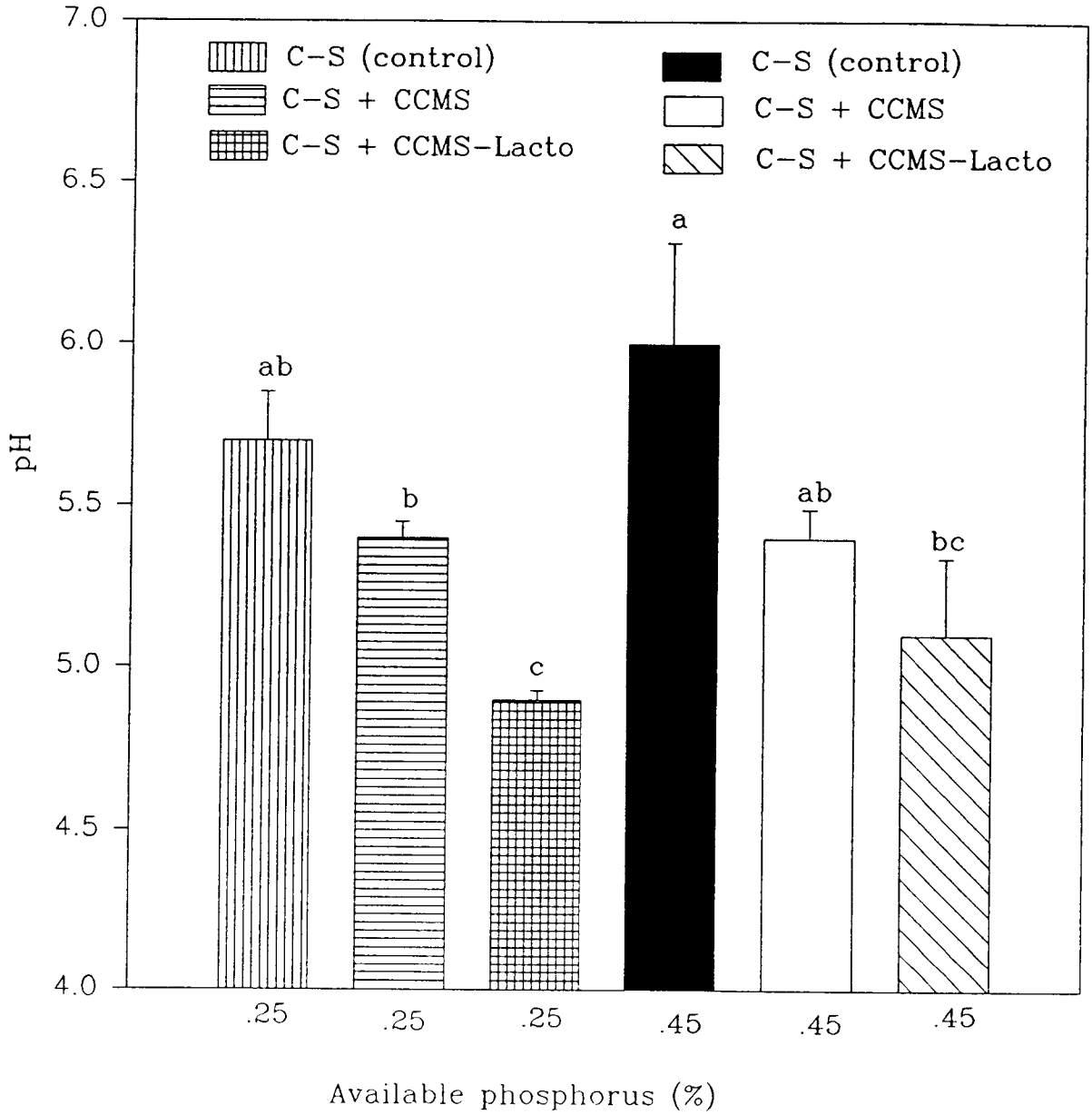

FIGURE VI.2. The pH of crop contents of Single Comb White Leghorn Layers fed corn-soybean meal (C-S) diets containing .25 and .45% available phosphorus (AP) with condensed cane molasses solubles (CCMS) and CCMS-*Lactobacillus* (Lacto) premix for nine 28-d periods. Mean values with no common letters differ significantly ($P \leq .05$).

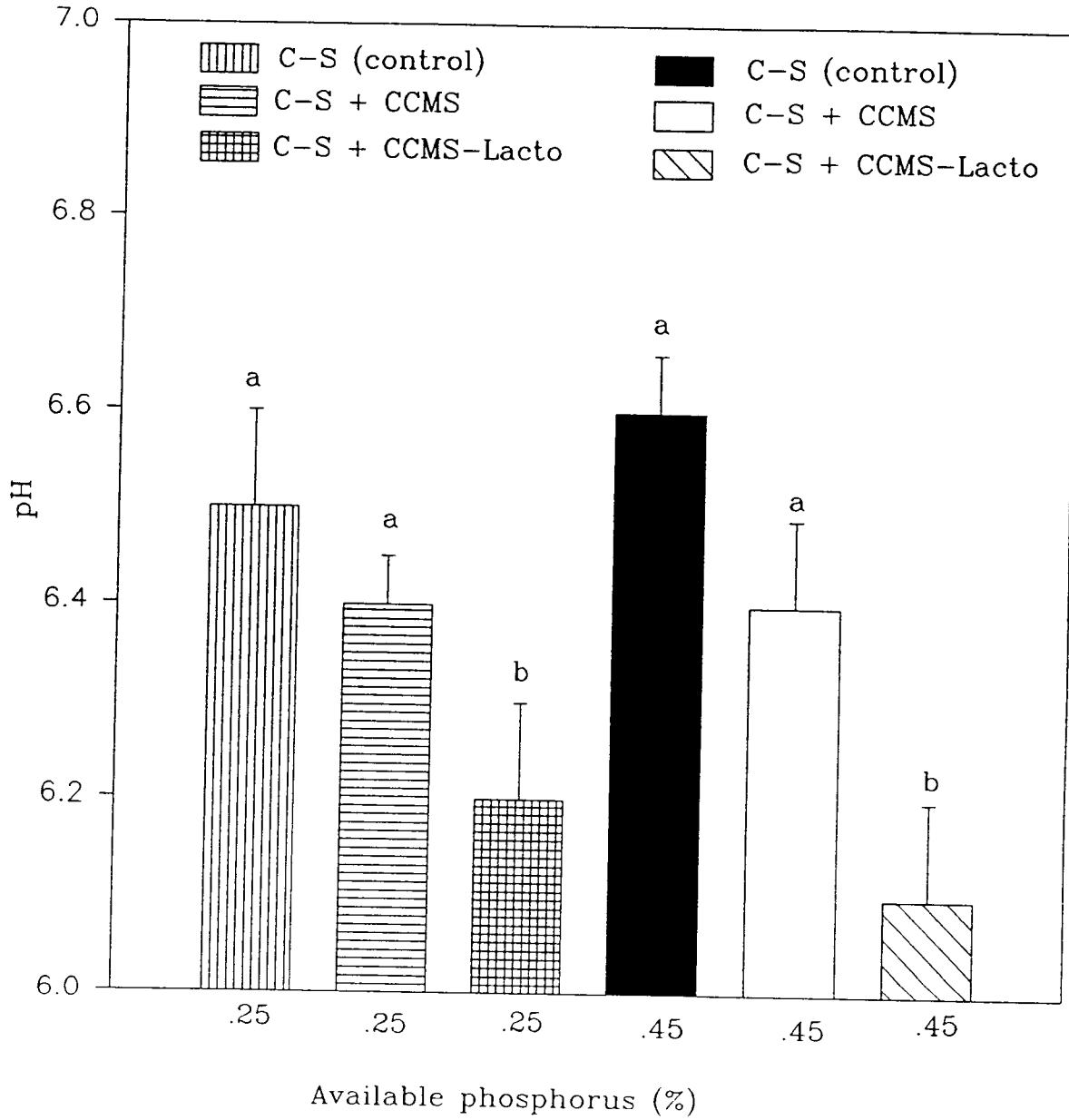

FIGURE VI.3. The pH of intestinal contents of Single Comb White Leghorn layers fed corn-soybean meal (C-S) diets containing .25 and .45% available phosphorus (AP) with condensed cane molasses solubles (CCMS) and CCMS-*Lactobacillus* (Lacto) premix for nine 28-d periods. Mean values with no common letters differ significantly ($P \leq .05$).

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CHAPTER VII

NUTRIENT RETENTION AND PRODUCTION VARIABLES OF SINGLE COMB WHITE LEGHORN LAYERS FED DIETS WITH VARYING CRUDE PROTEIN LEVELS AND SUPPLEMENTED WITH A DIRECT-FED MICROBIAL[1]

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ABSTRACT

A study was performed with Dekalb XL Single Comb White Leghorn layers to determine the effect of feeding 13.8, 14.3 and 15.3% crude protein (CP) diets with 1,100 mg *Lactobacillus* (Lacto)/kg diet (ppm) [4.4 x 10^7 cfu/mg Lacto] on the performance and on the fat (ether extract), nitrogen, calcium, and phosphorus retention of the layers. The dietary treatments were fed for eight 28-d periods and consisted of corn-soybean meal (C-S; control), C-S + condensed cane molasses solubles (CCMS), C-S + CCMS-1,100 ppm Lacto each containing 15.3% CP, C-S + CCMS-1,100 ppm Lacto with 14.3% CP and C-S + CCMS-1,100 ppm Lacto with 13.8% CP. Condensed cane molasses solubles were used as the carrier for the Lacto and the CCMS-Lacto premix was incorporated at 2% of the diet.

Egg masses and egg weights were better for layers fed the 15.3% CP diet with Lacto than without. Egg mass was less and yolk color was increased when layers were fed the 13.8% and the 14.3% CP Lacto diets than the 15.3% CP Lacto diet. No differences in hen-day egg production, feed conversion, daily feed consumption, egg weight, egg size, internal egg quality and egg specific gravity were noticed between the 14.3% CP Lacto and the 15.3% CP Lacto diets; however, there were differences between these parameters from hens fed the 13.8% CP Lacto and the 15.3% CP Lacto diets. Feed consumption and yolk color were higher for layers fed the 13.8% CP Lacto diet than layers fed the 15.3% CP Lacto diet. Lacto supplementation to the 15.3% and the 13.8% CP diets improved fat retention and Lacto supplementation to 14.3% CP diet improved fat and phosphorus retention. Fat, nitrogen, and calcium retentions were not different for layers fed the 15.3, 14.3 or 13.8% CP Lacto diets.

Under the conditions of this study, feeding 13.8, 14.3, and 15.3% CP diets with *Lactobacillus* improved fat and phosphorus retentions and did not affect layer performance.
(Key words: direct-fed microbials, *Lactobacillus*, crude protein, layer performance

INTRODUCTION

Proteinaceous feedstuffs are the most expensive components of poultry rations. Research has been carried out to study the reduction of dietary protein in poultry rations during the laying period (Penz and Jensen, 1991, and Keshavarz and Jackson, 1992). It is, however, difficult to reduce the protein levels of poultry rations without taking into consideration the amino acid balance, which can be the determining factor in layer performance.

Direct-fed microbials may facilitate digestion of complex feed ingredients (Prins, 1977) and improve metabolic efficiency when added into poultry feed. Sung *et al.* (1990) have reported that direct-fed microbials synthesize protein directly and indirectly by deconjugation of the bile salts and produce taurine and glycine which serve as sources of carbon and nitrogen .

The objective of the poultry producer is to increase meat and egg production with minimal cost. The reduction of protein level in the poultry diets and supplementing low protein feeds with direct-fed microbials without adversely effecting layer performance may be one way to reduce production costs.

Because there are no data available on the possible involvement of a direct-fed microbial source (*Lactobacillus*) on the utilization of protein by layers, the objective of this study was to determine the effect of feeding *Lactobacillus* diets containing 13.8, 14.3, and 15.3% crude protein (CP) on the layer performance and on the fat (ether extract), nitrogen, calcium and phosphorus retention.

MATERIALS AND METHODS

Dekalb XL Single Comb White Leghorn (SCWL) layers were fed five dietary treatments for eight 28-d periods beginning at 30.5 wk of age (WOA). The diets consisted of corn-soybean meal (C-S, control), C-S + condensed cane molasses solubles (CCMS), C-S + CCMS-1,100 mg *Lactobacillus* (Lacto)/kg diet (ppm) [4.4 x 10^7 cfu/mg Lacto] each with 15.3% crude protein (CP); C-S + CCMS-1,100 ppm Lacto with 14.3% CP, and C-S + CCMS-1,100 ppm Lacto with 13.8% CP. Condensed cane molasses solubles were used as the carrier for Lacto and the CCMS-Lacto premix was then incorporated at 2% of the diet. Diets were formulated to meet the nutrient requirements of the layers (NRC, 1984) and are presented in Table VII.1.

Dietary treatments were randomly assigned to rows of 24 individual cages [21 cm x 46 cm x 46 cm (966 cm^2 per layer)] with sloping wire floors in a stair-step arrangement per bank. Each row of 24 cages served as a replicate and each dietary treatment was replicated four times. The housing, lighting, feeding and watering conditions were similar to those described in Chapter III.

Five layers from each replicate were randomly selected and weighed individually prior to and at the end of the study. Egg production, egg weights, egg mass and egg size were determined at the end of every 28-d period according to the procedures described in Chapter III. Mortality was recorded as it occurred. Internal egg quality (Haugh units) and egg specific gravity were measured at the end of periods 1, 3, 5 and 8 as described in Chapter III.

During the 6th period, 2 layers from each replicate of each treatment group were randomly selected and fed their respective diets with the addition of .3% inert chromium oxide marker in order to determine fat (ether extract), nitrogen, calcium, and phosphorus retentions. These diets were fed for 7 d prior to 3 d of excreta collection. After collection,

excreta samples were homogenized and dried in an oven at 27 C for 24 h. Excreta samples from each replicate were ground separately in a Wiley mill with a 60 mesh screen. Chromium oxide levels in feed and excreta, and nitrogen, calcium, and phosphorus in the feed and excreta were determined using the procedures described in Chapter III. Fat was extracted using the Soxhlet apparatus (AOAC, 1980). Percent nutrient retentions were calculated using the formula described by Edwards and Gillis (1959).

Statistical Analysis

Percent data (egg production, egg size and nutrient retentions) were transformed to arc sine coefficients prior to analysis. All data were then subjected to analysis of variance with CCMS, Lacto and crude protein as main effects using the General Linear Model (GLM) procedure (SAS, 1988). All variables were analyzed using repeated measures with an exception of nutrient retentions. When significant ($P \leq .05$) F-values were observed, least significant difference (LSD) comparisons were used between treatment means for the main effects (Steele and Torrie, 1980).

RESULTS AND DISCUSSION

No significant CCMS x Lacto x CP x period interactions on the performance parameters were observed; therefore, the data were pooled and analyzed for treatment effects. The performance data are presented in Table VII.2. Hen-day egg production was not different for layers fed the 15.3% CP control diet and the 15.3, 14.3 and 13.8% CP CCMS-Lacto diets. Layers fed the 15.3% CP CCMS-Lacto diet produced more eggs than layers fed the 13.8% CP CCMS-Lacto diet. However, there were no differences in hen-day egg production between layers fed the 14.3% CP CCMS-Lacto and the 13.8% CP CCMS-Lacto diets and between layers fed 15.3% CP CCMS-Lacto and 14.3% CP CCMS-Lacto diets.

Daily feed consumptions were higher for layers fed the 15.3% CP diets with CCMS and CCMS-Lacto and the 14.3% CP CCMS-Lacto diet than the 15.3% CP control diet. Layers fed the 13.8% CP CCMS-Lacto diet consumed more feed than layers fed the 15.3% CP, 15.3% CP CCMS, 15.3% CP CCMS-Lacto and 14.3% CP CCMS-Lacto diets.

Feed conversions were better for layers fed 15.3 and 14.3% CP CCMS-Lacto diets than the 13.8% CP CCMS-Lacto diets. No differences in feed conversions were observed between the 15.3% CP CCMS-Lacto and 14.3% CP CCMS-Lacto diets. The less efficient feed conversion observed with layers fed the 13.8% CP CCMS-Lacto diet was, in part, due to an increase in feed consumption to compensate for the low dietary crude protein levels.

Egg mass was greater for layers fed the 15.3% CP CCMS-Lacto diet than the other dietary treatments while no differences in egg masses were noted between the 13.8% CP CCMS-Lacto and the 14.3% CP CCMS-Lacto diets.

Eggs from layers fed the CCMS-Lacto diets with 15.3% CP and 14.3% CP were heavier than the CCMS-Lacto diet with 13.8% CP. However, no differences in egg weights

were noticed between the 15.3% CP CCMS-Lacto and the 14.3% CP CCMS-Lacto diets and between the 15.3% CP CCMS diet and 15.3% CP diets.

Layers fed the 15.3% CP CCMS-Lacto diet laid larger eggs than those fed the 15.3% CP CCMS and the 13.8% CP CCMS-Lacto diets. No differences in internal egg quality were observed among treatment groups. Layers fed the 15.3%, 14.3% and 13.8% CP diets with CCMS-Lacto had better shell thickness than the 15.3% CP diets without CCMS-Lacto.

Egg yolk color was darker for layers fed the 13.8% CP CCMS-Lacto and the 14.3% CP CCMS-Lacto diets than the 15.3% CP, 15.3% CP CCMS and the 15.3% CP CCMS-Lacto diets. However, no difference was observed between the CCMS-Lacto diets with 13.8% CP and the 14.3% CP. The increase in yolk color intensity in layers fed the 13.8% CP diets may be due to a higher level of corn in this diet and also the increased feed consumption which may have resulted in higher consumption of xanthophylls than the other dietary treatments.

In analyzing nutrient retentions, no significant CCMS x Lacto x CP interactions were observed; therefore, data were pooled and analyzed for treatment effects. The retention of fat, nitrogen, calcium and phosphorus are presented in Table VII.3. Percent fat retentions were better for layers fed the CCMS-Lacto diets with the 15.3% CP, the 14.3% CP and the 13.3% CP diets than the 15.3% CP CCMS and the 15.3% CP diet. No difference in fat retentions were observed between the CCMS-Lacto diets regardless of the protein level.

Retention of nitrogen was improved for layers fed the 15.3% CP CCMS and the 15.3% CCMS-Lacto diets than the 15.3% CP diet. This finding is consistent with previously reported findings (Nahashon *et al.*, 1992, 1993). No differences were detected for the CCMS-Lacto diets regardless of the dietary protein levels.

Percent calcium retention was also improved for layers fed the 15.3% CP CCMS-Lacto diet than the 15.3% CP diet. However, no differences in calcium retention were observed between the 15.3% CP CCMS, the 15.3% CP CCMS-Lacto, 14.3% CP CCMS-Lacto and the 13.8% CP CCMS-Lacto diets. The increased calcium retention for the 15.3% CP CCMS-Lacto diet was in agreement with previous reports (Nahashon *et al.*, 1992, 1993).

Phosphorus retention was better for layers fed the 14.3% CP CCMS-Lacto diet than the 15.3% CP CCMS, the 15.3% CP CCMS-Lacto and the 15.3% CP diets. No differences in phosphorus retention were detected between the 15.3% CP diets and the CCMS-Lacto diets with 13.8% CP and 14.3% CP diets.

Under the conditions of this study, incorporating *Lactobacillus* in diets containing 13.8, 14.3% and 15.3 CP diets improved only fat and phosphorus retentions and did not affect layer performance.

Table VII.1. Composition of experimental diets

Ingredients and analyses	Lacto / CP	Dietary Lacto[1,2] and CP[3] levels				
		No Lacto 15.3	No Lacto 15.3	1100 15.3	1100 14.3	1100 13.8
		(%)				
Corn (yellow)		66.45	64.45	64.45	66.45	68.20
Soybean ml (47.5% CP)		18.50	18.50	18.50	16.50	14.75
Barley (8.7% CP)		5.00	5.00	5.00	5.00	5.00
Limestone flour (37% Ca)		4.00	4.00	4.00	4.00	4.00
Oyster shell (38% Ca)		3.80	3.80	3.80	3.80	3.80
Mono-dicalcium phosphate (16% Ca, 21% P)		1.70	1.70	1.70	1.70	1.70
CCMS[4]		----	2.00	----	----	----
CCMS-Lacto premix (55 g Lacto/kg)		----	----	2.00	2.00	2.00
Salt		.25	.25	.25	.25	.25
Trace mineral premix[5]		.05	.05	.05	.05	.05
Vitamin premix[6]		.20	.20	.20	.20	.20
D,L methionine (98%)		.05	.05	.05	.05	.05
Calculated analyses						
CP, %		15.3	15.3	15.3	14.3	13.8
ME, Kcal/kg		2844	2820	2844	2844	2844
Ca, %		3.60	3.60	3.60	3.60	3.60
Avail. P, %		.40	.40	.40	.40	.40
Total P, %		.65	.65	.65	.65	.65
Met., %		.30	.30	.30	.28	.27
Met + Cys., %		.60	.60	.60	.52	.52
Analyzed levels						
CP, %		15.2	15.3	15.1	14.0	13.7
Ca, %		3.6	3.4	3.3	3.4	3.3
Total P, %		.71	.65	.65	.63	.65

[1] *Lactobacillus*. [2] milligrams per kilogram. [3] Percent crude protein.

[4] Percent condensed cane molasses solubles.

[5] Provided per kilogram of diet: manganese, 60 mg; iodine, 1.2 mg; iron, 20 mg; copper, 2 mg; zinc, 20 mg; and cobalt, .2 mg.

[5] Provided per kilogram of diet: vitamin A (retinyl acetate), 3,300 IU; vitamin D_3, 1,100 ICU; dl-α-tocopheryl acetate, 1.10 IU; menadione bisulfite complex, .55 mg; vitamin B_{12}, 5.5 μg; riboflavin, 3.3 mg; pantothenic acid, 5.5 mg; niacin, 22 mg; choline chloride, 220 mg; folic acid, 220 μg; and ethoxyquin, 62.4 mg.

Table VII.2. Performance variables of Single Comb White Leghorn layers fed corn-soybean meal (C-S) diets containing *Lactobacillus* (Lacto)-condensed cane molasses solubles (CCMS) premix and 15.3, 14.3, and 13.8% crude protein (CP) for eight 28-d periods

Dietary CCMS, Lacto and CP levels			Hen-day egg production	Daily feed consumption	Feed conversion	Egg mass	Egg weight	Egg size ≥large	Internal egg quality	Egg specific gravity	Yolk color
CCMS (%)	Lacto (ppm)	CP (%)	(%)	(g/hen)	(kg/doz eggs)	(g/layer/day)	(g/egg)	(%)	(HU)[1]	(1.07--)	
0	0	15.3	88.6^{ab}	107^{c}	1.48^{b}	52.4^{b}	59.1^{c}	86.5^{ab}	80.5^{a}	77^{a}	9.8^{c}
2	0	15.3	89.5^{ab}	112^{b}	1.48^{b}	53.1^{b}	59.5^{bc}	85.8^{b}	79.6^{a}	66^{b}	9.7^{c}
2	1,100	15.3	89.1^{a}	112^{b}	1.49^{b}	53.9^{a}	60.4^{a}	88.9^{a}	79.3^{a}	67^{b}	9.8^{c}
2	1,100	14.3	88.3^{ab}	112^{b}	1.52^{b}	53.0^{b}	60.0^{a}	86.4^{ab}	80.3^{a}	67^{b}	10.1^{b}
2	1,100	13.8	88.1^{b}	114^{a}	1.57^{a}	52.5^{b}	59.6^{b}	85.1^{b}	80.5^{a}	65^{b}	10.5^{a}
Pooled SEM			1.2	1	.003	.9	.4	2.6	2.1	.0008	.2
Source of variation			Probabilities								
CCMS			NS	.05	NS	NS	NS	NS	NS	.05	NS
Lacto			NS	NS	NS	.05	.05	NS	NS	NS	NS
CP			.05	.01	.03	NS	NS	.05	NS	NS	.04
Period			.001	.001	.001	.001	.001	.001	.001	.001	.001

[a,b] Means within columns with no common superscript differ significantly ($P \leq .05$).

[1] Haugh units.

Table VII.3. Retention of fat (ether extract), nitrogen, calcium, and phosphorus by Single Comb White Leghorn layers fed corn-soybean meal (C-S) diets containing *Lactobacillus* (Lacto)-condensed cane molasses solubles (CCMS) premix and 15.3, 14.3, and 13.8% crude protein (CP) for eight 28-d periods

Dietary CCMS, Lacto and CP levels			Fat	N	Ca	P
CCMS (%)	Lacto (ppm)	CP (%)	----------(%)----------			
0	0	15.3	86.3[b]	30.3[b]	29.2[b]	19.4[c]
2	0	15.3	84.5[b]	41.1[a]	38.1[ab]	19.6[bc]
2	1,100	15.3	90.1[a]	42.4[a]	40.7[a]	20.2[bc]
2	1,100	14.3	90.1[a]	39.0[ab]	32.2[ab]	30.2[a]
2	1,100	13.8	90.2[a]	36.3[ab]	38.2[ab]	27.0[ab]
Pooled SEM			.9	3.4	3.1	2.5
Source of variation			----------Probabilities----------			
CCMS			NS	.05	NS	NS
Lacto			.04	NS	.05	NS
CP			NS	NS	NS	.05

[a,b] Means within columns with no common superscript differ significantly ($P \leq .05$).

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CHAPTER VIII

CONCLUSIONS AND POSTULATIONS

CONCLUSIONS

The observations from the six studies with Single Comb White Leghorn chickens were as follows:

1. Feeding 1,100 and 2,200 ppm Lacto corn-soybean meal (C-S) diets to layers stimulated appetite, improved egg production, egg mass, egg weight, egg size, internal egg quality and nitrogen, calcium and phosphorus retentions. Supplementing the Lacto diets with 1 and 3% fat reduced feed consumption, provided better feed conversion, egg production, egg mass, egg size, body weight gains, nitrogen, calcium and phosphorus retentions. The performance between layers fed either 1,100 or 2,200 ppm Lacto diets were not different.
2. Layers fed 1,100 ppm Lacto barley-corn-soybean meal (B-C-S) diet had improved body weight gains, fat and phosphorus retention and an increase in rate of passage of digesta in layers. Feeding Lacto to layers increased the cellularity of Peyer's patches which may stimulate mucosal immune system by secreting Immunoglobulin IgA.
3. When layers were fed 1,100 ppm Lacto corn-soybean meal diets with 15.3, 14.3 and 13.8% crude protein (CP) levels, and improvements in fat and phosphorus retention were observed regardless of the protein levels. No differences in performance of layers fed the 15.3 and 14.3% CP diets, implying that crude protein may be reduced from 15.3 to 14.3% in layer diets supplemented with 1,100 ppm Lacto without adversely affecting layer performance.

4. Feeding 1,100 ppm Lacto to pullets from 7 to 19 WOA improved daily feed consumption and body weight gains. Increased feed consumption, egg size, nitrogen and calcium retentions, stimulation of the mucosal immune system by increasing cellularity of Peyer's patches, and decreased length and weight of the intestines were observed when the same pullets continued to be fed the Lacto diets during the laying period (20-59 WOA).
5. Phytase activity in the CCMS-Lacto premix was higher than in the Lacto carrier (CCMS). Feeding CCMS-Lacto diets decreased the pH of the GI tracts, increased phytase activities in the diets and GI tract contents and tissues, and improved egg specific gravity and phosphorus retention. Layers fed C-S (control) diets containing .45 and .25% AP were not different in hen-day egg production, feed conversion, egg mass, egg weight, egg size and internal egg quality. Phosphorus retention was better for layers fed diets containing .25% AP with CCMS-Lacto than for layers fed .45% AP control diet.

Feeding *Lactobacillus* to pullets and layers stimulated appetite and improved body weight gains, and stimulated the mucosal immune system by increasing cellularity of Peyer's patches, improved egg production (in Experiment 1 only), egg mass, egg weight, egg size, egg specific gravity, the retentions of fat, nitrogen, calcium, phosphorus and manganese, decreased pH of the GI tract, increased phytase activity of GI crop and intestinal contents and tissues and increased the rate of passage of digesta through the GI tract of layers.

POSTULATIONS

The modes of action of direct-fed microbials in their animal hosts are very controversial. Most of the modes are hypothesized and need further research and confirmation. Direct-fed microbials of *Lactobacillus* (Lacto) *species* have been reported to improve the performance of chickens by enhancing health, growth and efficiency of utilizing feed by the chickens. Watkins and Kratzer (1984) reported that microbial cultures typically containing Lacto were successfully used as alternative to antibiotics. *Lactobacillus* colonizes the gut wall and is responsible for the suppression of pathogenic E. coli (Fuller, 1989; Watkins *et al.*, 1982 and Baba *et al.*, 1993) and *Salmonella* (Dunham *et al.*, 1993). The aggregation of direct-fed microbials in all areas of the GI tract inhibits access to adhesion of other microbes on the GI tract epithelium and thus considered potential inhibitors of microorganisms of the same or other species (Corthier *et al.*, 1985) by blocking receptors by microbial cells of the same or other species.

In this study, Lacto supplementation in layer diets induced mucosal immune system by increasing the cellularity of the peyer's patches, which are aggregates of lymphoid cells that produce immunoglobulin IgA. These proteins are capable of acting as antibodies, and they are the dominant antibodies in the gastrointestinal secretions and fluids bathing the organs and systems in contact with the outside world. They provide a first line of immunologic attack against bacterial invaders established on tissue surfaces. This finding confirms the theory of Naqi *et al.* (1984) that the most provocative mode of action of direct-fed microbials is immunostimulation. There is a possibility that the improvement in egg production, egg mass, egg weight, egg size and fat, nitrogen, calcium, phosphorus retentions were the result of the improved health of the gut by suppression of harmful microbes such as *E. coli* and

Salmonella. The improvement in layer performance may be due to the increased nutrient retention. Lacto supplementation in the layer diets may have improved the retention of other nutrients such as carbohydrates and vitamins, which were not examined in these studies.

Bailey (1987) documented that Lacto produce microbial metabolites such as lactic acid and VFA's (acetic acid in particular), which decreased the pH of the GI tract of broilers. The acidic environment of the GI tract suppresses the growth of harmful microbes which cannot survive the low pH levels. In this study, Lacto supplementation to layer diet decreased the pH of the crop and intestinal contents. The decreased pH of the GI tract was associated with improved calcium, and phosphorus retention by layers. Ashmead *et al.* (1985) cited evidence that minerals such as phosphorus and calcium salts require very low pH to solubilize. It is possible that the improvement in layer production performance was in part due to the decrease in the pH of the GI tract and the improvement in nutrient retentions of the layers.

Lacto have been shown to deconjugate bile acids (Gilliland and Speck, 1977) and this may have improved fat retention in layers fed diets supplemented with *Lactobacillus* in this study. Even though the deconjugated bile acids may be inhibitory to some fecal bacteria *in vitro* (Floch *et al.*, 1972), Lacto species are able to resist these acids better than potential pathogens such as *Clostridium* species and *enterococci*. Sung *et al.* (1990) have reported that Lacto deconjugate bile salts and the amino acids, taurine and glycine, which are products of the microbial bile acid deconjugation. These products serve as sources of nitrogen and carbon in the large intestine of the host animal.

Proteins are involved in the transport of minerals in the animal tissues. Therefore, the integrity of the intestinal mucosa and the turnover of the mucosal cells influences the absorption of nutrients by the animal. The improvement in the utilization of nitrogen may be involved in the enhancement of the retention of mineral elements in the animal tissues. It is,

however, difficult to monitor the utilization of microbial protein unless the Lacto is labelled with a radioactive isotope such as ^{15}N.

Phytase enzymes have been isolated from microbial sources such as *Pseudomonas* and *Bacillus subtilis* (Harland and Frolich, 1989) and from fungal sources such as *Saccharomyces cerevisiae* (Ullah, 1988). Cooper and Gowing (1983) isolated extremely low levels of phytase enzyme in the brush border region of mammalian small intestine. In these studies, the presence of phytase activity in Lacto was observed. The phytase activity in the Lacto source of these studies was associated with an increase in the phytase activities of the crop and intestinal contents and intestinal tissues.

Lactobacillus have also been reported to supply vitamins either from *de novo* synthesis or from sloughing off of the bacterial cells, which may be used by the host animal (Coates and Fuller, 1977). This may be a contributing factor to the improvement in the production performance of the layers fed the diets supplemented with the Lacto.

Although most of the modes of action of Lacto were documented as hypothetical, observations from these studies gave evidence that feeding Lacto stimulated the mucosal immune system of chickens by increasing the cellularity of Peyer's patches. The increase in mucosal immune system improved the health and productive performance of layers by reducing the level of harmful microbes such as *E. coli* and *Salmonella* and by maintaining a balanced intestinal microbial flora. In order to confirm this phenomenon of competitive exclusion of Lacto, the microorganisms of interest must be counted and quantified. The findings in this study confirm previous postulations on the possible involvement of Lacto on immunostimulation.

Lactobacillus may produce bacteriocin which possibly could inhibit the proliferation of other microorganisms in the host GI tract. It is not, however, clear whether these bacteriocin

and the acidic environment created by some Lacto species are selective to other Lacto species. There is also the possibility of inhibition of *Lactobacillus* growth by other microbes in the GI tract of the host. Sometimes, the failure of the layers to respond to Lacto treatment may have been caused by inhibitory factors among the GI tract microbes, including the Lacto because Lacto species differ in properties.

Lactobacillus may be involved in the production of enzymes or enzyme precursors which benefit the host animal with carbohydrate and protein digestion. According to these studies, Lacto produces the phytase enzyme which aids in the solubilization of phytin phosphorus. The free phosphate is utilized by both the Lacto and also the host. The host animal may benefit further by utilizing the Lacto as sources of carbon and nitrogen. It is also possible that these microbes utilize dietary phosphorus, protein and sugars and thus compete with the host animal for these nutrients. The *de novo* vitamin synthesis and bile acid deconjugation improved performance of the layers fed Lacto. To confirm the involvement of these microbes in digestion and competition in utilization of host nutrients, *in vivo* and *in vitro* experiments should be performed using diets and microbes enriched with ^{14}C and ^{15}N isotopes and then determine the incorporation of the isotopes in the layer tissues and microbial cells of interest.

The presence of phytase activity in Lacto and the decreased GI tract pH hydrolyzed the calcium, phosphorus and nitrogen complexes, increasing the retention of these nutrients by the layers. To clearly show that the phytase activity in Lacto is involved in the solubilization of organic phosphorus, however, the performance of layers fed diets without inorganic phosphorus should be evaluated.

Other possible contributions of Lacto in layer performance involve the increase in gastric motility, which was partially confirmed in this study by the increased feed consumption and rate of passage of digesta through the GI tract of the birds fed Lacto.

Based on the results of these studies, *Lactobacillus* seem to benefit layers by improving intestinal health by increasing mucosal immunity, increasing phytase activities in GI tract contents and tissues, decreasing the GI tract pH and as a result increasing nutrient retention and utilization and subsequently improve the performance of the layers.

IX. BIBLIOGRAPHY

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APPENDICES

APPENDICES

The following data provides more information regarding the studies described in previous Chapters. The data presented in Appendices A, B and C are additional data for Chapters III, IV and VI, respectively. The data presented in Appendix A consists of dietary treatments, performance and fat, nitrogen, calcium and phosphorus retentions of Single Comb White Leghorn Laying pullets fed corn-soybean meal (C-S) diets with varying crude protein levels and supplemented with *Lactobacillus* (Lacto) and fat. The data presented in Appendix B is for dry weight and length of the intestine of the laying pullets fed C-S diets supplemented with Lacto. Appendix C presents data on dietary treatments, performance and fat, nitrogen, calcium and phosphorus retentions of laying pullets fed C-S diets containing 1 and 3% fat and Lacto. Appendix D presents data on phytase activities in diets and amylase activities in CCMS, CCMS-Lacto premix, diets, intestine, liver and pancreatic tissues. The data in Appendix D was obtained by feeding Laying pullets .45% and .25% available phosphorus with *Lactobacillus* diets.

APPENDIX A

Table A.1. Composition of experimental diets

Ingredients and analyses	Dietary Lacto[1], fat[2] and CP[3] levels				
Lacto	0	1100	1100	1100	1100
Fat	0	0	3	0	3
CP	15.3	15.3	15.3	13.8	13.8
	(%)				
Corn (yellow)	66.45	64.45	59.55	68.20	64.50
Soybean ml (47.5% CP)	18.50	18.50	20.40	14.75	15.45
Barley (8.7% CP)	5.00	5.00	5.00	5.00	5.00
Poultry blended fat	----	----	3.00	----	3.00
Limestone flour (37% Ca)	4.00	4.00	4.00	4.00	4.00
Oyster shell (38% Ca)	3.80	3.80	3.80	3.80	3.80
CCMS[4]-Lacto premix (55 g Lacto/kg)	----	2.00	2.00	2.00	2.00
Mono-dicalcium phosphate (16% Ca, 21% P)	1.70	1.70	1.70	1.70	1.70
Salt	.25	.25	.25	.25	.25
Trace min. premix[5]	.05	.05	.05	.05	.05
Vitamin premix[6]	.20	.20	.20	.20	.20
D.L methionine (98%)	.05	.05	.05	.05	.05
Calculated analyses					
CP, %	15.3	15.3	15.3	13.8	13.8
ME, Kcal/kg	2855	2819	2947	2857	2998
Ca, %	3.60	3.60	3.60	3.60	3.60
Avail. P, %	.40	.40	.40	.40	.40
Total P, %	.65	.65	.65	.65	.65
Met., %	.30	.30	.30	.28	.27
Met. + Cys., %	.60	.60	.60	.52	.52
Linoleic acid, %	1.30	1.30	1.80	1.30	1.80
Analyzed levels					
CP, %	15.2	15.3	15.1	13.6	13.7
Ca, %	3.60	3.40	3.30	3.40	3.30
Total P, %	.71	.65	.65	.63	.65

[1] *Lactobacillus* (milligrams per kilogram). [2] percent.

[3] Crude protein (%). [4] condensed cane molasses solubles.

[5] Provided per kilogram of diet: manganese, 60 mg; iodine, 1.2 mg; iron, 20 mg; copper, 2 mg; zinc, 20 mg; and cobalt, .2 mg.

[6] Provided per kilogram of diet: vitamin A (retinyl acetate), 3300 IU; Vitamin D_3, 1100 ICU; dl-α-tocopheryl acetate, 1.10 IU; menadione bisulfite complex, .55 mg; vitamin B_{12}, 5.5 μg; riboflavin, 3.3 mg; pantothenic acid, 5.5 mg; niacin, 22 mg; choline chloride, 220 mg; folic acid, 220 μg; and ethoxyquin, 62.4 mg.

Table A.2. Performance and production variables of Single Comb White Leghorn Leghorn layers fed corn-soybean meal (C-S) diets containing *Lactobacillus* (Lacto)-condensed cane molasses solubles (CCMS) premix, 3% fat and 15.3 and 13.8% crude protein (CP) for seven 28-d periods

Dietary Lacto, CP, and fat levels			Hen-day egg production	Daily feed consumption	Feed conversion	Egg mass	Egg weight	Egg size ≥large	Internal egg quality	Egg specific gravity	Body weight gain
Lacto (ppm)	CP (%)	Fat (%)	(%)	(g/layer)	(kg/doz eggs)	(g/layer/day)	(g/egg)	(%)	(HU)[1]	(1.07--)	(g/hen)
0	15.3	0	85.9^{b}	115^{b}	1.61^{cd}	52.7^{bc}	61.3^{ab}	90.5^{ab}	80.4^{a}	72^{a}	255^{b}
1,100	15.3	0	86.8^{ab}	119^{a}	1.65^{b}	53.4^{ab}	61.5^{a}	90.9^{ab}	81.6^{a}	66^{a}	278^{b}
1,100	15.3	3	87.2^{a}	115^{b}	1.58^{d}	53.5^{a}	61.4^{a}	92.2^{a}	80.4^{a}	71^{a}	350^{a}
1,100	13.8	0	84.6^{c}	118^{a}	1.68^{a}	51.3^{d}	60.6^{c}	89.9^{b}	81.0^{a}	66^{a}	238^{b}
1,100	13.8	3	85.7^{bc}	114^{b}	1.61^{cd}	52.2^{c}	60.9^{bc}	91.6^{ab}	81.8^{a}	66^{a}	287^{ab}
Pooled SEM			.6	2	.01	.3	.2	1.0	1.3	.0005	.1
Source of variation			Probabilities								
Lacto			NS	.01	.01	NS	NS	NS	NS	NS	NS
CP			.04	.02	.01	.03	.05	NS	NS	NS	NS
Fat			NS	.01	.04	.05	NS	NS	NS	NS	.04
Period			.001	.001	.001	.001	.001	.001	.001	.001	.001

[a,b] Means within columns with no common superscript differ significantly ($P \leq .05$).

[1] Haugh units.

Table A.3. Retention of fat (ether extract), nitrogen, calcium, and phosphorus by Single Comb White Leghorn layers fed corn-soybean meal (C-S) diets containing *Lactobacillus* (Lacto)-condensed cane molasses solubles (CCMS) premix, 3% fat and 15.3 and 13.8% crude protein (CP) for seven 28-d periods

Dietary Lacto, CP and fat levels			Fat	N	Ca	P
Lacto (ppm)	CP (%)	Fat (%)	----------(%)----------			
0	15.3	0	81.7[c]	38.3[d]	44.1[c]	14.7[d]
1,100	15.3	0	83.2[c]	42.6[c]	60.1[a]	20.6[c]
1,100	15.3	3	91.5[b]	45.6[bc]	52.1[b]	20.2[c]
1,100	13.8	0	90.4[b]	47.1[b]	57.3[ab]	33.7[b]
1,100	13.8	3	94.5[a]	59.1[a]	63.0[a]	42.5[a]
Pooled SEM			.8	1.7	3.8	2.5
Source of variation			----------Probabilities----------			
Lacto			NS	.03	.01	.02
CP			.02	.05	NS	.05
Fat			.04	.03	.04	.05

[a,b] Means within columns with no common superscript differ significantly ($P \leq .05$).

APPENDIX B

Table B.1. Dry weight and length of the intestine of Single Comb White Leghorn laying pullets fed corn-soybean meal (C-S) diets containing condensed cane molasses solubles (CCMS) and *Lactobacillus* (Lacto)-CCMS premix for eight 28-d periods

Dietary CCMS and Lacto levels		Dry weight	Length
CCMS (%)	Lacto (ppm)	(g/kg body weight)	(cm)
0	0	9.1[a]	126[a]
2	0	8.7[a]	124[a]
2	1,100	9.2[a]	125[a]
Pooled SEM		0.7[a]	4.5[a]
Probabilities		NS	NS

[a] Means within columns with common superscript are not different (P > .05).

APPENDIX C

Table C.1. Composition of experimental diets

Ingredients and analyses	Lacto CCMS Fat	Dietary Lacto[1,2], CCMS[3] and fat[4] levels					
		No Lacto No CCMS 1	No Lacto No CCMS 3	No Lacto 2 1	No Lacto 2 3	1100 2 1	1100 2 3
		(%)					
Corn (yellow)		63.30	60.90	62.00	59.00	62.00	59.00
Soybean ml (47.5% CP)		21.93	22.30	21.28	22.21	21.28	22.21
Barley (8.7% CP)		3.00	3.00	3.00	3.00	3.00	3.00
Poultry blended fat		1.00	3.00	1.00	3.00	1.00	3.00
Limestone flour (37% Ca)		4.06	4.00	4.00	4.00	4.00	4.00
Oyster shell (38% Ca)		4.48	4.56	4.48	4.55	4.48	4.55
Mono-dicalcium phosphate (16% Ca, 21% P)		1.63	1.64	1.64	1.64	1.64	1.64
CCMS		----	----	2.00	2.00	----	----
CCMS-Lacto premix (55 g/kg)		----	----	----	----	2.00	2.00
Salt		.30	.30	.30	.30	.30	.30
Trace mineral premix[5]		.05	.05	.05	.05	.05	.05
Vitamin premix[6]		.20	.20	.20	.20	.20	.20
D,L methionine (98%)		.05	.05	.05	.05	.05	.05
Calculated analyses							
CP, %		15.3	15.3	15.3	15.3	15.3	15.3
ME, Kcal/kg		2861	2956	2833	2920	2833	2920
Calcium, %		3.60	3.60	3.60	3.6	3.6	3.6
Avail. P, %		.45	.45	.45	.45	.45	.45
Total P, %		.69	.69	.69	.69	.69	.69
Met., %		.32	.33	.32	.32	.32	.32
Met.+ Cys., %		.61	.62	.61	.61	.61	.61
Linoleic acid, %		1.46	1.85	1.44	1.81	1.44	1.81
Analyzed levels							
CP, %		15.1	15.4	15.3	15.1	15.2	15.1
Ca, %		3.52	3.55	3.54	3.6	3.5	3.6
Total P, %		.64	.62	.64	.66	.63	.66

[1] *Lactobacillus*. [2] milligrams per kilogram [3] Condensed cane molasses solubles (%)

[4] Percent

[5] Provided per kilogram of diet: manganese, 60 mg; iodine, 1.2 mg; iron, 20 mg; copper, 2 mg; zinc, 20 mg; and cobalt, .2 mg.

[6] Provided per kilogram of diet: vitamin A (retinyl acetate), 3,300 IU; vitamin D_3, 1,100 ICU; dl-α-tocopheryl acetate, 1.10 IU; menadione bisulfite complex, .55 mg; vitamin B_{12}, 5.5 μg; riboflavin, 3.3 mg; pantothenic acid, 5.5 mg; niacin, 22 mg; choline chloride, 220 mg; folic acid, 220 μg; and ethoxyquin, 62.4 mg.

Table C.2. Performance variables of Single Comb White Leghorn layers fed corn-soybean meal (C-S) diets containing 1,100 ppm *Lactobacillus* (Lacto)-Condensed cane molasses solubles (CCMS) premix and 1 and 3% fat for nine 28-d periods

Dietary CCMS, Lacto and fat levels			Hen-day egg production	Daily feed consumption	Feed conversion	Egg mass	Egg weight	Egg Size ≥medium	Internal egg quality	Egg specific gravity	Body weight gain
CCMS (%)	Lacto (ppm)	Fat (%)	(%)	(g/hen)	(kg/doz eggs)	(g/hen/day)	(g/egg)	(%)	(HU)[1]	(1.0---)	(g/hen)
0	0	1	88.7[a]	119a	1.60[b]	53.5[ab]	60.6[bc]	85.5[C]	86.6[a]	810[c]	350[a]
0	0	3	86.8[b]	114c	1.58[b]	52.4[c]	60.3[c]	88.9[ab]	86.2[a]	817[ab]	340[a]
2	0	1	88.0[ab]	120a	1.65[a]	53.7[ab]	60.9[b]	88.2[b]	86.8[a]	815[abc]	350[a]
2	0	3	87.9[ab]	117b	1.61[b]	54.2[a]	61.5[a]	90.9[a]	86.7[a]	815[abc]	300[a]
2	1,100	1	86.9[ab]	120a	1.67[a]	52.6[bc]	60.5[bc]	88.4[b]	86.9[a]	821[a]	340[a]
2	1,100	3	87.9[ab]	116b	1.60[b]	53.4[abc]	60.8[b]	89.0[ab]	85.8[a]	810[bc]	330[a]
pooled SEM			.6	.43	.01	.35	.07	.60	.35	.0002	21
Source of variation			----------	----------	----------	Probabilities	----------	----------	----------	----------	
CMS			NS	.001	.001	.02	.001	.001	NS	NS	NS
Lacto			NS	NS	NS	.02	.001	NS	NS	NS	NS
Fat			NS	.001	.001	NS	NS	.001	NS	NS	NS
Period			.001	.001	.001	.001	.001	.001	.001	.001	.005

[a,b] Mean values within columns with no common superscript differ significantly ($P \leq .05$).

[1] HU: Haugh units.

Table C.3. Retention of fat (ether extract), nitrogen, calcium, and phosphorus by Single Comb White Leghorn laying pullets fed corn-soybean meal (C-S) diets containing *Lactobacillus* (Lacto)-condensed cane molasses solubles (CCMS) premix and 1 and 3% fat for nine 28-d periods

Dietary CCMS, Lacto and fat			Fat	N	Ca	P
CCMS (%)	Lacto (ppm)	Fat (%)	----------(%)----------			
0	0	1	85.5^{c}	39.7^{a}	46.0^{bc}	18.2^{b}
0	0	3	90.7^{ab}	42.0^{a}	43.4^{c}	21.0^{b}
2	0	1	87.7^{abc}	41.1^{a}	50.3^{b}	31.2^{a}
2	0	3	92.0^{a}	39.8^{a}	48.8^{b}	30.1^{a}
2	1,100	1	86.6^{bc}	41.5^{a}	53.2^{a}	31.8^{a}
2	1,100	3	90.5^{ab}	44.6^{a}	42.0^{c}	31.5^{a}
Pooled SEM			1.6	2.0	1.5	1.3
Source of variation			----------Probabilities----------			
CCMS-Lacto			NS	NS	.004	.001
Fat			.009	NS	.001	NS

[a,b] Mean values with no common superscript differ significantly ($P \leq .05$).

APPENDIX D

Phytase and amylase activities in the CCMS, CCMS-Lacto premix and diets, intestine, liver and pancreas

Phytase activities were assayed according to the procedures described in Chapter VI. Amylase activities were assayed by the method of Bernfeld (1955) as modified by Gertler and Nitsan (1970). Reactions were terminated at 2 min intervals beginning at time 0 and ending at time 12 min. One unit of amylase activity was the change of 10^{-3} absorbance at 540 nm due to liberation of reducing groups from a solution of 1% starch after 2 min incubation.

The phytase activities of .25% and .45% AP diets with and without CCMS-Lacto are presented in Figures D.1, and D.2, respectively. Amylase activities of CCMS, CCMS-Lacto and .25% and .45% AP diets with and without CCMS-Lacto are presented in Figures D.3, D.4, and D.5. Table D.1 presents the amylase activities of the intestinal, liver and pancreatic tissues of Single Comb White Leghorn layers fed .25% and .45% AP diets supplemented with CCMS and CCMS-Lacto. The percentage retention of fat and nitrogen are presented in Table D.2.

Table D.1. Amylase activities of crop and intestinal contents, and intestinal and pancreatic tissues of Single Comb White Leghorn laying pullets fed corn-soybean meal (C-S) diets containing .45 and .25% available phosphorus (AP) with condensed cane molasses solubles (CCMS) and CCMS-*Lactobacillus* (Lacto) premix for nine 28-d periods

Dietary CCMS, Lacto and AP levels			Contents		Tissues	
			Crop	Intestine	Intestine	Pancreas
CCMS (%)	Lacto (ppm)	AP (%)	--------change of .001 absorbance/5 min---------			
0	0	.45	88^{b}	79^{e}	25^{b}	25^{a}
2	0	.45	23^{d}	94^{d}	14^{c}	22^{a}
2	1,100	.45	163^{a}	174^{a}	12^{c}	25^{a}
0	0	.25	19^{de}	83^{de}	21^{b}	24^{a}
2	0	.25	15^{e}	143^{c}	72^{a}	23^{a}
2	1,100	.25	53^{c}	156^{b}	74^{a}	25^{a}
Pooled SEM			5.3	4.1	2.6	3.2
Source of variation			----------Probabilities----------			
CCMS			NS	.03	.05	NS
Lacto			.01	.01	NS	NS
AP			.05	.05	.03	NS

[a,b] Means within columns with no common superscript differ significantly ($P \leq .05$).

Table D.2. Percent Fat (ether extract), and nitrogen retentions by Single Comb White Leghorn layers fed corn-soybean meal (C-S) diets containing .45 and .25% available phosphorus (AP) with condensed cane molasses solubles (CCMS) and CCMS-*Lactobacillus* (Lacto) premix for nine 28-d periods

Dietary CCMS, Lacto and AP levels			Fat	N
CCMS (%)	Lacto (ppm)	AP (%)	----------(%)----------	
0	0	.45	85.5[b]	39.7[bc]
2	0	.45	87.7[ab]	36.9[bc]
2	1,100	.45	86.6[b]	43.2[b]
0	0	.25	86.2[b]	33.5[c]
2	0	.25	84.9[b]	41.1[bc]
2	1,100	.25	90.0[a]	45.3[a]
Pooled SEM[1]			1.4	1.8
Source of variation			----------Probabilities----------	
CCMS			NS	NS
Lacto			.05	.05
AP			NS	NS

[a,b] Means within columns with no common superscript differ singificantly ($P \leq .05$).

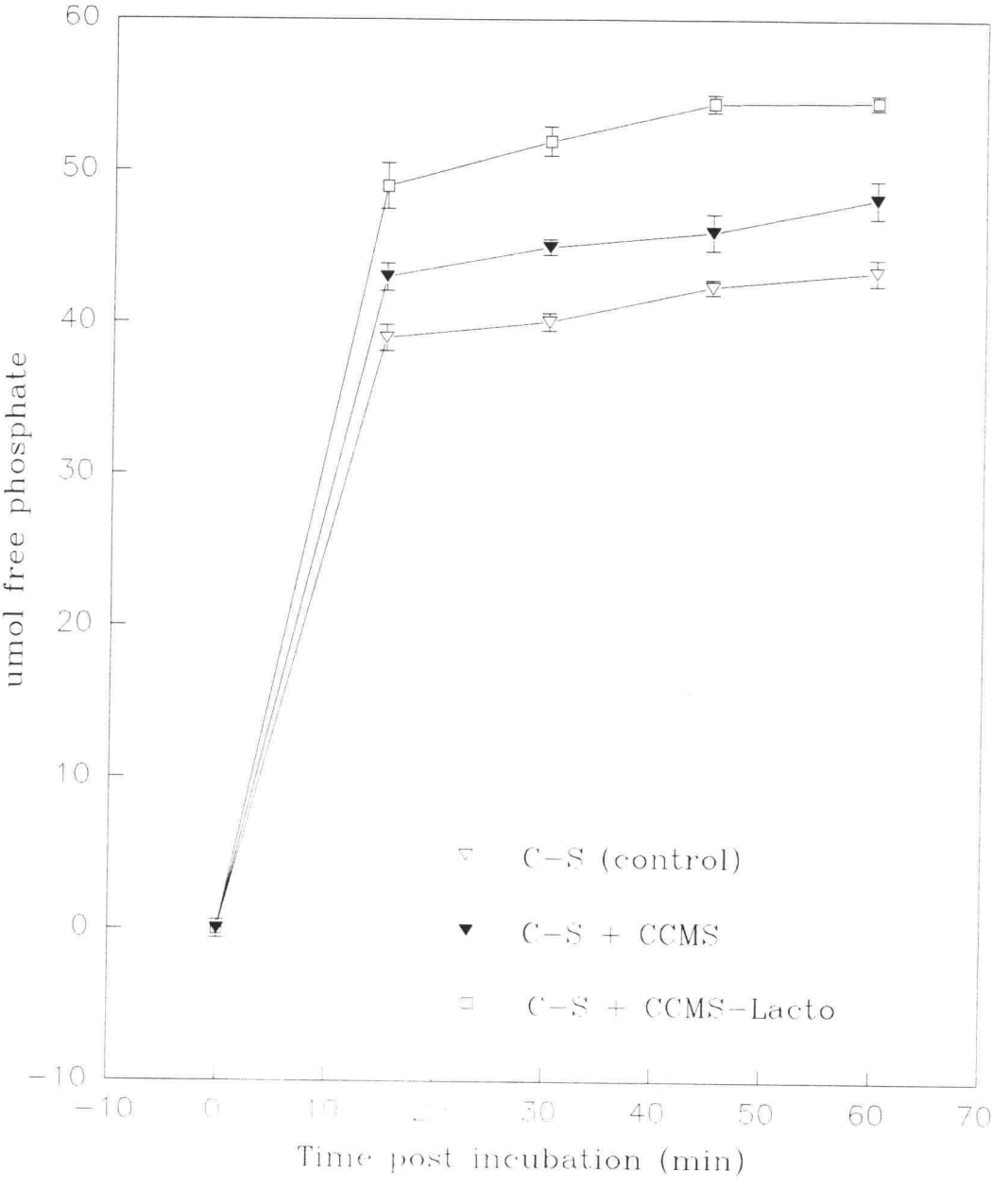

FIGURE D.1. Phytase activities of corn-soybean meal (C-S) layer diets containing .25% available phosphorus (AP) and supplemented with condensed cane molasses solubles (CCMS) and CCMS-*Lactobacillus* (Lacto) premix

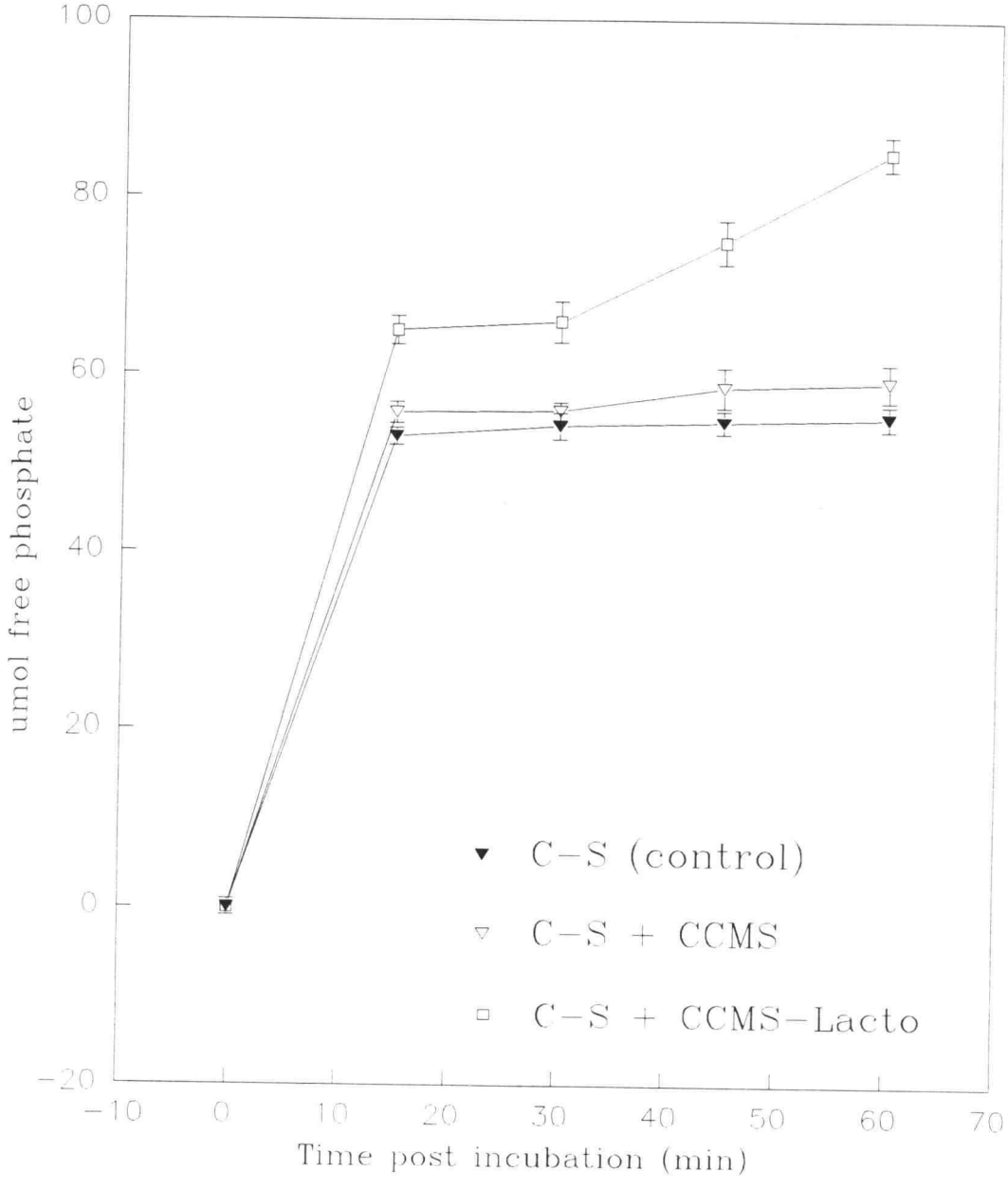

FIGURE D.2. Phytase activities of corn-soybean meal (C-S) layer diets containing .45% available phosphorus (AP) and supplemented with condensed cane molasses solubles (CCMS) and CCMS-*Lactobacillus* (Lacto) premix

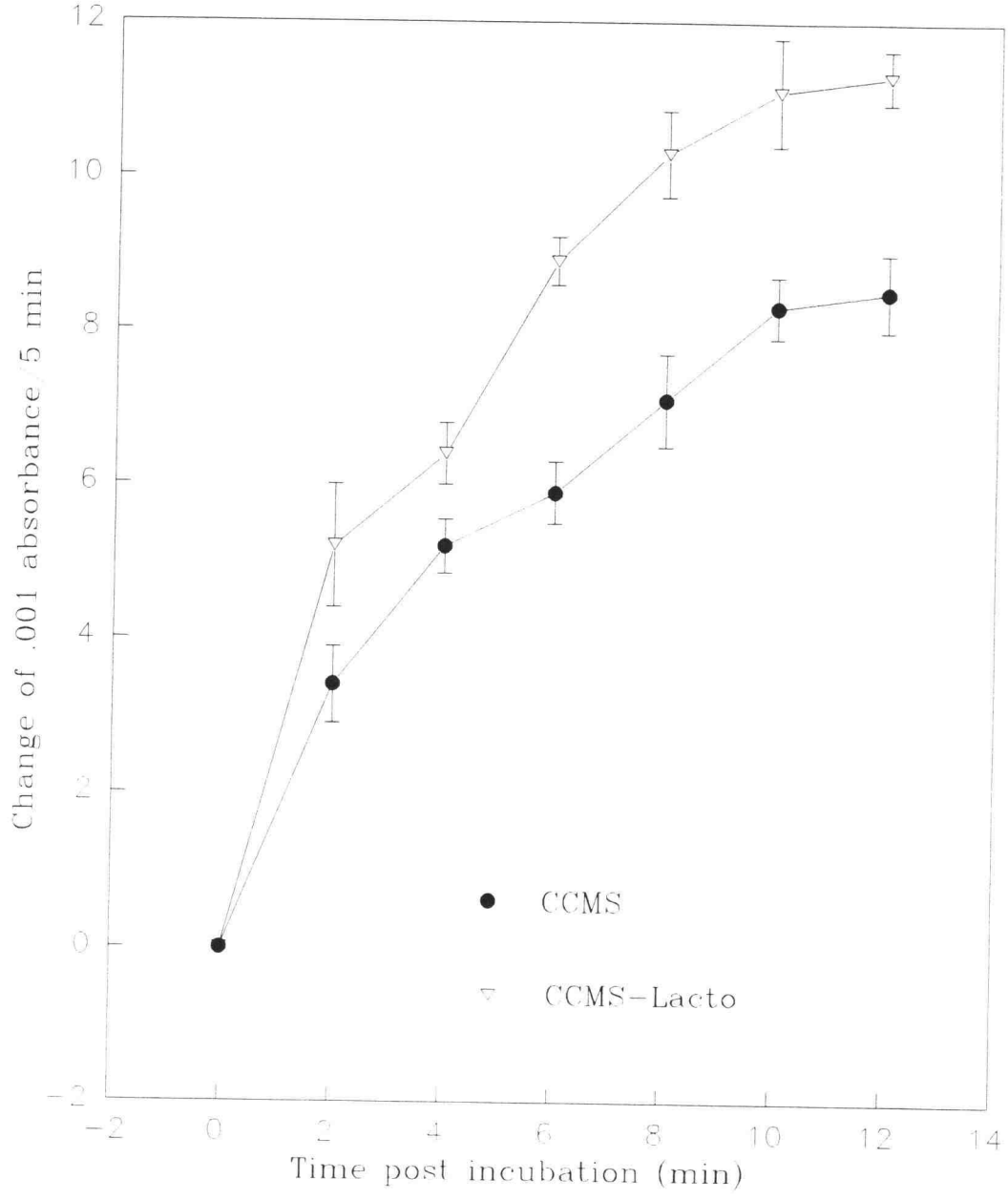

FIGURE D.3. Amylase activities of condensed cane molasses solubles (CCMS) and CCMS-*Lactobacillus* (Lacto) premix

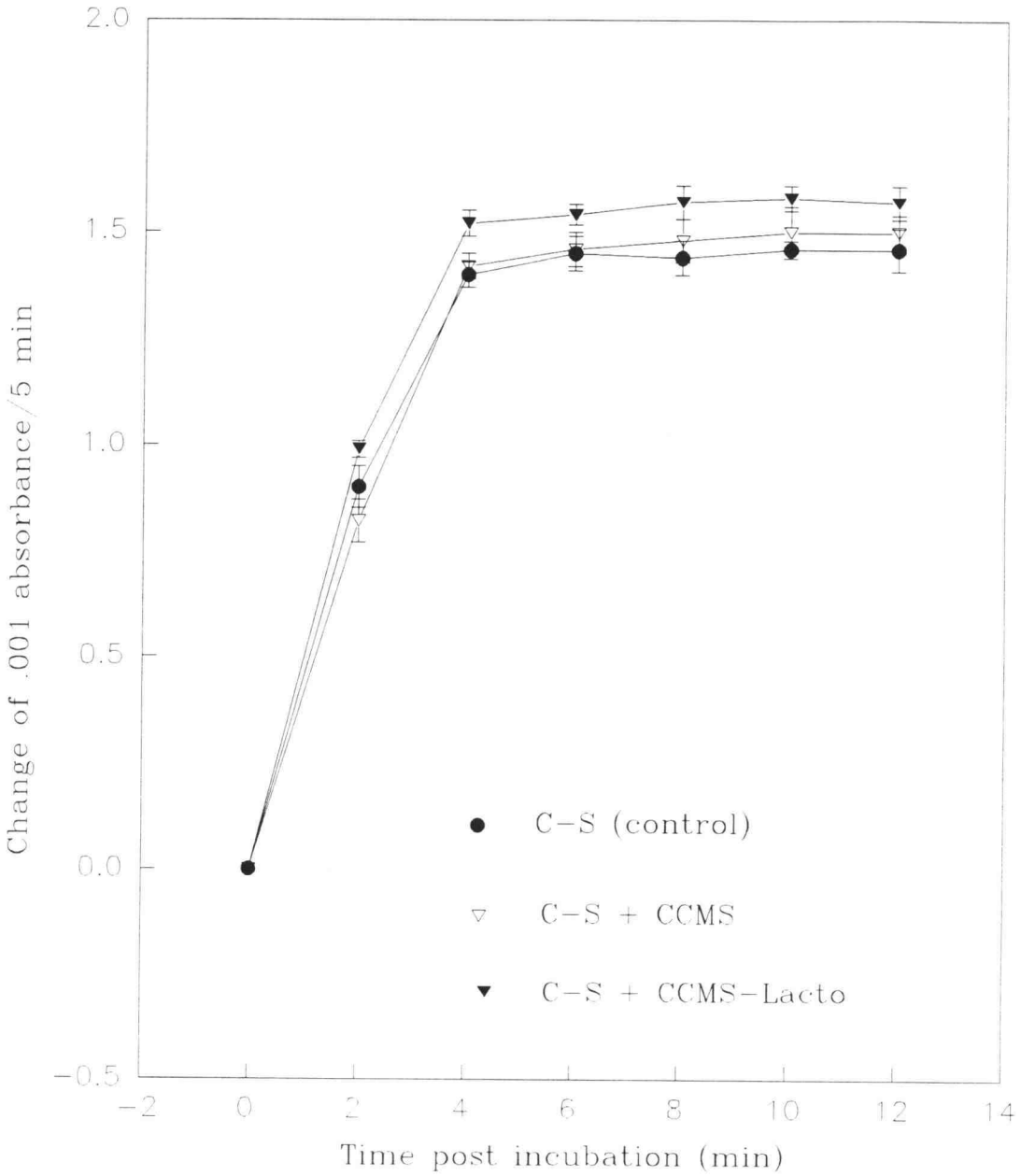

FIGURE D.5. Amylase activities of corn-soybean meal (C-S) layer diets containing .45% available phosphorus (AP) and supplemented with condensed cane molasses solubles (CCMS) and CCMS-*Lactobacillus* (Lacto) premix

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