EVALUATION OF GUAR MEAL AS A SOURCE OF PREBIOTIC GALACTOMANNANS FOR LAYING HENS

A Dissertation

by

CHENG ZHANG

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2004

Major Subject: Poultry Science

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Approved as to style and content by:

Christopher A. Bailey (Chair of Committee)	Aubrey Lee Cartwright (Member)
John B. Carey (Member)	Darrell A. Knabe (Member)
Alan R. Sams (Head of Department)	

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ABSTRACT

Evaluation of Guar Meal as a Source of Prebiotic Galactomannans for Laying Hens.

(August 2004)

Cheng Zhang, B.S., Beijing Agricultural University;

M.S., China Agricultural University

Chair of Advisory Committee: Dr. Christopher A. Bailey

Four experiments were conducted to evaluate guar meal as a source of prebiotic galactomannans for laying hens. In the 1st experiment, late phase laying hens were fed diets with 0, 5, 10% guar meal (GM) for 56 days or 15% GM for 28 days then switched to the 0% GM diet for the final 28 days. In the 2nd experiment, young pullets were fed guar germ (GG) or GM at 0, 2.5 or 5% for 20 weeks. In the 1st and 2nd experiments, egg production and feed consumption were not affected by feeding up to 5% guar by-products whereas feed efficiency was decreased by guar feeding. Feeding of GG or GM did not affect egg weight or shell quality, but decreased the egg yolk color and Haugh units. Guar increased absolute and relative liver weight, but did not affect the weights of the pancreas, spleen, or the incidence of fatty liver or liver hemorrhage. Feeding 10% GM depressed feed consumption and increased body weight loss. Feeding 15% GM severely depressed egg production followed by a recovery of production after returning to 0% GM feeding. In the 3rd and 4th experiments, late phase laying hens were induced to molt by feed withdrawal (FW) or feeding 15 or 20% GM with or without β-mannanase (Hemicell®). All hens except those fed 15% GM with enzyme obtained a complete

cessation of lay in 10 days. Compared to FW birds, hens fed GM had lower body weight reduction and mortality, while hens fed 20% GM with enzyme had higher post-molt egg production. *Salmonella enteritidis* (SE) present in 6 organs (crop, liver, spleen, ovary, oviduct and cecum), and SE in cecal contents were significantly reduced by 20% GM feeding with and without enzyme. The results showed that GG or GM can be safely fed to laying hens up to 5% without adverse effects on performance. An alternative molting method employing 20% GM with or without β-mannanase is preferable to FW because GM feeding results in a complete molt and decreases mortality, as well as enhances the resistance to SE of molted hens.

DEDICATION

I would like to express my love and gratitude to my wife, my parents, and my entire family for their continued encouragement, support, and sacrifices throughout my educational pursuits and daily life.

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CHAPTER I

INTRODUCTION

Guar, *Cyamopsis tetragonoloba,* is a drought-tolerant summer annual legume currently grown for its high concentration of galactomannan gum which is used as a thickener in food, cosmetics and pharmaceuticals as well as in oil well drilling mud, ore flotation, paper making, and even explosives. The vast majority of U.S. needs for guar gum are currently met by imports, primarily from India and Pakistan, as demand far exceeds the domestic supply. These imports are usually in the form of guar splits (unprocessed guar gum) rather than whole seeds as the by-product guar meal usually remains in-country as a source of protein for use in animal feeds. Efforts are underway to increase production of U.S. guar as it is an excellent drought tolerant rotational crop with cotton and sorghum that does not require irrigation. In the U.S. guar meal usually sells for almost half that of soybean meal and is most commonly used in cattle feedlot operations. Increased U.S. production of guar beans may offer expanded opportunities for use in least cost poultry feeds.

United States guar splitting plants produce both a high protein germ fraction and a lower protein psyllium husk fraction during the splitting process. These two fractions are traditionally recombined to create guar meal. The crude protein content of guar meal varies from 35 to 47.5% on a dry matter basis (Ambegaokar et al., 1969). Verma and

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This dissertation follows the format of Poultry Science.

McNab (1984b) reported that about 88% crude protein in guar meal was found to be present as true protein, and rich in arginine. However, methionine and lysine concentrations were comparatively lower than concentrations typically found in soybean meal (Verma and McNab, 1984a), and inadequate for optimum rat growth (VanEtten et al., 1961). Ambegaokar et al. (1969) suggested that tryptophan, methionine and threonine were the first three deficient amino acids of guar meal when compared to whole egg protein. The gross energy of raw and autoclaved guar meals were reported as 4.837 and 4.861 kcal/g while the N-corrected ME values of raw and autoclaved guar meals were 2.005 and 2.069 kcal/g respectively (Nagpal et al., 1971).

Excessive concentrations of guar meal in poultry diets cause diarrhea, depresses growth rate and increases mortality of broilers (Sathe and Bose, 1962; Couch et al., 1967b; Thakur and Pradhan, 1975b; Verma and McNab, 1982; Patel and McGinnis, 1985; Conner, 2002), and decreases egg production and feed efficiency of laying hens (Bakshi et al., 1964; Nagra et al., 1985; Patel and McGinnis, 1985; Nagra and Virk, 1986). Severe depression in egg production to cessation of lay were observed by Patel and McGinnis (1981) and Zimmermann et al. (1987) who fed laying hens 10 and 15% guar meal to induce a molt, and later obtained a satisfactory post-molt laying performance. The interior and exterior quality of eggs were reported not to be deleteriously affected by guar meal feeding (Couch et al., 1967b; Saxena and Pradhan, 1974; Patel and McGinnis, 1985; Zimmermann et al., 1987) except that the yolk color index decreased with the inclusion of guar meal in laying diets (Verma and McNab, 1984b).

Bakshi et al. (1964) proposed that guar meal contains two deleterious factors: trypsin inhibitor and guar gum residue. The trysin inhibitor was listed as a deleterious factor because the chicks fed guar meal had been reported to present pancreatic hypertrophy (Couch et al., 1967a) which can also be found in chickens fed un-heated soybean meal. However, the trypsin inhibitor was not universally accepted as a primary factor for the deleterious effects of feeding guar product to poultry (Anderson and Warnick, 1964; Vohra and Kratzer, 1964). Verma and McNab (1982) reported that neither heating the guar meal directly nor steam pelleting diets containing guar meal had much effect on the performance of the broiler chicks, which was in agreement with the findings of Nagpal et al. (1971) who reported autoclaving guar meal did not improve its gross protein value for chicks. The trypsin inhibitor activity in guar meal were reported to be significantly lower than in soybean meal commonly used in poultry feed (Verma and McNab, 1982; Conner, 2002), which indicates that the negative effects on performance of poultry when fed diets containing guar meal are not likely due to trypsin inhibitor activity.

Guar gum is a galactomannan polysaccharide consisting of a 1→ 4-linked β-D-mannopyranose backbone with branched 1→ 6- α-D-galactopyranose. The residual gum content of typical guar meals is approximately 18 to 20% (Bakshi et al., 1965; Nagpal et al., 1971). A series of feeding experiments conducted by Vohra and Kratzer (1964) demonstrated that as little as 1% guar gum in broiler chicken diets causes a depression of growth. When the diet contained 2% guar gum, the relative growth of broiler chickens was 61 to 67.4% of controls. Anderson and Warnick (1964) and Vohra and Kratzer

(1964) asserted that residual gum was chiefly responsible for the adverse effects on chicks, not trypsin inhibitor. Research by Lee et al. (2003a) also supported that residual gum was at least partially responsible for the effects seen when guar meal was fed.

Guar gum can dramatically alter the rheological properties of the contents of the gastro-intestinal tract resulting in significant physiological effects. High viscosity is generally connected with delayed gastric emptying and increased small intestinal transit time, hence inhibiting the absorption of nutrients (Blackburn and Johnson, 1981). However, the high viscosity of guar gum may contribute to some beneficial physiological functions as well, including decreasing plasma cholesterol (Favier et al., 1997a; Dario Frias and Sgarbieri, 1998; Favier et al., 1998; Moriceau et al., 2000; Yamamoto et al., 2000), decreasing postprandial serum glucose (Fairchild et al., 1996; Ou et al., 2001) and attenuating postprandial hypotension in type 2 diabetes patients (Groop et al., 1993; Russo et al., 2003). Guar gum has also been found to have putative protective effects against colonization by pathogenic bacteria, and their subsequent proliferation and resultant diarrhea, and to potentiate the effect of polar lipids in preventing microbial translocation within the gastro-intestinal tract (Bengmark, 1998). Guar gum is readily fermented by human fecal microbe and it has bifidogenic effects (Okubo et al., 1994), which may partially contribute to its prebiotic functions. Although no report on the effect of guar gum on immune function of animals or humans can be found, Duncan et al. (2002) successfully isolated a high-molecular-weight galactomannan, about 1.0 million Daltons, that enhances macrophage activation thus exhibiting immunostimulatory activity from the edible mushroom *Morchella esculenta*.

The relatively new concept of dietary fiber and prebiotics has changed the way we think about non-digestible carbohydrates in food and feed. Using guar by-products as a source of prebiotic galactomannans in poultry feed would be of great interest because of the desire to restrict the use of antibiotics as growth promoters. The objectives of this research are to determine the upper safe limit of guar meal in laying hen feed, to explore the possibility of using relatively high concentrations of guar meal to induce a full-fed molt as an alternative to conventional feed withdrawal, and to study the prebiotic functions of galactomannan residue in guar meal with respect to the *Salmonella* resistance during molting.

CHAPTER II

LITERATURE REVIEW

DIETARY FIBER AND PREBIOTICS

The American Association of Cereal Chemists (AACC) defined dietary fiber (DF) as "the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation" (American Association of Cereal Chemists, 2001). By this definition, galactomannans (guar and locust bean gum) as an indigestible non-starch polysaccharide (NSP) is a member of DF family. The definition of DF is similar to that of a prebiotic, which was putatively defined by Gibson and Roberfroid (1995) as "a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health". Whether or not a fiber is eligible to be a prebiotic depends on its fermentation type and its potential to benefit the host.

Dietary fibers and prebiotics exert their beneficial physiological functions by two mechanisms: 1) Promoting the growth and proliferation of "friendly" bacteria in the gastro-intestinal tract (GI tract); and 2) Prevent the adhesion of pathogens to epithelium of the GI tract. The "friendly" bacteria, referred to as *Bifidobacterium* and *Lactobacillus*

in general, are classified as probiotics. The fermentation of these probiotics produce high amounts of short chain fatty acids (SCFA), which decrease the luminal pH value and exert an inhibition effect on the growth and proliferation of pathogens. In addition to decreasing harmful bacteria and restoring a normal intestinal microflora, Tungland and Meyer (2002) summarized some other beneficial physiological functions of the SCFA produced by fermentation of probiotics: 1) Act as immunomodulators to enhance the immune functions of the host; 2) Decrease blood cholesterol and triglycerides; 3) Decrease food intolerances and food borne allergies; 4) Produce nutrients such as the B vitamins family, as well as some digestive enzymes; 5) Reduce liver toxins such as blood amines and ammonia; 6) Increase mineral absorption. Butyrate, a four-carbon short chain fatty acid (SCFA), is a prime energy substrate of the colonocyte, which provides 70% of the energy needed for growth and differentiation of the colonical epithelium. Butyrate enhances growth of the gut epithelium and promotes gut cell differentiation, improves immune functions of the host, induces apoptosis in colonic tumor cell lines and improves the intestinal barrier function (Brouns et al., 2002). In addition to producing SCFA, the probiotics improve the health of the host in many other ways including producing antibacterial compounds such as “bifidin”, altering enzyme activities and changing the enzymetic metabolism of microbes within the GI tract, competing against pathogens for nutrients and adhesion sites, and stimulating immune functions of the host (Fuller, 1989). Prebiotics and dietary fibers may also prevent the adhesion of pathogens to the GI tract by not only directly competing for adhesion sites

against pathogens, but also by binding to pathogens and deactivating them prior to excretion with the pathogens.

Potential prebiotics could be nondigestible carbohydrates such as resistant starch, non-starch polysaccharides, and oligosaccharides. Some proteins, peptides and lipids from milk could also be prebiotics. Galactomannan residues in guar meal (guar gum) which contain β-1,4- glycosidic bonds and can not digested by human and animals itself, are well fermented by the microbes in the GI tract, which produce high amounts of SCFA. The fermentability of guar gum and its beneficial physiological effects observed in human and animal studies qualified it as a pretiotic.

GUAR GUM

Guar gum is a highly viscous galactomannan polysaccharide similar to locust bean gum, consisting of a 1→ 4-linked β-D-mannopyranose backbone with branchpoints from their 6-positions linked to α-D-galactose (i.e. 1→ 6-linked-α-D-galactopyranose). The mannose: galactose residue ratio is about 1.5 to 2: 1. A guar gum molecule is made up of about 10,000 residues, which are non-ionic polydisperse rod-shaped polymers (longer than found in locust bean gum). The highly branching nature results in its high solubility in water. The high viscosity of guar gum results from both its high molecular weight and long chain structure.

Fermentation Properties of Guar Gum

Among the many factors influencing the extent of fermentation of fiber, the primary influence is its chemical structure and physicochemical nature. Cellulose, composed of β-1,4-linked D-glucose, is hardly fermented whereas those fibers having α-

1,4 and α-1,6 linked D-glucosyl residues are much more fermentable (Tungland and Meyer, 2002). The solubility of fiber also affects the fermentation ability. Fiber with lower water holding capacity (WHC) that contain higher levels of insoluble cellulosic material, such as wheat bran, tend to be unfermentable compared to those having high WHC such as gums or pectin (McIntyre et al., 1991).

Guar gum is cataloged as a "well-fermentable" fiber type which includes pectin, acacia (gum arabic), polydextrose, inulin and oligosaccharides (Tungland and Meyer, 2002). The end products of fermentation differ among different fiber types (Bourquin et al., 1996), as determined by their physicochemical properties. Poorly fermentable cellulose produce very little acid during its fermentation, most of which is only acetic acid whereas the easily fermentable fibers produce large quantities of SCFA, including propionic, butyric and acetic acids, in varying proportions. Guar gum was reported to promote propionate and butyrate-rich fermentation (Tulung et al., 1987; Moundras et al., 1994; Lu et al., 2000; Velazquez et al., 2000; Henningsson et al., 2002). In an experiment of in vitro incubation with fecal bacteria from adult male volunteers, Bourquin et al. (1996) reported that the molar ratios of acetate: propionate produced during fermentation varied considerably among substrates, ranging from 1.5 for guar gum to 12.6 for citrus pectin. Favier et al. (1997b) found that the SCFA concentration in the cecum of rats fed semi-purified diets with 8% guar gum (GG) or partially hydrolyzed guar gum (PHGG) were 130 ± 9 and 159 ± 10 mmol/L, respectively, compared to the 67 ± 6 mmol/L for control rats. The ratio of acetate: propionate: butyrate were 50:39:11, 40:50:10 and 63:27:10 for GG, PHGG and control rats, respectively. These results

demonstrate the different fermentabilities and pattern of fermentation of guar gum and its hydrolysates PHGG. The SCFA resulting from the fermentation process provide a certain amount of energy from their catabolism in the liver, and are the prime substrates for the energy metabolism in the colonocyte where they act as growth factors to promote a healthy epithelium.

Prebiotic Properties of Guar Gum

Improved Gastrointestinal Microbial Ecosystem. Although the effect of intact guar gum on the gut microbial ecosystem has not been studied, many investigations have demonstrated prebiotic functions of PHGG. In an in vivo experiment by Okubo et al. (1994), healthy adult human subjects were fed 21 g/d PHGG for 14 d to investigate the effects of PHGG intake on fecal microflora, bacterial metabolites, and pH. The count of *Bifidobacterium spp*. and the percentage of these species in the total count increased significantly during the PHGG intake periods. Another acid-forming bacteria genera, *Lactobacillus spp*. also increased. The fecal pH and fecal bacterial metabolites such as β-glucuronidase, putrefactive products and ammonia content were significantly decreased by PHGG intake. Two wk after the end of PHGG intake, the bacterial composition of microflora returned to the pre-treatment state. Ishihara et al. (2000) fed PHGG to pullets for 6 wk at 0, 0.025, 0.05 and 0.1%, then challenged pullets with 3.2×10^6 *Salmonella enteritidis* (SE), and found that the cecal *B. spp* and *L. spp* of pullets administered PHGG at 0.025% were significantly higher than those without PHGG. The numbers of SE incidence within internal organs and intestine of pullets were also significantly decreased by the 0.025% PHGG treatment. The decrease in SE incidence was attributed

to the increased excretion of SE in feces during the early stage of post inoculation. In a similar experiment with laying hens, Ishihara et al. (2000) observed decreased SE present in internal organs and intestine by 0.025% PHGG treatment. The SE incidence on the surface of eggs was also decreased by 0.025% PHGG inclusion in feed. However, higher concentrations of PHGG (0.05 and 0.1%) did not exhibit the same SE inhibition effect as 0.025% PHGG did.

Improved Immune Function. Yamada et al. (1999) compared the effect of dietary guar gum or partially hydrolyzed guar gum (PHGG) on the serum lipid level and immunoglobulin (Ig) production of Sprague-Dawley rats with that of water-insoluble cellulose. Both guar gum and PHGG feeding enhanced IgA productivity in the spleen and mesenteric lymph node lymphocytes, and an increase in serum IgA level was observed in the rats fed guar gum but not PHGG. In a similar study by the same group (Yamada et al., 2003), the IgA and IgG productivity in mesenteric lymph node (MLN) lymphocytes was significantly higher in the rats fed guar gum than in those fed cellulose. In addition, guar gum induced a significant increase of IgM productivity in MLN lymphocytes when compared to the cellulose group. Guar gum, as a polysaccharide of galactomannan, may also enhance macrophage activities. A high-molecular-weight galactomannan, about 1.0 million Daltons, isolated from the edible mushroom *Morchella esculenta*, was demonstrated to enhance macrophage activation thus exhibiting immunostimulatory activity (Duncan et al., 2002).

Inhibition of Colonic Cancer and Improved Colon Health. The health of the large intestine and colon is paramount because they, in addition to serving as a primary

site for water re-absorption, play an important role as a major immune organ and function as a barrier to prevent foreign materials from dietary or microbial origin from crossing into the internal body cavity. Heitman et al. (1992) reported that dietary supplementation with 10% guar gum (fed during the promotional stage of carcinogenesis) was found to suppress colon cancer incidence to a significant extent. The SCFA produced by colonic microbes with the substrate of soluble fibers has been proposed to play a key role in enhancing colon health by both stimulating cell division and regulating apoptosis (Wasan and Goodlad, 1996). Butyrate has been shown to increase apoptosis in human colonic tumor cell lines (Brouns et al., 2002). Apoptosis is a mechanism where excess or redundant cells are removed during development and restricted tissue size is maintained, thus serving as an innate cellular defense against carcinogenesis. Reduced intestinal pH by SCFA has a direct impact on carcinogenesis in the large intestine (Tungland and Meyer, 2002). Lowered colonic pH affects pH-dependent enzymatic reactions, for example, secondary bile acid formation. Short chain fatty acids can also decrease the amount of pathogenic bacteria in colon which results in a reduction in the production of carcinogenic substances. Dietary fiber could also reduce the amount of carcinogenic substances available to colonic mucosa by adsorption of the substances to the cell walls of the microbiota, by speeding up the intestinal transit time and by increasing colonic contents and thus diluting all solutes.

Hypoglycemic Effect. Well fermented viscous fiber, either as a part of food or as a supplementation in food, has been reported to be of great potential benefit in reducing glycemic response and increasing insulin sensitivity (Tungland and Meyer,

2002), although the mechanisms of dietary fiber's reducing postprandial glycemia and enhancing carbohydrate metabolism remain unclear. The most plausible explanation for these influences are those related to digesta viscosity in gastro-intestinal track and nutrient absorption, and systemic effects from colonic SCFA produced by microbial fermentation. Relative to postprandial glycemia, fibers that provide high viscosity in the small intestine (for example, guar gum and pectin) generally offer greater effects.

Guar gum has been reported to attenuate postprandial glycemia (Carter et al., 1998; Dario Frias and Sgarbieri, 1998; Williams et al., 2004), and attenuate the fall in blood pressure of type 2 diabetes patients after oral glucose (Russo et al., 2002). Serum insulin concentrations are also decreased (Fairchild et al., 1996; Gatenby et al., 1996; Sierra et al., 2001). Physicochemical characteristics of guar gum in the upper intestine may be important in determining blood glucose level (Nagata et al., 1996). Partially hydrolyzed guar gum, which is low in molecular weight and viscosity, has been reported to significantly decrease serum cholesterol, free fatty acid and glucose concentrations when administered to healthy young adults at 15 g/d, but the decrease was not significant when the dosage was 5 g/d (Yamatoya et al., 1997).

Cholesterol-Lowering Effect. Both guar gum and PHGG have been reported to significantly depress plasma and serum triglycerides, triglyceride-rich lipoprotein, total cholesterol, and apolipoprotein E levels (Moundras et al., 1994; Yamada et al., 1999). Increased bile acid excretion seems to be essential in the cholesterol-lowering effect of soluble fibers and related compounds. This effect is connected to induction of HMG CoA reductase and lowering concentrations of apolipoprotein E-containing particles

(Moundras et al., 1994). Favier et al. (1997b) studied the role of intestinal viscosity on the cholesterol-lowering effect of dietary fiber by comparing the cholesterol-effect of intact guar gum and PHGG. Compared with the low viscosity PHGG diet, the high viscosity intact guar gum diet was more potent in enhancing bile acid excretion and producing a potent cholesterol-lowering effect. Both PHGG and guar gum depressed plasma triglycerides whereas only the guar gum depressed low-density lipoprotein (LDL). The decreased efficiency of the PHGG diet to produce a hypocholesterolemic response resulted in a decreased stimulation of hydroxymethylglutaryl coenzyme A (HMG CoA) reductase, and this could explain the lesser efficiency of the PHGG diet to develop a cholesterol-lowering effect (Favier et al., 1997b). The hypocholesterolemic effect of dietary fibers might also be mediated by the SCFA from fiber fermentation. Propionate is reported to inhibit fatty acid metabolism which plays a key role in the synthesis of cholesterol (Nishina and Freedland, 1990; Wright et al., 1990; Demigne et al., 1995). Propionic acid has been reported to inhibit hepatic lipogenesis from acetate (Demigne et al., 1995).

Improved Mineral Bioavailability. Takahashi et al. (1994b) studied the effect of PHGG and intact guar gum (GG) on iron utilization in rats fed several iron-deficient diets and found the hemoglobin, serum iron and iron storage in the liver of rats fed iron-deficient diets as a control group (without PHGG and GG) significantly decreased, while those of the test group fed together with PHGG or GG were unchanged. In an iron balance test for 3 d, administration of PHGG or GG caused an increase in iron absorption. These results suggested that PHGG and GG increase the bioavailability of

dietary iron in deficiency. In studying the effect of dietary guar gum on mineral excretion of young male rats, Wood and Stoll (1991) found that dietary inclusion of guar gum significantly decreased fecal excretion of Ca, P, Mg, and Cu, and increased serum, but not bone, levels of these minerals with exception of P. Through their fermentation by colonic microbes and subsequent SCFA production, dietary fibers stimulate the proliferation of epithelial cells in the ceco-colon and reduce the luminal pH. The SCFA and lower pH may, in turn, dissolve insoluble mineral salts, especially calcium, magnesium and iron in the luminal content and increase their diffusive absorption via the paracellular route.

INDUCED MOLT

Molting is a periodic spontaneous process of shedding and replacement of feathers for adult avian species lasting up to 34 days, with a greatly reduced feed intake and a body weight reduction (BWR) of up to 50%, varying between species (Mrosovsky and Sherry, 1980). For most wild species of avian, molting involves reproductive rejuvenation with a cessation of oviposition as result of the regression of the reproductive tract (Berry, 2003). Commercial laying hens will naturally molt at various times of age which creates an economic problem, because the whole flock is not molting uniformly, and the flock's natural molt cycle may be extended over many weeks or even months (McDaniel and Aske, 2000). Artificial induction to molt of late phase layers is employed to accelerate the process of nature molting, stimulate multiple egg-laying cycles, and improve egg quality and desirable egg numbers, thus making the hens more

profitable. Holt (2003) estimated that in the U.S, the number of hens molted annually would be 192 to 204 million.

Although there are many methods available to induce molting and synchronize the second lay cycle for greater production efficiency, the most widely utilized commercial method is feed withdrawal. This method also involves day length or light intensity manipulation. A typical molting program involves a reduction of light and removal of feed for up to 14 d or until a BWR of 25 to 35% is obtained. This reduction in body weight is the result of ovarian and oviduct regression, depletion of fat and labile protein reserves, the loss of alimentary canal contents, and initial water intake reduction. Brake and Thaxton (1979) reported that a 12-d feed withdrawal resulted in a 25% weight loss and one fourth of this loss was attributed to liver, ovary, and oviduct weight reduction. Many researchers have recommended weight losses ranging from 25 to 35% (Baker et al., 1983; Zimmermann et al., 1987; Carey and Brake, 1989). Enhanced postmolt performance is related to the amount of regression and subsequent redevelopment of body organs and tissues. Lee (1982) found a positive and significant correlation between the length of the rest period and postmolt hen-day egg production. Fontana et al. (1991) reported similar results. Baker et al. (1983) looked at the relationship between BW loss and postmolt production performance of caged layers and came to the conclusion that a weight loss of 30% produced the optimum subsequent performance in terms of egg production and shell quality.

In recent years, the induction of molting by feed withdrawal has become the target of vigorous criticism with respect to both animal welfare and food safety

concerns. Two animal rights organizations, United Poultry Concerns and the Association of Veterinarians for Animal Rights, advocated the United States Department of Agriculture and the Food and Drug Administration ban the forced molting of laying birds in the United States, grounded on two issues: animal rights and food safety. Prolonged food deprivation was regarded as "extreme cruelty", and has been shown to severely stress the immune function and promote *Salmonella enteritidis* infection in laying birds. *Salmonella enteritidis* has been identified as a major contaminant in shell eggs, and has been scientifically linked to the practice of forced molting. The so called inhumane practice of withholding food from laying birds for days and even weeks at a time immunologically compromises them so significantly as to render the products derived from these birds a health risk to consumers of their products according to these two animal rights organizations.

Induced molting by feed withdrawal may have a negative effect on the cellular component of the immune system of the molted birds. In a study on the effect of feed withdrawal as a stressor on the humoral and cellular immune responses, Holt (1992a) reported that the lymphocyte numbers were lower in molted birds compared with nonmolted controls. The delayed hypersensitivity response to the skin sensitizer, dinitrofluorobenzene (DNFB), was depressed during the period of food withdrawal but recovered when the hens were returned to feed. Holt (1992b) also demonstrated that compared to the unmolted laying hens, fasted hens had significantly decreased CT4+ peripheral blood T cells as early as on the 3rd d of feed withdrawal. Feed withdrawal has also been demonstrated to decrease the number and function of peripheral blood

heterophils, which play a role in the increased susceptibility of molting hens to SE infections (Kogut et al., 1999).

As a result of the impaired immune functions, the fasted hens are much more vulnerable to the infection of pathogens, among which Salmonella is the most intensively investigated. In a study to compare the susceptibility to SE infection of laying hens molted by feed withdrawal with unmolted hens, Holt (1993) reported that molting hens infected with a 10^2 cfu of *S. enteritidis* shed 3 to 4 logs more of the organism at 7 days postinfection than the unmolted hens receiving a similar dose. The mean infectious dose (ID50) for *S. enteritidis* in unmolted hens ranged from 0.65×10^4 to 5.6×10^4 cfu, whereas in molting hens the ID50 was found to be less than 10^1 cfu, which indicates that hens molted by feed withdrawal were 100- to 1,000-fold more susceptible to infection by SE than unmolted hens.

Feed withdrawal has a profound effect on both intestinal and extraintestinal infection by *S. enteritidis*, and these effects occur within 24 h postinfection in the intestine and within 48 hr postinfection in the liver and spleen (Holt et al., 1995). By 24 h postinfection, *S. enteritidis* was most prevalent in the ceca and feces of unmolted hens, and this prevalence continued throughout the experimental period. In molted hens, however, *S. enteritidis* could be detected in a high percentage (90-100%) of colon, ceca, and feces samples at 24 to 96 h postinfection and in 67% or more of ileum samples at 48 to 96 h postinfection, indicating a much wider distribution of the *S. enteritidis* along the intestinal tract than in unmolted hens. The numbers of *S. enteritidis* recovered from these alimentary samples were also significantly higher in molted than unmolted hens.

Significantly more *S. enteritidis* was also recovered from the livers and spleens of the molted hens at 48, 72, and 96 h postinfection.

In addition to local and systemic colonization by Salmonella *enteritidis* in force-molted hens, forced molting encourages *S. enteritidis* to spread to other hens in the confined hoursing environment (Holt, 1995; Holt et al., 1998). *Salmonella enteritidis* of molted hens was transmitted more rapidly to the unchallenged hens than unmolted hens both via direct contact with infected hens or shedding and via the airborne route, and these molted hens shed significantly more of the organism than unmolted hens indicating that induced molting can have substantial effects on transmission of *S. enteritidis* to uninfected hens, which could affect the overall *S. enteritidis* status of a flock.

Many alternative methods to the complete deprivation of feed have been reported, which include the use of high levels of zinc, low levels of sodium or calcium, and using pharmaceuticals such as nicarbazin, progesterone, enheptin and others (Berry, 2003; Webster, 2003), among which high dietary zinc has been molst intensively studied.

Dietary Zn at a concentration of 20,000 ppm, usually in the form of ZnO, is reported to cause a complete cessation of egg production and induce a molt within 5 d (Scott and Creger, 1976; Creger and Scott, 1977; Berry and Brake, 1985, 1987), which is as effective as the feed withdrawal method (Roberson and Francis, 1979). A lower concentration of dietary Zn (10,000 ppm) as oxide, acetate or propionate was also reported capable of inducing a molt (Shippee et al., 1979; Park et al., 2004). Shippee et al. (1979) observed that 10,000 ppm Zn as oxide or acetate caused the egg production to

decline from 60 to 0% in 6 d. Park et al. (2004) reported that both Zn acetate and Zn propionate at a concentration of 10,000 ppm induced an ovary weight reduction similar to the feed withdraw method, and the overall egg production for the 12 wk post-molt period was not different for hens molted by these three methods. Although much evidence indicates that high concentrations of zinc (10,000 to 20,000 ppm) cause the cessation of lay primarily by depressing feed intake, Johnson and Brake (1992) demonstrated that the effectiveness of zinc to induce the cessation of lay is due, at least in part, to a direct inhibitory action on ovarian granulosa cell function both in differentiating and in preovulatory follicles. When combined with a low calcium diet, a relatively low level of Zn (2,800 ppm) has a suppressing effect on reproductive activity and induces an earlier reproductive regression than control hens fed only a low calcium diet (Breeding et al., 1992). However, inconsistent post-molt laying performance was observed by Bar et al. (2003) when laying hens were fed the same concentration of Zn (2,800 ppm) combined with a diet low in Ca and P.

Alternative methods of induction to molt by feeding diets low in calcium (0.05 to 0.3% Ca) (Douglas et al., 1972; Gilbert and Blair, 1975), low in sodium (0.04% Na) (Whitehead and Shannon, 1974; Dilworth and Day, 1976; Nesbeth et al., 1976a, b; Campos and Baiao, 1979; Ross and Herrick, 1981a, b) or high in iodine (2,500 to 5,000 ppm I) have been studied a few decades ago, and are reviewed by Berry (2003) and Webster (2003). However, these alternative molting techniques, with the exception of high Zn diets, have not proven to be as consistent in halting egg production.

Both feed withdrawal and providing nutritionally imbalanced diets to induce a molt have been criticized by animal welfare advocates. Providing laying hens with nutritionally balanced molt-inducing diets should be more humane and not subject to as much criticism with respect to animal well-being. Vermaut et al. (1998) reported restricted feed intake and subsequent induced molting of broiler breeders when fed a diet with 12% jojoba supplementation. Davis et al. (2002) reported that feeding diets containing 50% ground cottonseed was equivalently effective to complete feed withdrawal in reducing body weight, inducing molt, and post-molt laying performance. Feeding a grape pomace diet to laying hens was considered to be a preferable molting method to conventional FW in view of animal rights issues (Keshavarz and Quimby, 2002). Biggs et al. (2003) found that feeding laying hens corn or wheat middlings solely, particularly wheat middlings, was an effective non-feed removal method for molting hens. This group (Biggs et al., 2004) later concluded that feeding wheat middlings, corn, corn gluten feed, and wheat middlings:corn diets were effective non-feed removal methods for molting laying hens.

Guar meal has also been reported to induce cessation of egg production when fed to layers to at concentrations of 10 to 15% (Patel and McGinnis, 1981; Zimmermann et al., 1987). In Patel and McGinnis's study (1981), 36 wk old laying hens were fed either a control diet or a diet containing 10% raw or autoclaved guar meal for 33 d. The hens fed the raw and autoclaved guar meal laid at 0 and 8% hen-day egg production after one wk of guar meal feeding, and began to recover in egg production within one wk after they were switched to the control diet, and returned to over 85% within two wk. Thereafter

these hens consistently laid about 10 to 15% more eggs than those continuously fed control diet. The force molted hens laid at a rate of over 90% for a period of at least a week. Even though feeding raw guar meal for 57 d delayed the return to egg production, the peak in egg production of the following laying cycle was not lowered. Improved shell quality was also observed for the hens fed guar meal diets. In a study to compare several molt inducing methods, Zimmermann et al. (1987) fed 72-wk-old Single Comb White Leghorn hens with 10 or 15% guar meal substituting for corn. Egg production was arrested on d 8 and 10 for hens fed 15 and 10% guar meal diets, respectively. Hens obtained body weight reduction of 31.8% by d 28 of feeding 10% guar meal, and obtained body weight reduction of 30.5% by d 20 of feeding 15% guar meal. These hens obtained 50% egg production on the 50th day of post-molt, which was 2 to 5 d later than hens molted by various feed-restricting or fasting methods. However, the egg production of hens molted by guar meal feeding was comparable or superior to hens molted by the other methods, and significantly higher than unmolted hens. The Haugh units and shell quality of eggs from hens molted by guar meal feeding were also similar to hens molted by other methods, and were significantly improved when compared to unmolted hens.

These results suggest that feeding a relatively high concentration of guar meal could be an effective alternative to conventional feed withdrawal for molting hens. Because of the prebiotic properties of guar gum residue in guar meal, inhibition of SE colonization in laying hens molted by guar meal feeding may also be anticipated.

CHAPTER III

USE OF GUAR MEAL IN LATE PHASE LAYING HEN DIETS

INTRODUCTION

Guar, *Cyamopsis tetragonoloba,* is a drought-tolerant summer annual legume currently grown for its high concentration of galactomannan gum which is used as a thickener in food, cosmetics and pharmaceuticals as well as in oil well drilling mud, ore flotation, paper making, and even explosives. The vast majority of U.S. needs for guar gum are currently met by imports, primarily from India and Pakistan, as demand far exceeds the domestic supply. These imports are usually in the form of guar splits (unprocessed guar gum) rather than whole seeds as the byproduct guar meal usually remains in-country as a source of protein for use in animal feeds. Efforts are underway to increase production of U.S. guar as it is an excellent drought tolerant rotational crop with cotton and sorghum that does not require irrigation. In the U.S., guar meal usually sells for almost half that of soybean meal and is most commonly used in cattle feedlot operations. Increased U.S. production of guar beans may offer expanded opportunities for use in least cost poultry feeds.

United States guar splitting plants produce both a high protein germ fraction and a lower protein psyllium husk fraction during the splitting process. These two fractions are traditionally recombined to create guar meal. The crude protein content of guar meal varies from 35 to 47.5% on a dry matter basis (Ambegaokar et al., 1969). Verma and

McNab (1984b) reported that about 88% of the crude protein in guar meal was found to be present as true protein, and rich in arginine. However, methionine and lysine concentrations were lower than those found in soybean meal (Verma and McNab, 1984a), and inadequate for optimum rat growth (VanEtten et al., 1961). Ambegaokar et al. (1969) suggested that tryptophan, methionine and threonine were the first three deficient amino acids of guar meal when compared to whole egg protein. The gross energy of raw and autoclaved guar meals were reported as 4.837 and 4.861 kcal/g while the N-corrected ME values of raw and autoclaved guar meals were 2.005 and 2.069 kcal/g respectively (Nagpal et al., 1971).

Excessive concentration of guar meal in poultry diets causes diarrhea, depresses growth rate and increases mortality of broilers (Sathe and Bose, 1962; Couch et al., 1967b; Thakur and Pradhan, 1975b; Verma and McNab, 1982; Patel and McGinnis, 1985; Conner, 2002), and decrease egg production and feed efficiency of laying hens (Bakshi et al., 1964; Nagra et al., 1985; Patel and McGinnis, 1985; Nagra and Virk, 1986). Severe depression in egg production to cessation of lay were observed by Patel and McGinnis (1981) and Zimmermann et al. (1987) who fed laying hens 10 and 15% guar meal to induce a molt, and later obtained satisfactory post-molt laying performance. However, Verma and McNab (1984b) failed to obtain cessation of lay by feeding laying hens with diets containing up to 30% guar meal. The interior and exterior quality of eggs were reported not to be deleteriously affected by guar meal feeding (Couch et al., 1967b; Saxena and Pradhan, 1974; Patel and McGinnis, 1985; Zimmermann et al., 1987) except

that the yolk color index decreased with the inclusion of guar meal in laying diets (Verma and McNab, 1984b).

Bakshi et al. (1964) proposed that guar meal contains two deleterious factors: trypsin inhibitor and guar gum residue. However, the trypsin inhibitor was not universally accepted as a primary factor for the deleterious effects of feeding guar product to poultry (Anderson and Warnick, 1964; Vohra and Kratzer, 1964). Verma and McNab (1982) reported that neither heating the guar meal directly nor steam pelleting diets containing guar meal had much effect on the performance of the broiler chicks, which was in agreement with the findings of Nagpal et al. (1971) that autoclaving guar meal did not improve its gross protein value for chicks. The trypsin inhibitor activity in guar meal were reported to be significantly lower than in soybean meal commonly used in poultry feed (Verma and McNab, 1982), which indicates that the negative effects on performance of poultry when fed diets containing guar meal are not likely due to trypsin inhibitor activity.

The gum content of typical guar meals is approximately 18 to 20% (Bakshi et al., 1965; Nagpal et al., 1971). A series of feeding experiments conducted by Vohra and Kratzer (1964) demonstrated that as low as 1% guar gum depressed growth in broiler chickens. When the diet contained 2% guar gum, the relative growth of broiler chickens was 61 to 67.4% of controls. Anderson and Warnick (1964) and Vohra and Kratzer (1964) asserted that residual gum was chiefly responsible for the adverse effects on chicks, not trypsin inhibitor. Research by Lee et al. (2003a) also supported that residual gum was responsible for some deleterious effects seen when guar meal was fed.

Guar gum is a highly viscous galactomannan polysaccharide, consisting of a 1→4-linked β-D-mannopyranose backbone with branchpoints from their 6-positions linked to α-D-galactose (i.e. 1→6-linked-α-D-galactopyranose). There are 1.5 to 2 mannose residues for every galactose residue. Guar gum can dramatically alter the rheological properties of digesta, hence inhibiting the absorption of nutrients (Blackburn and Johnson, 1981). However, the high viscosity of guar gum may contribute to some beneficial physiological functions, including decreasing plasma cholesterol (Favier et al., 1997a; Dario Frias and Sgarbieri, 1998; Favier et al., 1998; Moriceau et al., 2000; Yamamoto et al., 2000), decreasing postprandial serum glucose (Fairchild et al., 1996; Ou et al., 2001) and attenuating postprandial hypotension in type 2 diabetes patients (Groop et al., 1993; Russo et al., 2003). Guar gum has also been found to have putative protective effects against colonization by pathogenic bacteria, and their subsequent proliferation and resultant diarrhea, and to potentiate the effect of polar lipids in preventing microbial translocation in the gastro-intestinal tract (Bengmark, 1998). Although no report on the effect of guar gum on immune function of animals or humans can be found, a high-molecular-weight galactomannan, about 1.0 million Daltons, isolated from the edible mushroom *Morchella esculenta,* was demonstrated to enhance macrophage activation thus exhibiting immunostimulatory activity (Duncan et al., 2002).

In addition to gum residue and trypsin inhibitor, other anti-nutritional factors such as saponins (Verma and McNab, 1984a; Curl et al., 1986) and polyphenols (Bajaj et al., 1978; Kaushal and Bhatia, 1982) were reported to be present in guar seed. Using soy bean saponin as a standard, Verma and McNab (1984a) estimated that the saponin

content in guar meal varies from 9.1 to 13.6%, which is 4 to 5 times the saponin content in alfalfa meal. The total phenols content in guar seed varies from 0.69 to 1.26% depending on the stage of maturity (Kaushal and Bhatia, 1982). The median lethal dose (LD_{50}) of the saponin to mice was found to be 200 mg/kg BW (Diwan et al., 2000). Very low amounts of saponin (Diwan et al., 2000) administered to rats or mice caused damage to liver, kidney, small intestine and thymus. These two toxic compounds could partially contribute to the adverse effects of feeding guar meal to poultry (Kumar, 1991; Diwan et al., 2000).

This experiment was conducted to establish an upper safe feeding level of U.S. guar meal for late phase laying diets and to explore the possibility of using guar meal to induce a "full-fed" molt.

MATERIALS AND METHODS

Hy-Line W-36 late phase hens (72-wk-old) procured from a commercial farm were placed in individual laying cages ($50 \times 30 \times 30$ cm^3) and initially fed a corn-soybean meal based laying hen diet while receiving a 16L:8D photo program. After 32 d, the top 100 hens based on the previous 12 d of egg production were selected from the flock. Hen-day egg production of hens selected was 78.0 ± 12.4%. Hens were assigned to 5 blocks of 4 experimental units per block. Each experimental unit consisted of 5 individual cages so that hen-day egg production of 5 birds averaged as close as possible to 78%. Immediately after the re-allocation, hens were fed one of 4 experimental diets formulated to an electrolyte balance of 200 meq/kg with 0 (Control), 5, 10, or 15% guar meal (Table 3-1) randomly assigned to the 4 units within each block. The nutrient

TABLE 3-1. Composition of experimental diets in experiment 1[1]

Ingredients	Guar meal (%)			
	0	5	10	15
	---------- (%) ----------			
Corn	69.15	67.62	65.47	63.31
Guar meal[2]	0.00	5.00	10.00	15.00
Dehulled soybean meal	17.93	14.13	10.44	6.76
DL-Methionine	0.10	0.11	0.12	0.12
L-Lysine HCl	0.04	0.08	0.12	0.17
L-Threonine	0.02	0.04	0.07	0.09
Fat (animal-vegetable blend)	1.00	1.44	2.09	2.73
Limestone	5.86	5.66	5.66	5.66
Oystershell	4.00	4.00	4.00	4.00
Mono-dicalcium PO_4	1.05	1.06	1.07	1.08
Sodium bicarbonate	0.54	0.56	0.67	0.78
Trace minerals[3]	0.05	0.05	0.05	0.05
Vitamins[4]	0.25	0.25	0.25	0.25

[1] Calculated analysis of all diets was as follows: crude protein, 15.0%; metabolizable energy, 2,850 kcal/kg; calcium, 3.98% for 0% guar diet and 3.90% for 5, 10 and 15% guar diets; available phosphorus, 0.32%; methionine, 0.35%; lysine, 0.74%; threonine, 0.57%; and tryptophan, 0.15-0.16%; Na^++K^+-Cl^-, 200 meq/kg.

[2] See Appendix I for the nutrient matrix.

[3] Trace minerals premix added at this rate yields: 27.50 mg sulphur, 150 mg manganese, 16.80 mg iron, 1.70 mg copper, 125.50 mg zinc, 0.25 mg selenium, 1.05 mg iodine, and 0.84 mg molybdenum per kilogram diet.

[4] Vitamin premix added at this rate yields: 11,023 IU vitamin A, 46 IU vitamin E, 3,850 IU vitamin D_3, 1.47 mg vitamin K, 2.94 mg thiamine, 5.85 mg riboflavin, 20.21 mg pantothenic acid, 0.55 mg biotin, 1.75 mg folic acid, 477.67 mg choline, 16.50 µg Vitamin B_{12}, 45.93 mg niacin, and 7.17 mg pyridoxine per kilogram of diet.

composition of the guar meal used in study was previously determined by Conner (2002) with amino acid analysis by Degussa-Huls Corporation.[1] Feed and water were provided ad libitum. Hens were fed continuously for two 28-d periods. After the 1st period, egg production in hens receiving the 15% guar meal diet essentially had ceased. Therefore, the 15% guar diet was replaced with the control diet (0% guar meal).

Egg production was recorded daily and eggs were collected on Wednesday of each week to measure egg weight, breaking force, Haugh units, and shell thickness. The breaking force of egg shells were measured by Instron[2] (Model 1101) using a 50 kg load cell with a load range of 10 kg and a crosshead speed of 50 mm/min. Albumin heights of broken-out eggs were measured with an AMES micrometer[3] (Model S-6428) at a point halfway between the yolk and the edge of the widest expanse of albumin (USDA, 2000). Haugh units was calculated by the equation:

$$\text{Haugh unit} = 100 \times \log (H + 7.57 - 1.7W^{0.37})$$

where H is albumin height in millimeters, and W is egg weight in grams (Panda, 1996). Egg shell thickness was measured with an AMES micrometer[3] (Model 25ME) at the middle part of eggs after the shell membrane was pealed away from the shell. Yolk color of eggs was measured at the beginning and 4th wk of the experiment by Minolta chromameter[4] (Model CR-200) with the CIELAB (L*, a*, b*) system which describes opponent-color scales, based on the opponent-color theory of human color vision, “a*”

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[1] Degussa-Huls Corporation, Applied Technology Chemical Group, Allendale, NJ.
[2] Instron Corporation, Canton, MA.
[3] AMES Pocket Thickness Measures, Waltham, MA.
[4] Minolta Camera Co. Ltd., 3-13, 2-Chome, Azuchi-Machi, Chuo-Ku, Osaka 541, Japan.

(redness) indicates redness when positive and greenness when negative, "b*" (yellowness) indicates yellowness when positive and blueness when negative, "L*" (luminosity) describes the relationship between reflected and absorbed light, with a value of 100 for white and 0 for black. At the end of the study, two birds selected randomly from each experimental unit were killed by CO_2 asphyxiation, and liver, pancreas, and spleen were excised and weighed. Livers were visually examined to detect incidence of hemorrhage and fat engorgement (Walzem et al., 1993).

Data were modeled as a randomized complete block design (Kuehl, 1999) and analyzed for variance using the General Linear Model procedure of SAS [5] software system with block as a fixed effect factor. Initial shell thickness prior to guar meal feeding was used as a covariate to analyze variance of shell thickness data. Duncan's multiple comparisons were conducted when a significant treatment effect was detected ($P < 0.05$).

RESULTS

Egg Production

During the 1st wk of the experiment, egg production decreased approximately 10% regardless of treatment (Table 3-2). Egg production continued to decline fairly rapidly for all treatments, including the control which declined to an average hen-day production of only 40% by wk 8 of the study. No significant differences in egg production were observed among birds fed 0, 5 and 10% guar meal diets, with an exception that in wk 3 birds receiving 10% guar meal had lower egg production than those receiving the 0 or

[5] The SAS System for Windows, Release 8.1 (TS1M0). SAS Institute Inc., Cary, NC.

TABLE 3-2. Hen-day egg production during 8 wk of feeding diets containing 0, 5, 10 or 15% guar meal [1]

Wk	Guar meal concentration (%)			
	0	5.0	10.0	15.0→0 [3]
	---------- (%) ----------			
0 [2]	78.0 ± 0.5	78.0 ± 0.5	77.7 ± 0.8	78.0 ± 0.5
1	69.7 ± 5.6	68.6 ± 4.5	68.6 ± 11.2	62.9 ± 4.0
2	65.1 ± 8.7 [a]	64.6 ± 8.5 [a]	56.4 ± 12.8 [a]	11.4 ± 7.3 [b]
3	62.9 ± 11.1 [a]	56.6 ± 11.3 [a]	42.1 ± 10.6 [b]	14.6 ± 6.4 [c]
4	53.1 ± 5.9 [a]	53.1 ± 5.9 [a]	46.4 ± 11.6 [a]	18.3 ± 9.6 [b]
5	51.4 ± 12.9 [a]	59.4 ± 11.1 [a]	51.6 ± 11.9 [a]	11.3 ± 6.1 [b]
6	39.4 ± 6.2	40.0 ± 7.3	32.6 ± 12.2	45.4 ± 17.3
7	42.9 ± 9.5 [b]	42.9 ± 7.3 [b]	37.6 ± 10.7 [b]	65.6 ± 8.3 [a]
8	38.9 ± 14.8 [b]	48.0 ± 13.8 [b]	34.9 ± 10.0 [b]	67.0 ± 11.8 [a]
Weekly means				
Wk 1 to 4	62.7 ± 5.5 [a]	60.7 ± 6.7 [ab]	53.1 ± 7.8 [b]	26.0 ± 5.7 [c]
Wk 5 to 8	44.9 ± 8.7	49.9 ± 9.3	41.5 ± 9.5	48.3 ± 10.7
Wk 1 to 8	53.8 ± 7.0 [a]	55.3 ± 7.7 [a]	47.3 ± 8.2 [a]	37.0 ± 7.3 [b]

[a-c] Means within a row lacking a common superscript are significantly different ($P < 0.05$).
[1] Experimental laying hens were 76 wk old at the beginning of experiment.
[2] The hen-day egg production of all treatment groups at the beginning of experiment was as close to 78% as possible.
[3] The 15% guar meal treatment was only fed for 4 wk at which time the 15% diet was replaced with the 0% guar meal diet (control).

5% diets. Interestingly, over the 2nd 28 d period, egg production of birds receiving the 5% guar meal diet were never lower than the egg production of control (0%) birds.

Egg production decreased dramatically in hens fed 15% guar meal to its lowest level (< 12%) 2 wk after guar meal was introduced. Egg production on the 15% guar diet was significantly lower than the other treatment groups. One week after the 15% guar meal feed was replaced with the 0% diet, egg production began to rapidly increase to 67% by wks 7 and 8. This level of egg production exceeded the egg production levels of all other treatment groups. The rapid decline of egg production in hens fed the 15% guar meal diet followed by a potentiated increase upon being fed 0% guar diet suggested that this regime could be used to potentially induce a "full-fed" molt.

Body Weight

Although body weight was not determined at the beginning of the 1st production period, body weight loss became obvious in hens fed the highest concentration of guar meal (Table 3-3). Body weights of laying hens fed 0, 5 and 10% guar meal diets were not significantly different at the end of the 1st 4 wk feeding period. However, during the following 4 wk hens fed 10% guar meal continued to lose body weight relative to those receiving the 0 and 5% diets. After 8 wk of the experiment, hens fed 10% guar meal had significantly lower body weight than 0 and 5% laying hens. Hens fed 5% guar meal maintained a body weight similar to 0% hens. After 4 wk of the experiment, the body weight of laying hens fed 15% guar meal was significantly lower than the 0% group, but the 15% fed hens gained weight by wk 8 after consuming the 0% diet for 4 wk of the 2nd period. These hens attained the highest body weight among all treatment groups by the

TABLE 3-3. Change in body weight and mean daily feed consumption of laying hens fed diets containing 0, 5, 10 or 15% guar meal for 2 production periods [1]

	Guar meal concentration (%)			
	0	5.0	10.0	15.0→0 [2]
Body weight	---------- (g) ----------			
0 d	N. D.[3]	N. D.	N. D.	N. D.
28 d	1441 ± 216 [a]	1408 ± 158 [a]	1396 ± 192 [a]	1286 ± 186 [b]
56 d	1394 ± 171 [ab]	1402 ± 186 [a]	1284 ± 191 [b]	1457 ± 219 [a]
56 d - 28 d [4]	- 48 ± 135 [bc]	- 6 ± 91 [b]	-112 ± 140 [c]	171 ± 136 [a]
Feed intake [5]	---------- (g/d per hen) ----------			
0 to 28 d	89.4 ± 5.6 [a]	83.3 ± 3.4 [ab]	77.0 ± 10.5 [b]	66.4 ± 8.8 [c]
29 to 56 d	75.4 ± 3.9 [b]	74.7 ± 4.7 [b]	68.0 ± 7.0 [c]	93.8 ± 5.1 [a]
0 to 56 d	82.4 ± 3.6 [a]	79.0 ± 3.5 [a]	72.5 ± 8.0 [b]	79.9 ± 6.2 [a]

[a-c] Means within a row lacking a common superscript are significantly different ($P < 0.05$).
[1] Experimental laying hens were 76 wk old at the beginning of experiment.
[2] The 15% guar meal treatment was only fed for 4 wk at which time the 15% diet was replaced with the 0% guar meal diet (control).
[3] No data, body weights were not recorded at the beginning of the study.
[4] Difference in body weight of hens at the end of experiment and at d 28 of experiment.
[5] Mean daily feed intake of hens during each experimental period and the whole study duration.

end of the study. The body weight difference between wk 4 and wk 8 showed that laying hens fed 15% guar meal gained weight after consuming the 0% feed for 4 wk, while those continuously fed 10% guar meal continued to lose body weight.

Feed Consumption

In the 1st period of the experiment, the control hens consumed significantly more feed than hens receiving the 10% guar meal diet, which consumed significantly more feed than hens receiving 15% guar meal (Table 3-3). Feed consumption of hens fed 5% guar meal was intermediary to those hens fed 0 or 10% guar meal, and was not significantly different from either of them. In the 2nd period of the experiment, after the 15% guar meal diet was replaced with the 0% guar meal diet, hens consumed significantly more feed than all other treatment groups (0, 5, and 10% guar meal diets). Feed consumption of hens fed 10% guar meal was significantly lower than those hens fed 0 or 5% guar meal diet. No feed consumption difference was observed between hens fed the 0 and 5% guar meal. For overall feed consumption during the whole experimental period, no significant difference in mean daily feed consumption was observed among hens receiving the 0 and 5% guar meal diets, and the 15 → 0% guar meal combination. However, overall feed consumption of hens fed the 10% guar meal diet was significantly lower than that of all other treatments.

Egg Quality

Mean egg weight (Mean ± SD) of all sampled eggs decreased from 62.6 ± 5.1 g at the beginning of the study to 58.5 ± 4.7 g at the end (See Appendix II). No significant difference on egg weight among hens fed different diets was observed at each time point

of measurement. The Haugh units (Mean ± SD) varied from 70.1 ± 13.8 to 86.7 ± 5.8, with no discernible pattern (See Appendix II). A significant difference in the Haugh units was detected only at the 2nd wk of study when hens fed the 5% guar meal diet had higher values than control hens. At the 1st wk of the experiment, the breaking force and shell thickness of eggs from hens fed 15% guar meal (Mean ± SD: 1.59 ± 0.56 and 0.303 ± 0.029) were significantly lower than that of eggs from hens fed the control diet (Mean ± SD: 2.76 ± 0.75 and 0.339 ± 0.023), however, at the 8th wk of experiment, the breaking force of eggs and shell thickness from those hens initially fed 15% guar meal then 0% guar meal (Mean ± SD: 2.95 ± 0.81 and 0.339 ± 0.021) were significantly higher than that of eggs from control hens (Mean ± SD: 1.99 ± 0.80 and 0.310 ± 0.031) (See Appendix II). No significant difference on egg breaking force and shell thickness was detected among other pairwise comparisons at each time point of measurement. The luminosity (L* value, Mean ± SD: 56.10 ± 1.63, $P = 0.2317$) and yellowness (b* value, Mean ± SD: 42.16 ± 2.57, $P = 0.5681$) of egg yolks among each treatment group were similar to each other at the beginning of experiment. However, by the 4th wk of the experiment, they had decreased linearly in relationship to the guar meal content of the diet (Figure 3-1). Significant differences in redness (a* value) of yolk among hens fed different diets for 4 wk were observed without recognizable pattern (See Appendix II).

Organ Weight and Incidence of Liver Hemorrhage

Absolute and relative liver weight for guar meal fed hens were higher than those for control hens (Table 3-4), but the increase was not incremental to guar meal concentration in the diets. No incidence of hepatic hemorrhage was observed, nor was

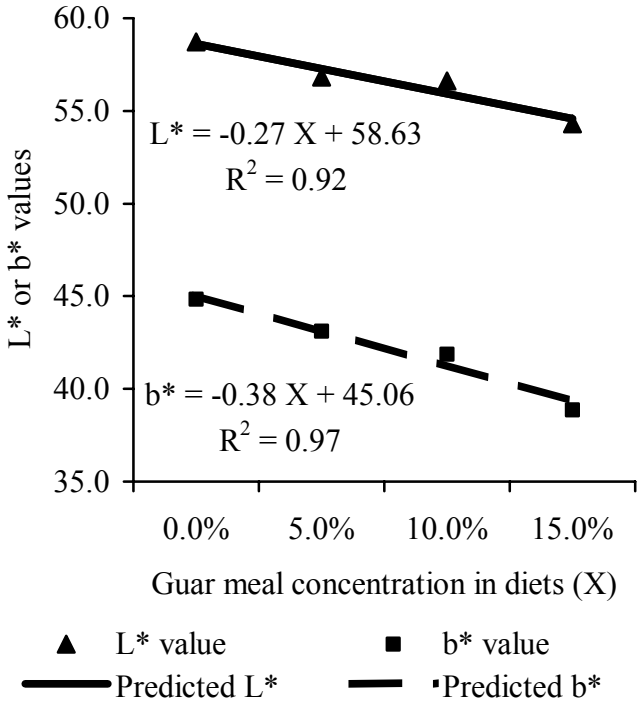


FIGURE 3-1. Egg yolk color (in CIELAB system) of hens after 4 wk of feeding diets containing 0, 5, 10 or 15% guar meal. Data for a* value were not shown as differences on a* value of egg yolk were observed among hens fed different diets without a certain pattern. ▲ and ■ are mean values of L* and b* over all eggs in each treatment sampled on Wednesday of wk 4. ——— and — — are predicted values of L* and b* for each treatment based on the regression equations. The slopes of equations are different from 0 ($P < 0.05$).

TABLE 3-4. Absolute and relative organ weight of hens after 8 wk of feeding diets containing 0, 5, 10 or 15% guar meal[1,2]

Organs		Guar meal concentration (%)			
		0	5.0	10.0	15.0→0 [3]
Liver					
	weight (g)	38.8 ± 7.5 [b]	47.9 ± 6.5 [a]	42.0 ± 6.7 [ab]	46.9 ± 9.7 [a]
	(%)	2.81 ± 0.49 [b]	3.47 ± 0.24 [a]	3.15 ± 0.48 [ab]	3.30 ± 0.40 [a]
Spleen					
	weight (g)	1.03 ± 0.29	1.10 ± 0.43	1.41 ± 0.89	1.06 ± 0.33
	(%)	0.075 ± 0.020	0.078 ± 0.023	0.104 ± 0.060	0.074 ± 0.017
Pancreas					
	weight (g)	2.97 ± 0.60	2.83 ± 0.70	3.20 ± 0.34	2.91 ± 0.51
	(%)	0.215 ± 0.042	0.203 ± 0.036	0.241 ± 0.034	0.207 ± 0.037

[a-b] Means within a row lacking a common superscript are significantly different ($P < 0.05$).
[1] Experimental laying hens were 84 wk old at the time of sampling.
[2] Mean ± SD, n = 10.
[3] The 15% guar meal treatment was only fed for 4 wk at which time the 15% diet was replaced with the 0% guar meal diet (control).

there any evidence of fatty liver. There was no difference of absolute and relative weight of spleen and pancreas among hens fed different guar meal diets.

Mortality

During the 1st 4 wk of experiment, there were 3 deaths among hens fed 10% guar meal, 1 death among hens fed 15% guar meal, and no deaths among hens fed the control and 5% guar meal diets. During the 2nd 4 wk period of the experiment, there was only one death which occurred in hens fed the 0% guar meal diet. Overall mortality was not significantly affected by treatment.

DISCUSSION

Egg production was poorer than expected for all treatments under evaluation including the control group which decreased about 10% during the first wk of the study. The hens were given a 32 d acclimation period after transport from the commercial farm to our research farm and the top 100 hens were laying at 78 ± 12.4% at this time. The birds were then re-allocated to different pens and treatment to begin the study. This sudden change in cage placement and feed may have contributed to the rather poor egg production over the 2 experimental periods. The specific composition of the experimental diets may have also played a significant role in the poor egg production. These diets were formulated on a total electrolyte basis where-by the Na^++K^+-Cl^- ratio was set at exactly 200 meq/kg. Specific concentration of Na^+ and Cl^- were not set and as a consequence the optimal least cost solution did not include NaCl. The resulting dietary Cl^- concentration was calculated to be 0.11% which is slightly lower than the 0.13% requirement specified by the 1994 NRC for hens consuming 100 g of feed per day.

While this potential Cl^- deficiency may have contributed to poor egg production it is interesting to note that the hens switched from the 15% guar diet to the control diet did very well during the 2nd period of production. Given the Cl^- deficit existed uniformly across all treatment groups we feel any conclusion drawn with respect to guar meal concentration still have validity.

There were no significant differences in egg production between hens fed the 0 and 5% guar meal diets throughout the study, which suggests that up to 5% guar meal can be incorporated into laying hen diets without detrimental effects on egg production. Egg production for birds fed 10% guar meal were about 5 percentage points lower than that for birds fed the 0% guar meal diet but no difference was observed between these two groups. These results on egg production agree with Nagra and Virk (1986) who reported that 10% guar meal in place of groundnut cake did not decrease egg production when compared to the control diet. However, other researchers (Bakshi et al., 1964; Couch et al., 1967a; Saxena and Pradhan, 1974; Patel and McGinnis, 1985) reported that 10% raw guar meal in the diet decreased egg production.

For hens fed 15% guar meal, egg production dropped rapidly from 78% to below 12% by the 2nd wk of feeding. Although feed consumption decreased, hens still consumed an average of 66 g/d. After the 15% guar meal diet was replaced with the 0% guar meal diet, egg production recovered and even surpassed that of the control hens. Hens fed 15% guar meal during the 1st 4 wk period and 0% guar meal diet during the 2nd 4 wk period weighed as much or more than any other treatment group by the end of the study. Only one of these particular hens died of the original 25. These facts suggest that

incorporation of 15% guar meal into laying diets may be a promising method to induce a molt without feed deprivation. This result agreed with the report by Patel and McGinnes (1981) who fed 10% raw or autoclaved guar meal (13% CP) to 36-wk-old hens to induce a molt. In their study egg production decreased to 0% and 8% after 1 wk of feeding. The authors also reported that 1 wk after the guar meal diets were replaced with the control diet, egg production began to recover and exceeded 85% within 2 wk. Egg production of hens molted by guar meal feeding exceeded non-molted hens by 10 to 15%. Zimmermann et al. (1987) also reported that feeding 15% guar meal caused a slow cessation of lay with acceptable post-molt laying performance.

Over the course of the study, feed consumption, egg weight and egg production for hens receiving 5% guar meal were similar to those of control hens. No significant differences suggesting trends in egg weight, Haugh units, or shell thickness, were observed in this study. Couch et al. (1967a) also reported that the egg weights were not reduced by 10% guar meal feeding. The 15% guar meal group had the lowest egg breaking force in the 1st wk of experiment and highest breaking force by the end of study, suggesting that using high concentrations of dietary guar meal to induce a molt may benefit egg shell quality, which agrees with Patel and McGinnis (1985) who indicated that egg shell quality was better for hens on the diet containing guar meal than for those on the control diet.

Verma and McNab (1984b) reported a decrease in yolk color index when laying hens were fed 10, 20 or 30% guar meal. In the present study, egg yolk color was affected by guar meal feeding in that the luminosity and yellowness of yolk were linearly

decreased by feeding different levels of dietary guar meal for 4 wk, whereas the redness was affected without a certain pattern. However, whether the decreases in measured values of luminosity and yellowness lead to a decrease in Roche color value, and whether the color change is visually perceivable by table-egg consumers are uncertain. By measuring the CIELAB values of a Roche color fan with the same chromameter used in this experiment, we observed linear relationships between Roche values and CIELAB values with R^2 of 0.96 to 0.99, in that when the Roche color value increases by 1, the L* value decreases by 0.73, the a* and b* values increase by 2.63 and 4.53 respectively. In a study by Roberson (2004), even though Minolta chromameter (Model 310) detected remarkable changes in L*, a* and b* values of egg yolks by feeding laying hens 15% corn dried distillers grains with solubles, the differences in Roche color value were less than 0.2 and without statistical significance.

Significant increases in relative weight of pancreas were observed when 10% raw or 30% heat processed guar meal (Couch et al., 1967a), or 2% guar gum (Vohra and Kratzer, 1964) were fed to broiler chickens. Contradictorily, Bakshi et al. (1964) and Brahma et al. (1982) reported no abnormalities or significant changes in relative weights of pancreas, liver and spleen when 10% raw or up to 16% toasted guar meal was fed to laying hens or broiler chickens respectively. Saponin (Verma and McNab, 1984a) and polyphenols (Kaushal and Bhatia, 1982) could contribute to the toxic effects of guar meal. Diwan et al. (2000) reported the median lethal dose (LD_{50}) of the saponin isolated from plant *Citrullus colocynthis* to mice as 200 mg/kg BW, and 100 mg/kg BW saponin administered to mice caused histopathological changes in the small intestine, liver and

kidneys. In the present study, guar meal at the concentrations tested did not appear to be toxic enough to affect the weight of pancreas or spleen, nor were there any evidence of liver hemorrhage. Although liver weights were increased by guar meal feeding, this was not proportional to the level of dietary guar meal.

From this study, we concluded that: 1) Guar meal can be incorporated into laying hen diets for up to 8 wk at concentrations up to 5% without adverse effects; 2) Guar meal at 10% of the diet decreases feed consumption and increases body weight loss; 3) Feeding 15% guar meal to laying hens depresses feed consumption and egg production, and may serve as a molt-inducing diet. After a 28-d molting period with 15% guar meal, laying hen performance was significantly improved; 4) Egg weight, Haugh units and shell quality is not affected by up to 15% dietary guar meal, but the values for luminosity and yellowness of egg yolk decrease linearly as total xanthophylls are diluted by the guar meal.

CHAPTER IV

EVALUATION OF GUAR BY-PRODUCTS IN HIGH PRODUCTION LAYING HEN DIET

INTRODUCTION

Guar (*Cyamopsis tetragonoloba*), a drought-tolerant legume, is primary used in the gum industry because its seeds contain high amounts of guar gum (galactomannan) which is used as a thickener in food, pharmaceuticals, paper and other industries. To produce gum, guar seeds are split, yielding both a high protein germ fraction and a lower protein husk fraction as by-products. These two fractions are usually recombined to create guar meal with a crude protein level of 35 to 47.5% on a dry matter basis depending on the relative concentration of the 2 fractions (Ambegaokar et al., 1969). Verma and McNab (1984a) reported about 88% of the nitrogen content was true protein. However, some essential amino acids such as methionine and lysine in guar meal had been reported to be inadequate for optimum rat growth (VanEtten et al., 1961), while arginine concentration in guar meal was about two times higher than in soybean meal (Verma and McNab, 1984a). Despite some disadvantages, the low price of guar meal makes it a potentially valuable protein source for the feed industry. Currently, the guar meal is sold at half the price of soybean meal, and is most commonly used in cattle feedlot operations.

The use of guar meal in poultry feed is limited because of reported adverse effects which includes diarrhea, depressed growth rate and increased mortality (Sathe and Bose,

1962; Couch et al., 1967b; Thakur and Pradhan, 1975a; Verma and McNab, 1982; Patel and McGinnis, 1985; Conner, 2002). Residual guar gum, a highly viscous galactomannan polysaccharide consisting of a 1→ 4-linked β-D-mannopyranose backbone with branched 1→6- α-D-galactopyranose, is probably the primary factor for the reported ill effects (Verma and McNab, 1982; Conner, 2002; Lee et al., 2003a), although there may be some other anti-nutritional factors such as saponins (Verma and McNab, 1984a; Curl et al., 1986) and polyphenols (Bajaj et al., 1978; Kaushal and Bhatia, 1982). Both saponins and polyphenols have been shown to cause damage to internal organs (liver, kidney, small intestine or thymus) of mice or rats (Berman et al., 1995; Diwan et al., 2000). While the anti-nutritional properties of guar meal are well established, numerous investigations have shown some beneficial physiological functions of galactomannans, such as guar gum, including decreased plasma cholesterol (Favier et al., 1997a; Dario Frias and Sgarbieri, 1998; Favier et al., 1998; Yamamoto et al., 2000; Maisonnier et al., 2001), decreased postprandial serum glucose (Fairchild et al., 1996; Ou et al., 2001), attenuating postprandial hypotension in type 2 diabetes patients (Groop et al., 1993; Russo et al., 2003), preventing colonization of pathogenic bacteria (Bengmark, 1998), and enhancing macrophage activation (Duncan et al., 2002).

Previous investigations on the effects of guar by-products on laying hen performance are sparse, and most of them were carried out on late phase laying hens (Saxena and Pradhan, 1974; Patel and McGinnis, 1981; Verma and McNab, 1984b; Patel and McGinnis, 1985; Nagra and Virk, 1986; Zimmermann et al., 1987). These generally agreed that 10% or higher concentration of guar meal incorporated into laying hen diets

deceased egg production and feed conversion efficiency, and had ill effect on egg yolk color. However, no study using lower concentrations of guar by-products such as germ meal in peaking pullets can be found. Therefore, the objective of the present study was to investigate the effects of relatively low concentration of guar germ and guar meal on egg production, egg interior and shell quality of high production laying hens.

MATERIALS AND METHODS

Experimental Design

This experiment followed a Latin square design with a linear arrangement of experimental units to diminish the experimental error associated with cage location. A total of 125 laying hen pullets (Lohmann LSL-classic, 19-wk-old) of similar BW were randomly assigned to 125 individual cages ($50 \times 30 \times 30$ cm^3) in one row in an open sided laying hen house. The 125 cages were divided into 5 blocks, with 25 cages per block. The 25 cages were divided into 5 experimental units, with 5 cages in each unit. After a 2-wk adaptation period, feeding a commercial type pre-lay pullet diet, five treatment diets were assigned to the 5 experimental units in each block using a Latin square design randomization. The dietary treatments consisted of 5 isocaloric, isoprotein laying hen diets with 0 (the control), 2.5 or 5.0% guar germ or guar meal (Table 4-1). The compositions of guar germ and meal fractions used in the study were previously determined by Conner (2002) with amino acid analysis by Degussa-Huls Corporation.[1] The gum residue in guar germ and guar meal were determined by HPLC (Hansen et al., 1992) to be 7.75% and 11.89% respectively, thus producing a calculated gum content in the 5 experimental diets of 0, 0.194, 0.297, 0.388, and 0.595%, respectively.

TABLE 4-1. Composition of experimental diets in experiment 2[1]

Ingredients (%)	Control	Guar germ (%)		Guar meal (%)	
		2.5	5.0	2.5	5.0
Corn	55.54	55.00	54.46	54.54	53.53
Guar germ fraction[2]	0	2.50	5.00	0	0
Guar meal[2]	0	0	0	2.50	5.00
Dehulled soybean meal	30.00	27.84	25.67	28.20	26.40
DL-Methionine	0.17	0.18	0.18	0.18	0.18
Fat (animal-vegetable blend)	2.23	2.42	2.62	2.53	2.82
Limestone	5.93	5.94	5.94	5.93	5.93
Oyster shells	4.00	4.00	4.00	4.00	4.00
Mono-dicalcium PO_4	1.46	1.46	1.47	1.46	1.46
Salt	0.36	0.36	0.36	0.36	0.36
Trace minerals[3]	0.05	0.05	0.05	0.05	0.05
Vitamins[4]	0.25	0.25	0.25	0.25	0.25

[1] Calculated analysis of all diets was as follows: crude protein, 19.6%; metabolizable energy, 2,800 kcal/kg; calcium, 4.10%; available phosphorus, 0.42%; methionine, 0.47-0.48%; lysine, 1.00-1.03%; threonine, 0.71-0.74%; and tryptophan, 0.23%.

[2] See Appendix I for the nutrient matrix.

[3] Trace minerals premix added at this rate yields: 27.5 mg sulphur, 150 mg manganese, 16.80 mg iron, 1.70 mg copper, 125.50 mg zinc, 0.25 mg selenium, 1.05 mg iodine, 0.84 mg molybdenum per kilogram diet.

[4] Vitamin premix added at this rate yields: 11,023 IU vitamin A, 46 IU vitamin E, 3,850 IU vitamin D3, 1.47 mg vitamin K, 2.94 mg thiamine, 5.85 mg riboflavin, 20.21 mg pantothenic acid, 0.55 mg biotin, 1.75 mg folic acid, 477.67 mg choline, 16.50 ug Vitamin B12, 45.93 mg niacin, and 7.17 mg pyridoxine per kilogram of diet.

Experimental feed and water were provided to all laying hens ad libitum. All hens received a 14L:10D photo program at 21 wk, and a 15 min/wk increase of light thereafter, until hens eventually received a 16L:8D photo program which was maintained for the remainder of the experiment.

Measurements

Individual BW of laying hens were measured at the beginning of the experiment and at the end of each 4 wk period for a total of 20 wk. Feed consumption and hen-day egg production were recorded weekly based on the 5 hens in each unit. All the eggs laid on Wednesday of each week were collected to measure egg weight individually. Total egg mass was calculated weekly by summing up the product of weekly mean egg weight and egg number in each experiment unit. Feed conversion ratio (FCR) was calculated as grams of total feed consumption per hen/total egg mass per hen. Egg shell quality (breaking force, thickness) and egg interior quality (yolk color, Haugh units) were measured on Wednesday of every other week using methods described previously (Chapter III). Specific gravity was measured on Wednesday of every other week by weighing the eggs immersed into tap water at room temperature, and calculated by equation:

Specific gravity = egg weight/loss of weight in water

Statistical Procedure

The experiment was modeled as a Latin square design (Kuehl, 1999). The data for one-time measured responses (BW gain, overall hen-day egg production, feed consumption, and feed conversion ratio, mean egg weight and total egg mass per hen)

were subjected to ANOVA directly. The data for repeated measured responses (BW, periodical egg production, egg weight, feed consumption, feed conversion ratio, Haugh units, yolk color, shell quality, and solid egg components) were first subjected to analysis of time trend effect and time × treatment interaction by the MANOVA procedure because the Sphericity test revealed that the correlations between each two time points were different ($P \leq 0.05$). The MANOVA analysis revealed that either time effects or time × treatment interaction or both existed ($P \leq 0.05$) in almost all of these responses so data were then analyzed for variance based on each time point of measurements. Analyses of variance were carried out using the GLM procedure of the SAS [5] software system with fixed effect factors of row and column in square, guar fraction and level. The PDIFF option within the GLM procedure was used to compare each mean of guar treatment to the control. Contrasts were constructed to exam main effects and the interaction of guar fraction and level. Means presented were LSmeans, and were deemed different when $P \leq 0.05$.

RESULTS

Body Weight

The mean BW of laying hens were almost the same for each treatment group (Table 4-2) at the beginning of experiment (20-wk of age), and increased at different rates during the study. The BW of each guar treated group was not different from the control in each period. Main effects of guar fraction and concentration as well as the fraction × concentration interaction were not significant with respect to any specific time during the study. However, the cumulative BW gain throughout the whole experimental

TABLE 4-2. Body weight, egg production and feed consumption of laying hens fed diets with 0 (control), 2.5 or 5% guar germ or guar meal [1]

Fraction	Level (%)	Body weight (g)							Hen-day egg production (%)						Feed consumption (g/d per hen)					
		20 wk	24 wk	28 wk	32 wk	36 wk	40 wk	Gain	21-24 wk	25-28 wk	29-32 wk	33-36 wk	37-40 wk	Overall	21-24 wk	25-28 wk	29-32 wk	33-36 wk	37-40 wk	Overall
Control	0.0	1462	1505	1539	1575	1582	1607	145	89.00	99.00	96.71	98.43	97.29	96.09	92.7	101.3	101.0	100.0	100.7	99.2
Germ	2.5	1467	1533	1571	1603	1609	1630	170	90.43	95.79‡	96.58	97.32	92.36	94.43	96.9	104.3	102.5	100.9	101.3	101.2
Meal	2.5	1459	1552	1598	1638	1656	1670	219‡	89.86	97.86	95.87	95.80	93.14	94.48	101.4‡	104.5	105.1	102.1	102.0	103.0
Germ	5.0	1461	1513	1585	1612	1602	1635	174	85.71	97.71	96.85	95.86	95.57	94.34	95.3	106.6	104.1	99.1	102.4	101.5
Meal	5.0	1457	1498	1530	1540	1528	1580	123	90.71	97.57	98.57	98.00	96.72	96.31	99.0‡	103.7	103.8	102.4	105.9	103.0
Pooled MSE		137	125	141	159	165	170	107	3.94	1.43	2.09	2.58	5.15	1.50	3.0	3.3	3.3	4.0	4.8	3.1
Main effect																				
Fraction																				
Germ		1464	1523	1578	1608	1606	1633	172	88.07	96.75	96.72	96.59	93.96	94.39	96.1	105.5	103.3	100.0	101.9	101.4
Meal		1458	1525	1564	1589	1592	1625	171	90.29	97.72	97.22	96.90	94.93	95.40	100.2	104.1	104.5	102.3	104.0	103.0
Level																				
2.5%		1463	1543	1585	1621	1633	1650	195	90.14	96.82	96.23	96.56	92.75	94.46	99.2	104.4	103.8	101.5	101.7	102.1
5.0%		1459	1506	1558	1576	1565	1608	149	88.21	97.64	97.71	96.93	96.14	95.33	97.2	105.2	104.0	100.8	104.2	102.3
Probability																				
Fraction × level		0.941	0.500	0.153	0.095	0.079	0.173	0.023#	0.140	0.110	0.218	0.138	0.940	0.179	0.784	0.321	0.353	0.577	0.541	0.890
Fraction		0.821	0.918	0.633	0.584	0.680	0.827	0.977	0.234	0.158	0.602	0.793	0.683	0.159	0.010*	0.381	0.439	0.237	0.345	0.254
Level		0.900	0.140	0.346	0.189	0.056	0.266	0.048	0.296	0.225	0.138	0.754	0.167	0.216	0.158	0.620	0.924	0.708	0.265	0.921

‡ Mean of the treatment was significantly different from the control ($P \leq 0.05$).
# Interaction of guar fraction × level existed ($P \leq 0.05$).
* Significant main effect was detected ($P \leq 0.05$).
[1] Experimental laying hens were 21 wk old at the beginning of experiment.

period for hens fed 2.5% guar meal were significantly higher than the controls. A guar fraction × concentration interaction was detected, in that the BW gain decreased from 219 to 123 g when the concentrations of guar meal increased from 2.5 to 5% while the BW gain remained the same when guar germ level increased from 2.5 to 5%. Hens fed guar germ had numerically higher BW gain than hens fed the control.

Laying Hen Performance

Hen-Day Egg Production. Egg production of the whole experimental flock was 74.17% for the first week of the experiment (21-wk-old), and reached a peak (98.05%) at 27 wk of age. All groups had excellent egg production during the study (Figure 4-1). For periodical egg production, significant differences existed only between 2.5% guar germ fed hens and the control hens in the 2nd period (25 to 28 wk, Table 4-2). For the overall egg production during the experiment, each guar treated group was not significantly different from the control, although hens fed 2.5 or 5% guar germ, and 2.5% guar meal diets were about 1.6% lower. No main effects and interactions of guar fraction and level were detected on any periodical and overall egg production.

Feed Consumption. Numerically hens fed diets with guar fractions consumed more feed than hens fed the control diet, however, the statistically significant difference existed only in the first period of egg production (21 to 24 wk) in which hens fed guar meal consumed more feed than hens fed the control diet (Table 4-2). In this period, main effects of guar fraction were also presented in that guar meal fed hens had higher feed consumption than guar germ treated hens. Thereafter, no difference between each guar treatment group and the control was observed, neither were main effects and interactions

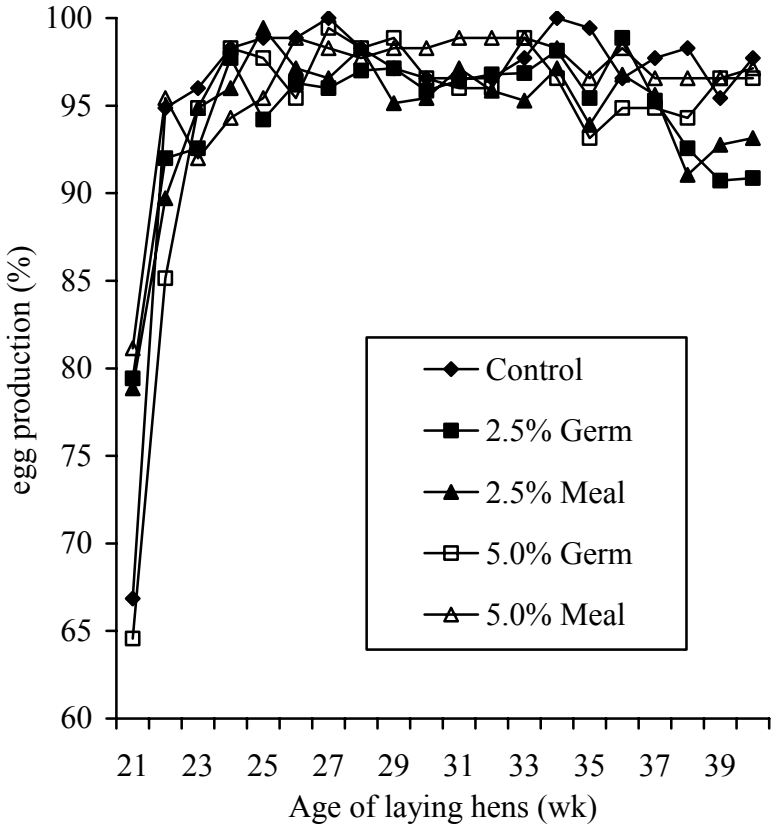


FIGURE 4-1. Hen-day egg production of hens fed diets with 0 (control), 2.5, or 5.0% guar germ or guar meal.

of guar fraction and level. For the pooled feed consumption, no difference was observed between hens fed guar fractions and the control, although guar germ and meal fed hens had about 2 and 4 g/d per hen higher feed intake than the control. No main effects and interactions of guar fraction and level were detected related to overall feed consumption.

Egg Weight. Egg weight increased as the age of hens increased. However, no significant difference between each guar diet fed group and the control was observed in each period and in the overall mean egg weight (Table 4-3). Nor were there significant main effects and interactions of guar fractions and concentrations in each period. However, main effects were detected in overall mean egg weight between hens fed 2.5 and 5% guar fractions, with the latter higher than the former about 1.3 g.

Total Egg Mass. The 2.5% guar meal fed group had significantly lower total egg mass per hen than the control while the other guar treated groups were not different from the control (Table 4-3). An interaction of guar fraction × level was detected in that when the level of guar meal in diets increased from 2.5 to 5%, the total egg mass per hen increased from 7,422 to 7,856 g, but no increase was observed when the level of guar germ in diets increased from 2.5 to 5%.

Feed Conversion Ratio (FCR). For periodical FCR, significant differences existed only between the 2.5% guar meal group and the control during the 3rd, 4th and 5th periods (29 to 40 wk) when the former had higher FCR than the latter, while other guar treated groups were not different from the control during any period (Table 4-3). An interaction of guar fraction × level was detected during the 3rd and 4th periods in that the FCR decreased when guar meal level increased but it remained unchanged or increased

TABLE 4-3. Egg weight, total egg mass and feed conversion ratio of laying hens fed diets with 0 (control), 2.5 or 5% guar germ or guar meal [1]

Fraction	Level (%)	Egg weight (g)						Total egg mass (g/hen)	FCR					
		21-24 wk	25-28 wk	29-32 wk	33-36 wk	37-40 wk	Overall		21-24 wk	25-28 wk	29-32 wk	33-36 wk	37-40 wk	Overall
Control	0.0	52.3	56.7	58.4	59.5	59.7	57.5	7,711	2.002	1.808	1.792	1.710	1.724	1.798
Germ	2.5	52.9	56.5	59.1	59.5	60.6	57.4	7,652	2.040	1.927	1.792	1.736	1.816	1.856
Meal	2.5	52.1	55.7	57.2	57.4	59.0	56.3	7,422‡	2.190	1.921	1.923‡	1.862‡	1.867‡	1.940‡
Germ	5.0	53.5	57.1	59.2	59.4	61.0	58.1	7,663	2.098	1.916	1.816	1.746	1.761	1.855
Meal	5.0	53.4	57.3	59.5	60.0	61.0	58.3	7,856	2.067	1.855	1.769	1.741	1.798	1.834
Pooled MSE		3.0	3.3	3.3	3.6	3.4	3.1	155	0.133	0.072	0.062	0.067	0.070	0.044
Main effect														
Fraction														
Germ		53.2	56.8	59.2	59.4	60.8	57.8	7,658	2.069	1.922	1.804	1.741	1.788	1.856
Meal		52.8	56.5	58.3	58.7	60.0	57.3	7,639	2.129	1.888	1.846	1.801	1.832	1.887
Level														
2.5%		52.5	56.1	58.1	58.4	59.8	56.9	7,537	2.115	1.924	1.857	1.799	1.841	1.898
5.0%		53.5	57.2	59.4	59.7	61.0	58.2	7,760	2.082	1.885	1.793	1.743	1.780	1.845
Probability														
Fraction × level		0.555	0.396	0.094	0.056	0.213	0.278	0.010#	0.153	0.407	0.007#	0.050#	0.824	0.019#
Fraction		0.515	0.635	0.197	0.293	0.229	0.446	0.791	0.331	0.313	0.157	0.067	0.183	0.133
Level		0.130	0.099	0.067	0.099	0.099	0.028*	0.007	0.591	0.245	0.037	0.091	0.071	0.017

‡ Mean of the treatment was significantly different from the control ($P \leq 0.05$).
# Interaction of guar fraction × level existed ($P \leq 0.05$).
* Significant main effect was detected ($P \leq 0.05$).
[1] Experimental laying hens were 21 wk old at the beginning of experiment.

when guar germ level increased. No main effect of guar fraction or level was observed. The consistently higher FCR of 2.5% guar meal group contributed to its higher overall FCR when compared to the control, while other guar treated groups were not different from the control. The decrease of overall FCR from 1.940 to 1.834 when guar meal level increased from 2.5 to 5% led to the interaction of guar fraction and level, since guar germ did not affect FCR as the level increased from 2.5 to 5%.

Egg Quality

Haugh Units. The Haugh units of eggs from control hens were higher than those from the 5% guar meal fed hens in wk 27, 29, 31 and 39, and higher than the 2.5% guar meal fed hens in wk 37 (Table 4-4). No main effect of guar fraction or level was detected. The interactions of guar fraction and concentration were detected only in wk 31 and 33, in which the Haugh units increased when the guar germ concentration increased while it decreased when guar meal concentration increased.

Yolk Color. The luminosity values of egg yolk of guar treated groups were similar to the control with a few exceptions. The 5% guar meal group was higher than the control in wk 21 and lower than the control in wk 25, and the 5% guar germ treated group was lower than the control in wk 25 and 33 (Table 4-5). Mean luminosity values of egg yolk for guar germ and guar meal treated hens were similar. However, main effects of the concentration of guar product were detected at 5 of 10 measurements with a pattern that the luminosity decreased as the concentration increased.

The redness values of the egg yolk of guar treated groups were similar to the control with a few exceptions that had no discernable pattern. Unlike the luminosity

TABLE 4-4. Haugh units of eggs of laying hens fed diets with 0 (control), 2.5 or 5% guar germ or guar meal [1]

Fraction	Level (%)	Age of laying hens (wk)									
		21	23	25	27	29	31	33	35	37	39
Control	0.0	89.5	88.5	84.5	86.1	85.6	79.1	81.8	83.2	87.9	82.2
Germ	2.5	87.8	87.7	84.2	84.0	82.7	75.4	78.6	82.6	85.5	79.2
Meal	2.5	86.9	86.7	83.7	84.1	83.6	78.7	79.9	81.8	84.1‡	82.1
Germ	5.0	86.2	88.7	84.5	83.4	84.5	78.5	82.3	82.2	87.1	79.4
Meal	5.0	85.7	87.9	84.2	81.9‡	81.6‡	75.2‡	78.7	80.8	84.9	78.3‡
Pooled MSE		6.2	5.3	6.6	5.7	5.4	6.0	5.9	5.4	4.7	5.6
Main effect											
Fraction											
Germ		87.0	88.2	84.4	83.7	83.6	77.0	80.5	82.4	86.3	79.3
Meal		86.3	87.3	84.0	83.0	82.6	77.0	79.3	81.3	84.5	80.2
Level											
2.5%		87.4	87.2	84.0	84.1	83.2	77.1	79.3	82.2	84.8	80.7
5.0%		86.0	88.3	84.4	82.7	83.1	76.9	80.5	81.5	86.0	78.9
Probability											
Fraction × level		0.714	0.954	0.804	0.465	0.088	0.009#	0.023#	0.674	0.667	0.101
Fraction		0.716	0.530	0.788	0.525	0.403	0.900	0.519	0.505	0.095	0.387
Level		0.406	0.308	0.869	0.290	0.967	0.993	0.276	0.545	0.261	0.184

‡ Mean of the treatment was significantly different from the control ($P \leq 0.05$).
# Interaction of guar fraction × level existed ($P \leq 0.05$).
* Significant main effect was detected ($P \leq 0.05$).
[1] Experimental laying hens were 21 wk old at the beginning of experiment.

TABLE 4-5. Egg yolk color (L*, a*, and b* values) of laying hens fed diets with 0 (control), 2.5 or 5% guar germ or guar meal [1]

		Age of laying hens (wk)									
		21	23	25	27	29	31	33	35	37	39
Fraction	Level (%)	---------- Luminosity (L* value) ----------									
Control	0.0	56.08	58.00	60.54	59.61	59.99	58.61	59.84	59.58	59.27	58.02
Germ	2.5	57.55	58.04	60.23	59.93	60.25	59.14	60.17	59.48	59.70	58.29
Meal	2.5	57.16	57.73	59.73	59.65	60.01	58.49	60.04	59.49	59.64	58.19
Germ	5.0	56.98	57.21	59.49‡	59.26	59.56	58.05	58.84‡	58.83	58.42	57.51
Meal	5.0	57.89‡	57.64	59.10‡	59.26	59.72	58.30	59.34	59.15	58.84	57.61
Pooled MSE		2.33	1.67	1.45	1.54	1.41	1.44	1.27	1.47	1.39	1.48
Main effect											
Fraction											
Germ		57.26	57.62	59.86	59.59	59.91	58.60	59.51	59.16	59.06	57.90
Meal		57.52	57.69	59.41	59.45	59.86	58.39	59.69	59.32	59.24	57.90
Level											
2.5%		57.35	57.88	59.98	59.79	60.13	58.82	60.10	59.49	59.67	58.24
5.0%		57.43	57.42	59.29	59.26	59.64	58.17	59.09	58.99	58.63	57.56
Probability		---------- *P*-value ----------									
Fraction × level		0.345	0.343	0.863	0.527	0.468	0.130	0.237	0.634	0.445	0.783
Fraction		0.722	0.726	0.144	0.730	0.858	0.474	0.497	0.649	0.508	0.987
Level		0.912	0.171	0.032*	0.121	0.083	0.043*	0.001*	0.120	0.002*	0.039*
Fraction	Level (%)	---------- Redness (a* value) ----------									
Control	0.0	-0.54	-2.33	-3.65	-3.34	-3.94	-3.90	-4.10	-4.16	-4.07	-2.47
Germ	2.5	-1.18	-2.49	-3.42	-3.27	-3.69	-3.62	-3.56‡	-3.87‡	-3.78	-2.43
Meal	2.5	-1.07	-2.69	-3.69	-3.41	-4.02	-4.19‡	-4.33	-4.17	-4.07	-2.58
Germ	5.0	-1.27	-2.59	-3.59	-3.32	-3.85	-3.72	-3.78‡	-4.06	-3.86	-2.57
Meal	5.0	-1.16	-2.64	-3.72	-3.65	-4.28‡	-4.20‡	-4.06	-4.35	-4.15	-3.03‡
Pooled MSE		0.91	0.60	0.51	0.61	0.51	0.45	0.47	0.43	0.43	0.58
Main effect											
Fraction											
Germ		-1.23	-2.54	-3.50	-3.29	-3.77	-3.67	-3.67	-3.96	-3.82	-2.50
Meal		-1.12	-2.67	-3.70	-3.53	-4.15	-4.19	-4.19	-4.26	-4.11	-2.80

TABLE 4-5. Continued

		Age of laying hens (wk)									
		21	23	25	27	29	31	33	35	37	39
Level											
2.5%		-1.13	-2.59	-3.55	-3.34	-3.85	-3.90	-3.95	-4.02	-3.93	-2.50
5.0%		-1.22	-2.62	-3.65	-3.49	-4.06	-3.96	-3.92	-4.20	-4.00	-2.80
Probability		---------- *P*-value ----------									
Fraction × level		0.719	0.586	0.521	0.413	0.634	0.755	0.009[#]	0.895	0.888	0.268
Fraction		0.500	0.330	0.074	0.064	0.001*	0.000*	0.000	0.001*	0.004*	0.018*
Level		0.546	0.911	0.311	0.237	0.052	0.387	0.974	0.050*	0.466	0.015*
Fraction	Level (%)	---------- Yellowness (b* value) ----------									
Control	0.0	44.73	41.57	42.46	43.45	40.59	41.76	40.77	40.09	38.49	42.53
Germ	2.5	44.98	42.27	42.80	44.09	40.53	41.37	41.58	39.69	38.19	42.12
Meal	2.5	44.48	42.07	42.30	44.01	40.50	42.43	41.60	39.47	38.39	42.33
Germ	5.0	45.02	42.34	43.28	43.71	40.96	42.14	40.69	39.30	38.80	41.81
Meal	5.0	44.48	42.86	42.98	43.89	40.26	41.65	41.67	39.64	38.75	42.66
Pooled MSE		0.83	0.54	0.45	0.50	0.50	0.46	0.55	0.54	0.49	0.67
Main effect											
Fraction											
Germ		45.00	42.31	43.04	43.90	40.75	41.75	41.13	39.49	38.49	41.96
Meal		44.48	42.46	42.64	43.95	40.38	42.04	41.63	39.56	38.57	42.50
Level											
2.5%		44.73	42.17	42.55	44.05	40.52	41.90	41.59	39.58	38.29	42.23
5.0%		44.75	42.60	43.13	43.80	40.61	41.89	41.18	39.47	38.77	42.24
Probability		---------- *P*-value ----------									
Fraction × level		0.712	0.540	0.777	0.716	0.508	0.080	0.388	0.539	0.740	0.631
Fraction		0.592	0.764	0.338	0.879	0.450	0.466	0.373	0.810	0.972	0.402
Level		0.744	0.398	0.172	0.552	0.858	0.960	0.441	0.741	0.324	0.955

[‡] Mean of the treatment was significantly different from the control ($P \leq 0.05$).
[#] Interaction of guar fraction × level existed ($P \leq 0.05$).
* Significant main effect was detected ($P \leq 0.05$).
[1] Experimental laying hens were 21 wk old at the beginning of experiment.

value, the redness value of yolks was not affected by the concentration of guar product, but the type of fraction had significant main effects in wk 29 and thereafter with a pattern that guar meal treatments had lower redness values than guar germ treatments.

For the yellowness value, no difference between guar treated groups and the control, nor main effects and interaction of guar fraction and level were detected during the study.

Egg Shell Quality. With respect to egg shell quality (breaking force, thickness and specific gravity), differences between guar treated groups and the control, as well as main effects of guar fraction and concentration were detected sparsely (Table 4-6). Interaction of guar fraction and level on shell quality was not observed.

Egg Components

The percentage of water in eggs was very steady with average water content of all eggs tested of 69.6%, which led to an increasing mean dry egg weight from an average of 15.8 in 22 wk to an average of 18.6 g in 40 wk, and fresh egg weight increased from an average of 51.8 in 22 wk to an average of 60.9 g in 40 wk (Table 4-7). With respect to egg solids, the mean yolk weight of all eggs measured increased from 5.67 to 8.09 g when hens aged from 22 to 40 wk, contributing to an increasing egg yolk content from 36.0 to 43.5% (Table 4-8). The dry weight of albumin was also very steady at about 4.7 g of average over all eggs measured, which led to a decreasing mean albumin percentage from 29.4 to 25.0% from wk 22 to 40 (Table 4-9). The mean dry weight of egg shell had a tiny but recognizable increase from 5.5 g in wk 22 to 5.9 g in wk 40, which was not comparable to the increase of dry egg weight, and led to a

TABLE 4-6. Shell quality of eggs of laying hens fed diets with 0 (control), 2.5 or 5% guar germ or guar meal [1]

		Age of laying hens (wk)									
		21	23	25	27	29	31	33	35	37	39
Fraction	Level (%)	---------- Breaking force (kg) ----------									
Control	0.0	3.996	4.318	4.162	4.061	3.992	3.952	3.083	3.896	3.320	3.491
Germ	2.5	4.149	4.216	4.166	3.832	3.706	3.993	3.553	3.892	3.228	3.662
Meal	2.5	4.260	4.527	4.390	4.060	4.085	4.412	3.433	3.898	3.574	4.164‡
Germ	5.0	4.724‡	4.140	4.195	4.110	3.917	3.821	3.488	3.816	3.333	3.807
Meal	5.0	3.937	4.297	3.878	4.131	3.891	3.688	3.145	3.796	3.712	3.610
Pooled MSE		0.940	1.108	1.080	0.990	1.100	0.900	0.768	0.924	0.760	0.972
Main effect											
Fraction											
Germ		4.437	4.178	4.180	3.971	3.811	3.907	3.521	3.854	3.280	3.734
Meal		4.099	4.412	4.134	4.095	3.988	4.050	3.289	3.847	3.643	3.887
Level											
2.5%		4.205	4.371	4.278	3.946	3.895	4.203	3.493	3.895	3.401	3.913
5.0%		4.330	4.218	4.037	4.121	3.904	3.754	3.316	3.806	3.522	3.709
Probability		---------- *P*-value ----------									
Fraction × level		0.063	0.784	0.229	0.618	0.525	0.143	0.435	0.804	0.880	0.094
Fraction		0.135	0.385	0.844	0.537	0.372	0.498	0.183	0.928	0.037*	0.472
Level		0.635	0.551	0.297	0.441	0.842	0.025*	0.242	0.776	0.433	0.304
Fraction	Level (%)	---------- Thickness (mm) ----------									
Control	0.0	0.369	0.371	0.358	0.367	0.366	0.365	0.354	0.355	0.341	0.361
Germ	2.5	0.373	0.380	0.366	0.367	0.363	0.370	0.356	0.362	0.348	0.368
Meal	2.5	0.374	0.375	0.358	0.367	0.364	0.363	0.349	0.356	0.342	0.364
Germ	5.0	0.384	0.373	0.364	0.373	0.365	0.368	0.350	0.357	0.339	0.361
Meal	5.0	0.379	0.380	0.369	0.382‡	0.374	0.380	0.363	0.365	0.352	0.373
Pooled MSE		0.025	0.024	0.022	0.020	0.019	0.028	0.023	0.024	0.022	0.023
Main effect											
Fraction											
Germ		0.378	0.376	0.365	0.370	0.364	0.369	0.353	0.359	0.343	0.364
Meal		0.376	0.378	0.363	0.374	0.369	0.372	0.356	0.361	0.347	0.368

TABLE 4-6. Continued

		Age of laying hens (wk)									
		21	23	25	27	29	31	33	35	37	39
Level											
2.5%		0.373	0.377	0.362	0.367	0.363	0.367	0.353	0.359	0.345	0.366
5.0%		0.381	0.377	0.366	0.377	0.370	0.374	0.357	0.361	0.345	0.367
Probability		---------- *P*-value ----------									
Fraction × level		0.578	0.263	0.157	0.235	0.412	0.114	0.069	0.206	0.064	0.108
Fraction		0.706	0.723	0.730	0.311	0.214	0.625	0.463	0.704	0.459	0.407
Level		0.292	0.875	0.375	0.015*	0.121	0.210	0.422	0.692	0.943	0.906
Fraction	Level (%)	---------- Specific gravity ----------									
Control	0.0	1.0918	1.0902	1.0881	1.0859	1.0856	1.0881	1.0796	1.0786	1.0851	1.0841
Germ	2.5	1.0942	1.0910	1.0886	1.0869	1.0865	1.0877	1.0802	1.0816	1.0856	1.0850
Meal	2.5	1.0949	1.0912	1.0898	1.0877	1.0880	1.0888	1.0798	1.0801	1.0861	1.0842
Germ	5.0	1.0935	1.0909	1.0864	1.0870	1.0857	1.0863	1.0786	1.0783	1.0835	1.0831
Meal	5.0	1.0940	1.0914	1.0886	1.0869	1.0879	1.0879	1.0795	1.0800	1.0878	1.0860
Pooled MSE		0.0048	0.0046	0.0075	0.0043	0.0043	0.0045	0.0047	0.0049	0.0047	0.0045
Main effect											
Fraction											
Germ		1.0938	1.0909	1.0875	1.0870	1.0861	1.0870	1.0794	1.0799	1.0846	1.0840
Meal		1.0945	1.0913	1.0892	1.0873	1.0879	1.0884	1.0796	1.0801	1.0870	1.0851
Level											
2.5%		1.0946	1.0911	1.0892	1.0873	1.0872	1.0883	1.0800	1.0808	1.0859	1.0846
5.0%		1.0937	1.0911	1.0875	1.0870	1.0868	1.0871	1.0790	1.0792	1.0856	1.0845
Probability		---------- *P*-value ----------									
Fraction × level		0.891	0.819	0.744	0.655	0.650	0.847	0.608	0.184	0.065	0.062
Fraction		0.593	0.655	0.260	0.676	0.036*	0.135	0.793	0.779	0.014*	0.221
Level		0.504	0.956	0.362	0.697	0.792	0.211	0.366	0.087	0.868	0.943

‡ Mean of the treatment was significantly different from the control ($P \leq 0.05$).
# Interaction of guar fraction × level existed ($P \leq 0.05$).
* Significant main effect was detected ($P \leq 0.05$).
[1] Experimental laying hens were 21 wk old at the beginning of experiment.

TABLE 4-7. Dry weight and water content of eggs of laying hens fed diets with 0 (control), 2.5 or 5% guar germ or guar meal [1]

Fraction	Level (%)	Dry egg weight (g)										Water content (%) of fresh egg									
		22 wk	24 wk	26 wk	28 wk	30 wk	32 wk	34 wk	36 wk	38 wk	40 wk	22 wk	24 wk	26 wk	28 wk	30 wk	32 wk	34 wk	36 wk	38 wk	40 wk
Control	0.0	15.21	16.68	16.84	17.61	17.53	17.71	17.64	17.43	18.30	18.17	70.13	69.56	69.61	69.72	70.08	69.59	69.92	70.10	69.81	69.60
Germ	2.5	15.98‡	16.93	17.03	17.17	18.17	17.91	17.98	17.86	18.38	18.71	69.31‡	69.36	69.54	69.60	69.67	69.78	69.83	69.65	69.48	69.30
Meal	2.5	15.80	16.34	17.08	17.43	17.53	17.61	17.42	17.38	18.13	18.39	69.22‡	69.05	69.45	69.02	69.34	69.27	69.50	69.57	69.32	69.35
Germ	5.0	15.79	16.64	17.05	17.77	17.93	17.80	17.71	17.63	18.10	18.83	69.76	69.67	69.99	69.34	69.88	69.93	70.04	70.34	69.99	69.41
Meal	5.0	16.03‡	16.66	17.40	17.80	18.26	17.99	17.89	17.77	18.24	18.84	69.19‡	69.55	69.52	69.65	69.49	69.74	70.15	70.16	69.65	69.50
Pooled MSE		0.96	1.09	1.05	1.05	1.07	0.97	1.16	1.14	1.13	1.15	0.93	1.01	0.98	1.05	1.04	1.14	1.07	1.10	1.00	0.97
Main effect																					
Fraction																					
Germ		15.88	16.79	17.04	17.47	18.05	17.85	17.85	17.75	18.24	18.77	69.54	69.51	69.76	69.47	69.77	69.85	69.93	69.99	69.74	69.35
Meal		15.91	16.50	17.24	17.61	17.89	17.80	17.66	17.57	18.19	18.62	69.21	69.30	69.48	69.33	69.41	69.51	69.83	69.87	69.48	69.42
Level																					
2.5%		15.89	16.64	17.06	17.30	17.85	17.76	17.70	17.62	18.26	18.55	69.27	69.20	69.49	69.31	69.50	69.52	69.67	69.61	69.40	69.33
5.0%		15.91	16.65	17.22	17.78	18.09	17.89	17.80	17.70	18.17	18.83	69.48	69.61	69.75	69.49	69.68	69.84	70.09	70.25	69.82	69.45
Probability																					
Fraction × level		0.339	0.266	0.573	0.645	0.031#	0.213	0.108	0.236	0.402	0.556	0.323	0.828	0.361	0.057	0.878	0.434	0.277	0.999	0.717	0.883
Fraction		0.803	0.351	0.310	0.473	0.423	0.750	0.398	0.477	0.694	0.403	0.204	0.344	0.187	0.499	0.141	0.111	0.545	0.611	0.190	0.745
Level		0.943	0.909	0.572	0.033*	0.209	0.514	0.686	0.910	0.801	0.210	0.366	0.165	0.191	0.404	0.492	0.228	0.082	0.010*	0.083	0.621

‡ Mean of the treatment was significantly different from the control ($P \leq 0.05$).
# Interaction of guar fraction × level existed ($P \leq 0.05$).
* Significant main effect was detected ($P \leq 0.05$).
[1] Experimental laying hens were 21 wk old at the beginning of experiment.

TABLE 4-8. Dry egg yolk weight and yolk content of eggs of laying hens fed diets with 0 (control), 2.5 or 5% guar germ or guar meal [1]

Fraction	Level (%)	Dry yolk weight (g)										Yolk percentage of dry egg (%)									
		22 wk	24 wk	26 wk	28 wk	30 wk	32 wk	34 wk	36 wk	38 wk	40 wk	22 wk	24 wk	26 wk	28 wk	30 wk	32 wk	34 wk	36 wk	38 wk	40 wk
Control	0.0	5.36	6.17	6.65	7.23	7.09	7.60	7.66	7.69	7.89	7.85	35.21	37.02	39.50	41.02	40.47	42.93	43.39	44.11	43.10	43.19
Germ	2.5	5.79‡	6.56	6.73	7.03	7.47	7.68	7.89	7.85	8.07	8.20	36.24	38.74	39.51	40.84	41.10	42.88	43.85	43.97	43.85	43.83
Meal	2.5	5.70	6.44	6.91	7.27	7.23	7.59	7.79	7.73	7.93	8.04	36.06	39.39‡	40.43	41.66	41.22	43.10	44.65	44.46	43.65	43.65
Germ	5.0	5.60	6.32	6.70	7.28	7.34	7.61	7.82	7.66	7.86	8.34‡	35.44	37.94	39.27	40.93	40.87	42.73	44.17	43.46	43.46	44.29
Meal	5.0	5.91‡	6.36	6.94	7.37	7.56‡	7.77	7.84	7.74	7.75	8.02	36.79	38.05	39.93	41.37	41.41	43.19	43.80	43.58	42.50	42.58
Pooled MSE		0.51	0.57	0.57	0.65	0.65	0.60	0.70	0.62	0.67	0.65	2.21	2.11	2.10	2.17	2.34	2.13	2.25	2.07	2.16	2.01
Main effect																					
Fraction																					
Germ		5.69	6.44	6.72	7.15	7.40	7.65	7.85	7.75	7.96	8.27	35.84	38.34	39.39	40.89	40.98	42.80	44.01	43.72	43.66	44.06
Meal		5.81	6.40	6.92	7.32	7.40	7.68	7.81	7.74	7.84	8.03	36.43	38.72	40.18	41.51	41.31	43.14	44.23	44.02	43.08	43.11
Level																					
2.5%		5.74	6.50	6.82	7.15	7.35	7.64	7.84	7.79	8.00	8.12	36.15	39.06	39.97	41.25	41.16	42.99	44.25	44.21	43.75	43.74
5.0%		5.76	6.34	6.82	7.32	7.45	7.69	7.83	7.70	7.81	8.18	36.12	38.00	39.60	41.15	41.14	42.96	43.99	43.52	42.98	43.43
Probability																					
Fraction × level		0.082	0.537	0.825	0.681	0.097	0.341	0.638	0.486	0.898	0.538	0.146	0.696	0.793	0.807	0.734	0.854	0.223	0.646	0.411	0.071
Fraction		0.312	0.860	0.083	0.232	0.799	0.774	0.774	0.822	0.344	0.056	0.290	0.433	0.088	0.200	0.612	0.392	0.599	0.585	0.244	0.023*
Level		0.912	0.340	0.902	0.233	0.418	0.596	0.977	0.411	0.225	0.564	0.987	0.071	0.376	0.745	0.931	0.953	0.613	0.142	0.112	0.525

‡ Mean of the treatment was significantly different from the control ($P \leq 0.05$).
# Interaction of guar fraction × level existed ($P \leq 0.05$).
* Significant main effect was detected ($P \leq 0.05$).
[1] Experimental laying hens were 21 wk old at the beginning of experiment.

TABLE 4-9. Dry egg albumin weight and albumin content of eggs of laying hens fed diets with 0 (control), 2.5 or 5% guar germ or guar meal [1]

Fraction	Level (%)	Dry albumin weight (g)										Albumin percentage of dry egg (%)									
		22 wk	24 wk	26 wk	28 wk	30 wk	32 wk	34 wk	36 wk	38 wk	40 wk	22 wk	24 wk	26 wk	28 wk	30 wk	32 wk	34 wk	36 wk	38 wk	40 wk
Control	0.0	4.61	4.92	4.73	4.73	4.87	4.67	4.59	4.48	4.62	4.59	30.27	29.54	28.03	26.88	27.77	26.30	25.96	25.73	25.23	25.22
Germ	2.5	4.69	4.81	4.70	4.61	4.84	4.64	4.59	4.51	4.52	4.61	29.39	28.43	27.69	26.90	26.66	25.97	25.55	25.25	24.59	24.62
Meal	2.5	4.62	4.59	4.72	4.62	4.69	4.59	4.44	4.39	4.49	4.61	29.27	28.09	27.62	26.51	26.82	26.05	25.52	25.27	24.80	25.08
Germ	5.0	4.69	4.90	4.88	4.80	4.90	4.70	4.58	4.59	4.59	4.67	29.71	29.49	28.68	27.02	27.39	26.44	25.85	26.02	25.41	24.76
Meal	5.0	4.57	4.72	4.77	4.70	4.80	4.60	4.55	4.52	4.59	4.72	28.46‡	28.41	27.40	26.39	26.30	25.59	25.40	25.41	25.13	25.07
Pooled MSE		0.39	0.39	0.40	0.40	0.43	0.39	0.42	0.41	0.39	0.46	1.89	1.83	1.74	1.87	2.09	1.80	1.70	1.67	1.72	1.87
Main effect																					
Fraction																					
Germ		4.69	4.86	4.79	4.71	4.87	4.67	4.59	4.55	4.56	4.64	29.55	28.96	28.18	26.96	27.02	26.21	25.70	25.64	25.00	24.69
Meal		4.59	4.65	4.74	4.66	4.75	4.60	4.49	4.45	4.54	4.67	28.86	28.25	27.51	26.45	26.56	25.82	25.46	25.34	24.97	25.07
Level																					
2.5%		4.66	4.70	4.71	4.62	4.77	4.61	4.52	4.45	4.50	4.61	29.33	28.26	27.66	26.70	26.74	26.01	25.54	25.26	24.70	24.85
5.0%		4.63	4.81	4.83	4.75	4.85	4.65	4.56	4.55	4.59	4.69	29.08	28.95	28.04	26.71	26.85	26.02	25.62	25.72	25.27	24.91
Probability																					
Fraction × level		0.852	0.890	0.421	0.408	0.717	0.879	0.445	0.768	0.891	0.762	0.226	0.363	0.105	0.600	0.187	0.256	0.592	0.460	0.522	0.904
Fraction		0.371	0.047*	0.610	0.668	0.187	0.292	0.252	0.287	0.678	0.880	0.143	0.108	0.074	0.236	0.347	0.261	0.454	0.437	0.840	0.337
Level		0.574	0.306	0.213	0.106	0.307	0.657	0.630	0.305	0.299	0.372	0.489	0.192	0.295	0.912	0.852	0.979	0.833	0.220	0.136	0.933

‡ Mean of the treatment was significantly different from the control ($P \leq 0.05$).
# Interaction of guar fraction × level existed ($P \leq 0.05$).
* Significant main effect was detected ($P \leq 0.05$).
[1] Experimental laying hens were 21 wk old at the beginning of experiment.

decreasing mean egg shell percentage from 34.6 to 31.5% from wk 22 to 40 (Table 4-10). Significant differences between guar treated groups and the control, and main effects and interaction of guar fraction and concentration on solid egg components were detected sparsely without a discernable pattern.

Mortality

The mortality data were not subjected the statistical analysis. Among the 25 laying hens for each treatment, only 2 in the 2.5% guar germ treatment and 2 in the 2.5% guar meal treatment group died during the study.

DISCUSSION

In the present study, the BW and BW gain of hens fed guar fractions were similar to or higher than the control, indicating that feeding up to 5% guar by-products did not have adverse effects on the growth of these young laying hens. Feeding guar meal at a level higher than 5% may retard the growth of young laying hens as indicated by the observation that the BW gain of hens receiving 5% guar meal were significantly lower than for hens receiving only 2.5% guar meal.

No effect of guar by-product feeding was observed on overall hen-day egg production, which suggested that both guar germ and guar meal can be incorporated into high production laying hen diets at a level up to 5% without deleterious effects on egg production, which is in agreement with previous work in our lab (Chapter III). Interestingly, an approximate 2% increase of overall egg production was observed when guar meal level increased from 2.5 to 5%. Substantially higher levels of guar meal may not be desirable in laying hen diets since decreased egg production was observed when

TABLE 4-10. Dry egg shell weight and shell content of eggs of laying hens fed diets with 0 (control), 2.5 or 5% guar germ or guar meal [1]

Fraction	Level (%)	Dry egg shell weight (g)										Egg shell percentage of dry egg (%)									
		22 wk	24 wk	26 wk	28 wk	30 wk	32 wk	34 wk	36 wk	38 wk	40 wk	22 wk	24 wk	26 wk	28 wk	30 wk	32 wk	34 wk	36 wk	38 wk	40 wk
Control	0.0	5.25	5.58	5.47	5.65	5.57	5.45	5.40	5.25	5.79	5.74	34.52	33.44	32.47	32.10	31.76	30.77	30.65	30.16	31.67	31.59
Germ	2.5	5.50	5.56	5.59	5.53	5.86	5.59	5.50	5.50	5.80	5.90	34.37	32.83	32.80	32.26	32.25	31.15	30.60	30.78	31.56	31.55
Meal	2.5	5.48	5.32	5.46	5.54	5.61	5.43	5.19	5.26	5.71	5.75	34.67	32.52	31.95	31.83	31.97	30.85	29.83	30.27	31.55	31.27
Germ	5.0	5.50	5.42	5.46	5.69	5.69	5.48	5.31	5.39	5.64	5.82	34.85	32.56	32.06	32.04	31.74	30.83	29.98	30.52	31.12	30.95
Meal	5.0	5.57‡	5.58	5.68	5.74	5.89‡	5.61	5.51	5.51	5.91	6.09‡	34.75	33.54	32.67	32.24	32.28	31.22	30.80	31.01	32.36	32.36
Pooled MSE		0.41	0.46	0.43	0.38	0.42	0.40	0.38	0.45	0.44	0.40	1.51	1.49	1.46	1.55	1.49	1.54	1.54	1.62	1.55	1.41
Main effect																					
Fraction																					
Germ		5.50	5.49	5.53	5.61	5.77	5.53	5.41	5.44	5.72	5.86	34.61	32.70	32.43	32.15	31.99	30.99	30.29	30.65	31.34	31.25
Meal		5.52	5.45	5.57	5.64	5.75	5.52	5.35	5.39	5.81	5.92	34.71	33.03	32.31	32.03	32.13	31.03	30.31	30.64	31.96	31.81
Level																					
2.5%		5.49	5.44	5.52	5.54	5.73	5.51	5.35	5.38	5.75	5.83	34.52	32.68	32.37	32.04	32.11	31.00	30.21	30.52	31.56	31.41
5.0%		5.53	5.50	5.57	5.71	5.79	5.55	5.41	5.45	5.77	5.96	34.80	33.05	32.36	32.14	32.01	31.03	30.39	30.76	31.74	31.65
Probability																					
Fraction × level		0.663	0.080	0.066	0.750	0.010#	0.077	0.002#	0.081	0.071	0.020#	0.533	0.098	0.021#	0.327	0.188	0.283	0.019#	0.182	0.067	0.007#
Fraction		0.729	0.785	0.542	0.704	0.774	0.865	0.439	0.604	0.420	0.566	0.775	0.381	0.745	0.710	0.597	0.894	0.950	0.915	0.067	0.048
Level		0.691	0.556	0.679	0.030*	0.370	0.706	0.463	0.630	0.783	0.102	0.396	0.331	0.980	0.746	0.901	0.958	0.616	0.537	0.571	0.432

‡ Mean of the treatment was significantly different from the control ($P \leq 0.05$).
# Interaction of guar fraction × level existed ($P \leq 0.05$).
* Significant main effect was detected ($P \leq 0.05$).
[1] Experimental laying hens were 21 wk old at the beginning of experiment.

laying hens were fed diets with 10% raw guar meal (Bakshi et al., 1964; Couch et al., 1967b).

The higher feed consumption of guar treated hens indicated that unfavorable characteristics of guar by-product does not necessarily decrease feed consumption of laying hens when added at low levels, which is also supported by previous experimentation (Chapter III). Feed conversion efficiency for hens fed 2.5% guar meal was significantly worse than the control whereas other guar by-product treated groups had similar feed conversion efficiency to the control. A decreased feed conversion efficiency of hens fed 10% raw guar meal diet was reported by Bakshi et al. (1964).

The egg weights of hens fed guar by-products were not different from the control throughout the study. Similar results with regards to egg size were reported by Couch et al. (1967b) who observed that 10% guar meal did not affect egg weight. Contradictorily, Bakshi et al. (1964) reported a decreased egg size of hens fed diets containing 10% of raw guar meal. The consistently marginal lower egg weight of hens fed 2.5% guar meal resulted in a significantly lower total egg mass. Guar gum residue in the diet is unlikely to be the factor for the adverse effect on total egg mass and numerically reduced feed efficiency, because hens fed diets containing higher guar gum had higher egg mass and improved feed efficiency.

The Haugh units of eggs by control hens were consistently higher than those eggs from guar treated hens, and significant differences were detected between the control hens and 5% guar meal-fed hens after 7 wk of feeding, which suggested that long term feeding of guar meal at 5% might decrease Haugh units of eggs. In a previous study in

our lab (Chapter III), feeding guar meal up to 10% to late phase hens for 8 wk did not significantly depress Haugh units.

The luminosity of egg yolk for hens fed 2.5% guar by-products were not different from the control, whereas those fed 5% guar meal or guar germ fraction were significantly lower than control at 2 out of 10 times of measurement. Significant main effects of guar by-production concentration in diets on luminosity were detected at 5 measurement times in that 5% guar by-products groups had lower luminosity of egg yolk than 2.5% guar by-products groups. This might be anticipated as the dietary corn content was decreased slightly in the 5% guar by-products diet. Unlike luminosity, the redness of egg yolk was affected by guar fraction at 5 of the 10 measurement times, and generally unrelated to the added level with 2 exceptional measurement times. After 9 wk of treatment (29 wk old hens), egg yolks from guar meal-fed hens had lower redness values than guar germ-fed hens, which indicated that the factors responsible for redness-lowering may be distributed differently in guar germ and meal. For example, guar germ contains less residual guar gum than guar meal. The yellowness (b*) of egg yolk was not affected by either guar fraction or by added level. However, whether the decreases in measured values of luminosity and redness lead to a detectable decrease in Roche color value, and whether the color change is visually perceivable by table-egg consumers are uncertain. By measuring the CIELAB values of a Roche color fan with the same chromameter used in this experiment, we observed linear relationships between Roche values and CIELAB values with R^2 of 0.96 to 0.99, in that when the Roche color value increases by 1, the L* value decreases by 0.73, the a* and b* values increase by 2.63 and

4.53 respectively. Therefore, the tiny although significant differences in lightness and redness are unlikely to change the Roche color value of egg yolks. In a study by Roberson (2004), even though Minolta chromameter (Model 310) detected remarkable changes in L*, a* and b* values of egg yolks by feeding laying hens 15% corn dried distillers grains with solubles, the differences in average Roche color value were less than 0.2 and without statistical significance.

The shell quality, indicated by breaking force, shell thickness and specific gravity, was not affected by the feeding the guar by-products. This confirmed the observations in a previous study conducted by our lab that feeding 5% guar meal did not affect shell quality (Chapter III).

In the present study, the egg components were affected only by the age of laying hens, and not affected by the feeding of guar by-products at the tested levels. The water content in fresh egg was very steady over the age of hens, which is consistent with the finds of Yannakopoulos et al. (1994) who reported that age of bird had no significant effect on water content of whole egg. The yolk weight increased over age which is consistent with reports by (Yannakopoulos et al., 1994; Rossi and Pompei, 1995), whereas the albumin weight remained unchanged throughout the experiment which contradicts to previous report (Yannakopoulos et al., 1994; Rossi and Pompei, 1995). An increasing trend of shell weight was observed in the present study. However, the shell weight decreased during wk 32 to 36 of age, in which the hens experienced a very high ambient temperature in summer time. A decreased specific gravity was also observed during this period. High ambient temperature was reported to cause inferior egg quality,

including shell weight, shell thickness, shell breaking strength and specific gravity (Hsu et al., 1998). However, the breaking force and shell thickness were not affected by the ambient temperature in the present study. Egg components were reported to be affected by contents of dietary fat (Whitehead et al., 1991; Grobas et al., 1999a; Grobas et al., 1999b), crude protein (Penz and Jensen, 1991; Keshavarz and Nakajima, 1995), lysine (Prochaska et al., 1996), and methionine (Shafer et al., 1996; Shafer et al., 1998). However, the calculated contents of these nutrients were identical for each experimental diet of present study except for the dietary fat, which explains the unaffected egg components by dietary treatments.

In conclusion, the results of this study suggests that, both guar germ and guar meal can be fed to high production laying hens at up to 5% without unfavorable effects on egg production, feed consumption, egg weight, egg shell quality and solid egg components. However, feed efficiency, Haugh units and some aspects of egg yolk color may be decreased by feeding guar meal by-products.

CHAPTER V

APPLICATION OF GUAR BY-PRODUCTS AS A FULL-FED MOLTING ALTERNATIVE APPROACH TO FEED WITHDRAWAL

INTRODUCTION

Molting is a spontaneous process for adult avian species lasting up to 34 d, with a greatly reduced feed intake and a BWR of up to 50% varying between species (Mrosovsky and Sherry, 1980). For most wild species of avian, molting involves reproductive tissue rejuvenation with a cessation of oviposition as a result of the regression of the reproductive tract (Berry, 2003). In the table-egg industry, artificially inducing a molt of late phase layers is employed to accelerate the process of natural molting, stimulate multiple egg-laying cycles, and improve egg quality and desirable egg numbers thus making the hens more profitable. A typical molting program involves a reduction of light and removal of feed for up to 14 d or until a BWR of 25 to 35% is obtained. Many alternative methods to the complete deprivation of feed have been reported, which include the use of high concentrations of zinc, low levels of sodium or calcium, and using pharmaceuticals such as nicarbazin, progesterone, enheptin and others (Berry, 2003; Webster, 2003). Both feed withdrawal and providing nutritional imbalanced diets to induce a molt have been criticized by animal welfare advocators. In addition, the stress associated with long term FW impairs the immunological function of laying hens and increases the susceptibility of layers to *Salmonella* (Holt, 2003; Ricke,

2003), thus increasing the risk of human salmonellosis from *Salmonella enteritidis*-contaminated eggs. Providing laying hens with nutritional balanced molt-inducing diets should be more humane and not subject to criticism with respect to animal well-being.

Vermaut et al. (1998) reported restricted feed intake and subsequent induced molting of broiler breeders when fed a diet with 12% jojoba supplementation. Davis et al. (2002) reported that feeding diets containing 50% ground cottonseed was equivalently effective to complete feed withdrawal in reducing body weight, inducing molt, and post-molt laying performance. Feeding a grape pomace diet to laying hens was considered to be a preferable molting method to conventional FW in view of animal rights issues (Keshavarz and Quimby, 2002). Biggs et al. (2003) found that feeding laying hens corn or wheat middlings solely, particularly wheat middlings, was an effective non-feed removal method for molting hens. This group (Biggs et al., 2004) later concluded that feeding wheat middlings, corn, corn gluten feed, and wheat middlings:corn diets were effective non-feed removal methods for molting laying hens.

Guar (*Cyamopsis tetragonoloba*) meal, a by-product of the guar gum industry, contains between 35 to 47.5% CP on a dry matter basis (Ambegaokar et al., 1969), 2.005 kcal/g AMEn (Nagpal et al., 1971), and 18 to 20% gum residue (Bakshi et al., 1965; Nagpal et al., 1971). Guar gum is a polysaccharide of β-galactomannan consisting of a 1→ 4-linked β-D-mannopyranose backbone with branched 1→ 6- α-D-galactopyranose. The gum residue has been reported to cause ill effects, including diarrhea, depressed growth rate and increased mortality of broiler chickens (Sathe and Bose, 1962; Couch et al., 1967b; Thakur and Pradhan, 1975a; Verma and McNab, 1982; Patel and McGinnis,

1985; Conner, 2002; Lee et al., 2003a). Many investigators have shown that 10% or higher concentration of GM incorporated into laying hen diets deceased egg production and feed conversion efficiency, and had negative effects on egg yolk color (Saxena and Pradhan, 1974; Patel and McGinnis, 1981; Verma and McNab, 1984b; Patel and McGinnis, 1985; Nagra and Virk, 1986; Zimmermann et al., 1987). Guar meal has also been fed to layers to induce a molt (Patel and McGinnis, 1981; Zimmermann et al., 1987) at concentrations of 10 and 15% for 20 to 33 d, with acceptable post-molt laying performance observed afterwards. Our lab has observed a rapid decrease in egg production when late phase laying hens were fed 15% GM, and after the GM diet was replaced with a standard laying hen diet, egg production returned and exceeded hens continuously fed the control laying hen diet (Chapter III).

As a dietary fiber, gum residue in GM may have some beneficial effects in improving the health status of animals. Guar gum has been found to have putative protective effects against colonization by pathogenic bacteria, and their subsequent proliferation and resultant diarrhea, and to potentiate the effect of polar lipids in preventing microbial translocation in the gastro-intestinal tract (Bengmark, 1998). Although no reports on the effects of guar gum on the immune function of animals or humans can be found, a high-molecular-weight galactomannan, about 1.0 million Daltons, isolated from the edible mushroom *Morchella esculenta*that has a function of enhancing macrophage activation thus exhibiting immunostimulatory activity (Duncan et al., 2002).

Our objective for this study was to compare alternative molt-inducing methods by feeding laying hens high levels (15 and 20%) of guar meal with and without β-mannanase (Hemicell®). The enzyme was supplemented in guar meal diets to exam the role of galactomannan, oligosaccharide, and viscosity in a full fed guar molt.

MATERIALS AND METHODS

Experimental Design and Molting Procedure

The experimental animal care and treatment in the study was in accordance with the AUP 2003-0256 approved by the Institutional Agricultural Animal Care and Use Committee (IAACUC). A total of 125 Bovan white late phase laying hens (64-wk-old) at similar BW were obtained from a commercial poultry farm and allocated into 25 cages in two rearing batteries in an environmentally controlled room, and allowed to acclimate for 1 wk. Eggs laid in this wk were collected daily and measured a few hours after collection for egg weight, breaking force, Haugh units, yolk color, shell thickness and specific gravity as pre-molt data. At the end of the acclimation period, hens were weighed and re-allocated into 25 cages so that the pooled weight of 5 hens in each cage were within 30 grams of each other. Then the hens were subjected to five different molt-inducing procedures for 14 d. The five molt-inducing approaches consisted of feed withdrawal (denoted as FW, control) and full-fed diets with 15 or 20% GM (Table 5-1) with or without supplementation of 250,000 units/kg β-mannanase (Hemicell®), which are denoted as 15% GM, 15% GM+E, 20% GM and 20% GM+E. The nutrient composition of guar meal used in study was previously determined by Conner (2002) with amino acid analysis by Degussa-Huls Corporation. [1] The 5 treatments were

TABLE 5-1. Composition of molt-inducing diets containing 15 or 20% guar meal[1]

	GM (%)	
Ingredients (%)	15	20
Corn	74.39	72.29
Guar meal[2]	15.00	20.00
Dehulled soybean meal	3.54	0.00
DL-Methionine	0.10	0.10
L-Lysine HCl	0.17	0.21
Fat (animal-vegetable blend)	0.00	0.59
Limestone	4.51	4.52
Mono-dicalcium PO_4	1.47	1.48
Salt	0.51	0.51
Trace minerals[3]	0.05	0.05
Vitamins[4]	0.25	0.25

[1] Calculated analysis of all diets was as follows: crude protein, 14.00 and 14.06% for 15 and 20% GM diets; metabolizable energy, 2,893 kcal/kg; calcium, 2.00%; available phosphorus, 0.40%; methionine, 0.32%; lysine, 0.68%; threonine, 0.44 and 0.42% for 15 and 20% GM diets; and tryptophan, 0.13%.
[2] See Appendix I for the nutrient matrix.
[3]Trace minerals premix added at this rate yields: 27.5 mg sulphur, 150 mg manganese, 16.80 mg iron, 1.70 mg copper, 125.50 mg zinc, 0.25 mg selenium, 1.05 mg iodine, 0.84 mg molybdenum per kilogram diet.
[4]Vitamin premix added at this rate yields: 11,023 IU vitamin A, 46 IU vitamin E, 3,850 IU vitamin D3, 1.47 mg vitamin K, 2.94 mg thiamine, 5.85 mg riboflavin, 20.21 mg pantothenic acid, 0.55 mg biotin, 1.75 mg folic acid, 477.67 mg choline, 16.50 ug Vitamin B12, 45.93 mg niacin, and 7.17 mg pyridoxine per kilogram of diet.

assigned to laying hens rotationally to minimize the experimental error associated with the location of cages. Feed consumption was recorded every other day and the day all hens in a particular treatment ceased laying was recorded. From the 9th d of treatment on, pooled BWs of 5 hens in each cage subjected to FW were monitored. During the 21 d of acclimation and molting, all hens received a 8L:16D light program with free access to water. Full-fed hens received experimental diets ad libitum. Feed consumption for each cage was recorded every other day.

After 14 d of treatment, pooled BW of 5 hens in each cage was measured and all hens were transferred to an open sided laying hen house and re-allocated to 125 individual cages ($50 \times 30 \times 30$ cm^3) divided into 5 blocks in a complete randomized block design. All hens were fed the same commercial type corn-soybean based laying hen feed ad libitum except that in the first 3 d after transfer, all hens received only 200 g/d per 5 hens of each treatment. All hens received a 16L:8D light program and free access to water. Egg production was recorded daily through the 11th wk of post-molt, and feed consumption was recorded bi-weekly through wk 8 of post-molt. Individual egg weights were measured weekly using all eggs laid on Wednesday of each wk through the 8th wk of post-molt, and egg interior (Haugh units, yolk color in L*, a*, b* system) and exterior shell quality (breaking force, thickness and specific gravity) were measured in wk 3, 5 and 7 of post-molt with all eggs laid on Wednesday, using the method described previously (Chapter III). Total egg mass per hen and feed conversion ratio (FCR, g feed/g egg mass) were calculated. Deaths of laying hens were recorded as they occurred and used to adjust egg production and feed consumption data.

Statistical Procedure

The days to 0 egg production were not subjected to statistical analysis because of the small sample size and lack of replications. Feed consumption, initial and ending BW and BWR during the molting period were subjected to ANOVA using the GLM procedure of the SAS System [5] for a complete randomized design. Data for post-molt feed consumption, hen-day egg production, egg weight and egg interior and shell quality were first subjected to a test of time effect. Because time effect or time × treatment interactions were detected for most of these parameters, these data were then subjected to ANOVA based on each time point of measurement by the GLM procedure for a complete randomized block design, with fixed effect factors of block and treatment. Data for mortality of laying hens were first subjected to an arcsine-square root transformation (asin(sqrt y) in Microsoft® Excel). Transformed data were then analyzed for variance by the GLM procedure of the SAS System [5] for a complete randomized block design. The PDIFF option within the GLM procedure was used to compare means of each full-fed GM group to the FW group, and to compare each mean of post-molt data to pre-molt data. Contrasts were constructed to test the main effect and interaction of GM level and enzyme supplementation. Significance level was set at $P \leq 0.05$.

RESULTS

Responses in Molting Period

Days To 0 Egg Production. The data for days needed to obtain 0% egg production were not subjected to statistical analysis (Table 5-2). Fasted hens stopped laying at the 6th d of FW, which was at least 2 d earlier than full-fed hens. Hens full-fed

TABLE 5-2. Days to 0 egg, BW and BWR, and feed consumption of guar molted laying hens [1]

Molting methods	GM level	Enzyme	Days to 0 egg [2]	Feed consumption (g/d per hen)	BW		
					Initial (g)	Ending (g)	BWR (%)
FW			6	ND[4]	1475	1035	29.84
Full-fed	15%	–	10	40.7	1478	1296‡	12.29‡
Full-fed	15%	+	NA[3]	57.9	1472	1374‡	6.70‡
Full-fed	20%	–	8	39.5	1473	1290‡	12.44‡
Full-fed	20%	+	9	51.6	1474	1336‡	9.35‡
SD				3.1	3	30	2.06
Main effect							
GM level							
	15%			49.3	1475	1335	9.49
	20%			45.6	1473	1313	10.90
Enzyme							
	–			40.1	1475	1293	12.37
	+			54.8	1473	1355	8.03
Probability							
GM level × enzyme				0.416	0.303	0.597	0.549
GM level				0.241	0.533	0.461	0.503
Enzyme				0.000*	0.407	0.051	0.048*

‡ Mean of the treatment was significantly different from FW ($P \leq 0.05$).
* Significant main effect was detected ($P \leq 0.05$).
[1] Experimental laying hens were 65 wk old at the beginning of experiment.
[2] Days needed for laying hens to obtain 0 egg were not subjected to statistical analysis.
[3] Hens fed 15% GM with Hemicell® failed to cease laying during the 14 d molting period.
[4] No data.

15% GM + E failed to completely cease laying during the 14-d molting period, while hens fed 15% GM without enzyme, or fed 20% GM with or without enzyme stopped laying at the 8th to 10th d of feeding.

Feed Consumption. Full-fed hens consumed 40 to 60 g/d of feed with a 10 to 30 g decrease compared to the pre-molt acclimation period (68.5 ± 13.8 g/d per hen) suggesting an abnormally high level of stress. Supplementation of 250,000 units Hemicell® /kg increased the feed consumption of laying hens fed both 15 and 20% GM diets (Table 5-2). No main effect of GM concentration or interaction was observed.

Body Weight. The mean initial BW of laying hens was almost the same for each treatment group (Table 5-2). However, after 14 d of treatment, fasted laying hens lost significantly more BW than full-fed hens. Hens fed GM diets with enzyme supplementation had lower BWR regardless of the GM level. The level of GM inclusion did not affect the BWR and ending BW of laying hens.

Post-Molt Laying Hen Performance

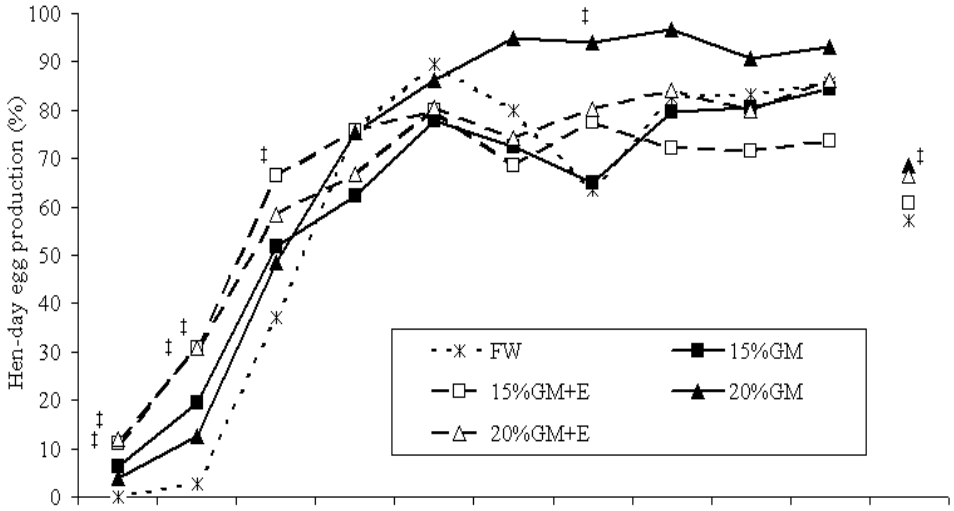
Feed Consumption. The recovery of feed consumption of fasted hens was slower than hens full-fed GM diets, especially those fed 20% GM with enzyme (Table 5-2). By the 4th wk, all hens returned to "normal" feed intake. Post-molt feed consumption of hens fed the 15% GM + E diet, which failed to cease laying during the molting period, was significantly lower than fasted hens after wk 4 of post-molt. The main effects and interactions of GM concentration and enzyme inclusion were not significant.

Hen-Day Egg Production. Fasted hens resumed laying in the 2nd wk of post-molt, and reached 50% production in the 4th wk, while full-fed hens resumed laying in

the 1st wk of post-molt and reached 50% production in the 3rd wk (Figure 5-1). During the first 3 wk of post-molt, hens full-fed GM diets supplemented with enzyme had significantly higher egg production than the FW group. However, the difference faded away after the 4th wk of post-molt as a result of the rapid increase in production of FW hens. After the 6th wk of post-molt, egg production of hens molted by feeding 20% GM with enzyme was consistently higher, and significantly different than FW in wk 7. Hens molted by 20% GM laid from 10 to 16% more eggs than hens full-fed 15% GM after the 6th wk, with significant differences detected in wk 8 and 11. Significantly higher egg production of hens fed GM diets with versus without enzyme inclusion was detected only in wk 9 to 10. For overall hen-day egg production, full-fed hens were not different to the fasted hens except for the hens molted by feeding 20% GM without enzyme. Neither main effects nor interaction of GM level and enzyme inclusion on overall egg production was detected.

Egg Weight. Post-molt egg weight increased as the age of hens increased (Table 5-3). After the 2nd wk of post-molt, all groups had higher egg weight when compared to the pre-molt period. Significant differences existed between FW hens and hens full-fed 20 and 15% GM without enzyme after the 4th and 5th wk of post-molt respectively. Neither main effects nor interaction of GM level and enzyme supplementation was detected with respect to egg weight.

Total Egg Mass Per Hen. No difference in total egg mass per hen were detected (Table 5-3).



Main effect	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8	Wk 9-10	Wk 11	Wk 1-11
GM level											
15%	8.65	25.25	59.05	68.93	78.95	70.42	71.25	76.01	75.98	79.11	60.69
20%	8.00	21.69	53.45	71.07	83.36	84.50	87.07	90.29	85.29	89.71	67.43
Enzyme											
–	5.10	16.07	50.08	68.79	82.07	83.57	79.50	88.17	85.59	88.83	64.59
+	11.55	30.87	62.42	71.21	80.23	71.35	78.82	78.13	75.67	79.99	63.54
Probability											
GM level × enzyme	0.626	0.613	0.774	0.236	0.531	0.275	0.117	0.662	0.840	0.653	0.781
GM level	0.838	0.599	0.491	0.816	0.492	0.075	0.064	0.023*	0.058	0.026*	0.085
Enzyme	0.054	0.040*	0.143	0.792	0.772	0.119	0.933	0.096	0.045*	0.058	0.781

FIGURE 5-1. Post-molt hen-day egg production of laying hens induced-molt by feed withdrawal (FW, –∗–), full-fed diets of 15% GM (—■—), 15% GM with 250,000 units/kg Hemicell® (15% GM+E, –□–), 20% GM (—▲—) or 20% GM with 250,000 units/kg Hemicell® (20% GM+E, –△–). Significant difference ($P \leq 0.05$) between full-fed group and FW was denoted by ‡ in Figure. A probability value with * denoted that significant main effect was detected ($P \leq 0.05$).

TABLE 5-3. Post-molt egg weight, total egg mass, feed consumption, FCR and mortality of guar molted laying hens[1]

Molting Methods	GM level	Enzyme	Egg weight (g)								Egg mass	Feed consumption				FCR	Mortality
			Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8		Wk 1-2	Wk 3-4	Wk 5-6	Wk 7-8		
Pre-molt period			58.93	58.93	58.93	58.93	58.93	58.93	58.93	58.93	(g/hen)	---------- (g/d per hen) ----------					(%)
FW			ND	ND	68.40†	73.63†	71.88†	73.00†	75.69†	74.88†	2206	69.2	127.4	132.5	139.3	3.202	64
Full-fed	15%	–	62.18	65.68†	66.44†	70.00†	67.24‡†	67.65‡†	68.44‡†	68.25‡†	2063	86.9	120.0	112.9	126.1‡	3.056	21‡
Full-fed	15%	+	60.58	63.82†	66.79†	68.76‡†	68.30†	69.92†	69.66‡†	70.62†	2254	77.4	106.5	108.1‡	124.8‡	3.076	32‡
Full-fed	20%	–	58.50	63.45†	65.16†	67.07‡†	67.91‡†	67.56‡†	67.68‡†	68.87‡†	2417	83.2	120.0	117.7	133.4	2.641	16‡
Full-fed	20%	+	61.91	66.71†	68.00†	70.22†	69.48†	69.46†	70.63‡†	71.23†	2369	100.1‡	124.7	120.3	131.2	2.816	8‡
SD			2.64	1.66	1.89	1.93	1.58	1.54	1.72	2.03	198	13.0	9.9	9.2	4.7	0.245	11
Main effect																	
GM level																	
	15%		61.38	64.75	66.62	69.38	67.77	68.79	69.05	69.44	2158	82.2	113.2	110.5	125.4	3.066	27
	20%		60.21	65.08	66.58	68.64	68.69	68.51	69.15	70.05	2393	91.7	122.4	119.0	132.3	2.729	12
Enzyme																	
	–		60.34	64.56	65.80	68.53	67.57	67.61	68.06	68.56	2240	85.1	120.0	115.3	129.7	2.849	19
	+		61.25	65.26	67.40	69.49	68.89	69.69	70.14	70.93	2311	88.8	115.6	114.2	128.0	2.946	20
Probability																	
GM level × enzyme			0.811	0.091	0.357	0.079	0.824	0.867	0.440	0.996	0.461	0.322	0.319	0.632	0.923	0.695	0.753
GM level			0.642	0.826	0.981	0.550	0.411	0.803	0.927	0.637	0.658	0.472	0.316	0.279	0.115	0.624	0.119
Enzyme			0.183	0.640	0.237	0.441	0.244	0.064	0.065	0.070	0.156	0.778	0.625	0.889	0.680	0.103	0.829

‡ Mean of the treatment was significantly different from FW ($P \leq 0.05$).
† Mean of the treatment was significantly different from pre-molting mean ($P \leq 0.05$).
[1] Experimental laying hens were 67 wk old in wk 1 of post-molt.

Feed Conversion Ratio (FCR). Difference in FCR were not statistically significant (Table 5-3).

Mortality During the Whole Experiment

Unfortunately, mortality was unusually high over the course of this experiment with 64% of the FW hens dying by the end of the wk 8 of the study (Table 5-3). In contrast only 8% of the birds receiving 20% guar meal with enzyme died over the study period. Similar mortality to the FW hens was observed among the 25 extra laying hens (13 deaths) not subjected to any treatment suggesting a problem with this particular flock of spent laying hens not related to treatment. In retrospect we believe these hens were exposed to mycoplasma although we cannot say for certain as the hens were not tested by a licensed veterinarian. No interaction of GM level and enzyme supplementation was observed on mortality.

Egg Quality

Haugh Units. Compared to the pre-molt period, the Haugh units of eggs from hens full-fed 20 and 15% GM without enzyme inclusion were higher in wk 3 and 5, respectively (Table 5-4). In wk 7, all full-fed hens had higher Haugh units compared to pre-molt eggs. The main effects of GM level and enzyme were not significantly different nor was there a significant interaction between the two.

Yolk Color Index. The differences in brightness (L*) and redness (a*) between post-molt eggs and pre-molt eggs were inconsistent, while all treatments had lower egg yellowness (b* value) compared to pre-molt hens (Table 5-4). No difference between full-fed hens and fasted hens existed with respect to egg yolk color indices, except that

TABLE 5-4. Haugh units and egg yolk color (L*a*b*) of guar molted laying hens [1]

Molting methods	GM level	Enzyme	Haugh units			Brightness (L*)			Redness (a*)			Yellowness (b*)		
			Wk3	Wk5	Wk7	Wk3	Wk5	Wk7	Wk3	Wk5	Wk7	Wk3	Wk5	Wk7
Pre-molt			67.1	67.1	67.1	58.33	58.33	58.33	-3.23	-3.23	-3.23	44.08	44.08	44.08
FW			75.5	69.9	68.8	56.24	58.05	57.76	-2.35†	-3.90†	-3.38	39.20†	39.30†	41.08†
Full-fed	15%	–	71.8	73.7†	74.8†	57.28	56.78†	57.09†	-3.05	-3.75	-3.32	40.99†	39.04†	40.91†
Full-fed	15%	+	73.6	72.8	74.0†	58.25	58.05	56.97†	-3.05	-3.72	-3.40	40.92†	40.18†	40.09†
Full-fed	20%	–	77.0†	71.8	75.2†	57.40	58.15	57.66	-2.71†	-3.78	-3.67	40.12†	40.08†	40.14†
Full-fed	20%	+	69.5	71.4	75.1†	58.38	58.11	57.66	-2.68†	-3.70	-3.31	43.41‡	40.09†	42.18†
SD			5.6	4.5	3.9	1.15	0.82	0.73	0.49	0.34	0.32	1.64	1.12	0.98
Main effect														
GM level														
15%			72.7	73.2	74.4	57.76	57.42	57.03	-3.05	-3.73	-3.36	40.95	39.61	40.50
20%			73.3	71.6	75.2	57.89	58.13	57.66	-2.70	-3.74	-3.49	41.76	40.09	41.16
Enzyme														
–			74.4	72.7	75.0	57.34	57.47	57.38	-2.88	-3.76	-3.49	40.55	39.56	40.52
+			71.6	72.1	74.5	58.31	58.08	57.31	-2.87	-3.71	-3.36	42.16	40.14	41.14
Probability														
GM level × enzyme			0.088	0.927	0.885	0.996	0.172	0.901	0.949	0.909	0.296	0.038#	0.386	0.026#
GM level			0.843	0.534	0.765	0.822	0.137	0.184	0.145	0.984	0.525	0.313	0.466	0.301
Enzyme			0.296	0.812	0.854	0.085	0.200	0.897	0.962	0.787	0.514	0.047	0.379	0.333

‡ Mean of the treatment was significantly different from FW ($P \leq 0.05$).
† Mean of the treatment was significantly different from pre-molting mean ($P \leq 0.05$).
# Interaction of GM level × enzyme existed ($P \leq 0.05$).
[1] Experimental laying hens were 67 wk old in wk 1 of post-molt.

in wk 3 of post-molt, hens molted by feeding 20% GM with enzyme had higher yellowness values than controls. No main effects of GM level and enzyme inclusion were detected with respect to yolk color index. An interaction of the two factors was detected only on yolk yellowness in wk 3 and 7. The inclusion of enzyme increased the egg yolk yellowness of hens fed 20% GM, while no such increase was observed when enzyme was included in the 15% GM diets.

Egg Shell Quality. Post-molt eggs of all treatment groups had higher breaking force, shell thickness and specific gravity when compared to pre-molt eggs, with a few exceptions (Table 5-5). The main effects of GM level and enzyme supplementation were not significant in regard to egg shell quality nor was there a significant interaction.

DISCUSSION

Post-molt egg production has been reported to be positively correlated to the length of rest period (Lee, 1982; Fontana et al., 1991; Hurwitz et al., 1995) and a rest period of 14 to 21 d was proposed by Hurwitz et al. (1995) to obtain maximum improvement in egg production and egg shell quality. A BWR of molted hens from 25 to 35% has also been recommended by many investigators (Zimmermann et al., 1987; Carey and Brake, 1989). In the present study, however, hens full-fed 20% GM without enzyme supplementation ceased laying 2 days later than fasted hens with a BWR of only 12%, but had 11% higher post-molt hen-day egg production than the FW group. Other full-fed groups, even those which failed to completely cease laying, with BWR of 6.7 to 12.4%, had similar post-molt egg production to fasted hens. Body weight loss of 8 to 18% for hens fed 94% wheat middlings, 94% corn, 71% wheat middlings with 23%

TABLE 5-5. Egg shell quality of guar molted laying hens[1]

Molting methods	GM level	Enzyme	Breaking force (kg)			Thickness (mm)			Specific gravity		
			Wk3	Wk5	Wk7	Wk3	Wk5	Wk7	Wk3	Wk5	Wk7
Pre-molt period			2.24	2.24	2.24	0.321	0.321	0.321	1.0742	1.0742	1.0742
FW			2.69	4.38†	2.98	0.355	0.403†	0.388†	1.0827†	1.0880†	1.0872†
Full-fed	15%	–	3.01†	3.51†	3.41†	0.366†	0.389†	0.394†	1.0854†	1.0844†	1.0886†
Full-fed	15%	+	3.23†	2.88‡	3.37†	0.386†	0.369†	0.374†	1.0875†	1.0824†	1.0849†
Full-fed	20%	–	3.35†	3.66†	3.57†	0.393†	0.401†	0.388†	1.0892†	1.0873†	1.0879†
Full-fed	20%	+	2.69	3.22†	3.42†	0.374†	0.380†	0.382†	1.0866†	1.0848†	1.0853†
SD			0.38	0.60	0.53	0.027	0.020	0.019	0.0045	0.0035	0.0032
Main effect											
GM level											
	15%		3.12	3.20	3.39	0.376	0.379	0.384	1.0865	1.0834	1.0867
	20%		3.02	3.44	3.49	0.384	0.390	0.385	1.0879	1.0861	1.0866
Enzyme											
	–		3.18	3.59	3.49	0.380	0.395	0.391	1.0873	1.0858	1.0882
	+		2.96	3.05	3.40	0.380	0.374	0.378	1.0870	1.0836	1.0851
Probability											
GM level × enzyme			0.247	0.787	0.873	0.133	0.972	0.549	0.276	0.918	0.797
GM level			0.788	0.482	0.767	0.559	0.351	0.940	0.520	0.198	0.962
Enzyme			0.560	0.129	0.785	0.984	0.078	0.278	0.920	0.279	0.129

‡ Mean of the treatment was significantly different from FW ($P \leq 0.05$).
† Mean of the treatment was significantly different from pre-molting mean ($P \leq 0.05$).
[1] Experimental laying hens were 67 wk old in wk 1 of post-molt.

corn, 47% wheat middlings with 47% corn, 95% corn gluten or 94% distillers dried grains with solubles for 28 d were observed by Biggs et al (2003, 2004) but the low BWR did not result in lower post-molt egg production. Zimmermann et al. (1987) observed a slow cessation of laying occurred in hens fed 15% GM, but the post-molt egg production of these birds were similar or superior to hens molted by other methods. From these studies, we might propose that for a "full-fed" alternative molting method, the recommendation of a 25 to 35% BWR is not suitable. Zimmermann et al. (1987) also reported that hens molted by GM feeding were slow to return to lay, which was contradicted by this study.

The various molting procedures in this study all increased egg weight, improved egg Haugh units and shell quality, but deceased the yellowness of egg yolk when compared to the pre-molt period. However, the pre-molt period egg weight was lower than we expected, possibly because of the low feed intake (68.5 ± 13.8 g/d per hen) of pre-molt hens. Improved egg shell quality after molting has been observed in many studies (Hurwitz et al., 1975; Garlich et al., 1984; Berry and Brake, 1991; Albatshan et al., 1994; Burke and Attia, 1994; Hurwitz et al., 1998; Bar et al., 2001). The improvements have been attributed to increased duodenal calcium uptake (Albatshan et al., 1994) and increased amounts of calbindin in the shell gland and duodenum of molted hens (Berry and Brake, 1991). No difference on yolk color among various molting method was observed while all molted hens had lower yellowness value of yolk when compared to pre-molt period. In previous studies, luminosity and yellowness values (Chapter III) and luminosity and redness values (Chapter IV) were decreased by feeding

guar by-product, while difference was observed on yellowness value in this study between pre- and post-molt hens. However, whether the decreases in measured values of yellowness lead to a detectable change in Roche color value, and whether the color change is visually perceivable by table-egg consumers are uncertain. By measuring the CIELAB values of a Roche color fan with the same chromameter used in this experiment, we observed linear relationships between Roche values and CIELAB values with R^2 of 0.96 to 0.99, in that when the Roche color value increases by 1, the L* value decreases by 0.73, the a* and b* values increase by 2.63 and 4.53 respectively. Therefore, the tiny although significant differences yellowness value, coupled with unchanged values of brightness and redness, are unlikely to change the Roche color value of egg yolks. In a study by Roberson (2004), even though Minolta chromameter (Model 310) detected remarkable changes in L*, a* and b* values of egg yolks by feeding laying hens 15% corn dried distillers grains with solubles, the differences in average Roche color value were less than 0.2 and without statistical significance.

Supplementation of enzyme to GM diets increased the feed consumption of hens in the molting period. This is most likely the result of degradation of polysaccharide galactomannan (guar gum), which is an anti-nutritional factor in GM diets. The loss of body weight was apparently affected by the nutrient availability during the molting period. Hens fed the β-mannanase lost less BW than birds not on the enzyme suggesting less viscosity (Lee et al., 2003a, b) and as a consequence greater nutrient availability. However, no positive effect of enzyme inclusion was observed with respected to post-molt laying performance and egg quality in this study. Using a matrix-assisted laser

desorption ionization time-of-flight (MALDI-TOF) mass spectrometer [6], we determined that the hydrolysates of guar gum by β-mannanase (Hemicell®) include every possible combination of hydrolysate products ranging from 6-carbon monomers consistent with mannose and galactose to oligosaccharide exceeding 10 units and more. Greenberg (2000) reported that partially hydrolyzed guar gum (PHGG), which is produced by the partial enzymatic hydrolysis of guar gum, has an average molecular weight of 25,000 Daltons, which was higher than the majority of the product detected in our MALOI-TOF analysis. As a prebiotic, the galactomannan and its degradation products may have some beneficial physiology functions, which is beyond the scope of this experiment. Dietary mannan-oligosaccharide, which is a possible hydrolysate from guar gum, was reported to increase cecal *Bifidobacterium spp.* and *Lactobacillus spp.* numbers, while decreasing members of Enterobacteriaceae and *Enterococcus spp*., and reduced susceptibility in young chickens to colonization by *S. enteritidis* (Spring et al., 2000; Fernandez et al., 2002). Ishihara et al. (2000) reported similar effects of PHGG on promoting the growth of *B. spp* and *L spp.*, improving the resistance to *S. enteritidis* of laying hens. Galactomannans isolated from mushrooms (*Morchella esculenta*) have been demonstrated to enhance macrophage activation thus exhibiting immunostimulatory activity (Duncan et al., 2002). It is interesting to note that all of the guar fed hens experienced significantly less mortality than the FW hens suggesting a health benefit of molting laying hens with guar meal with and without β-mannanase, which could result

[6] Texas A&M University Laboratory for Biological Mass Spectrometry, Department of Chemistry, College Station, TX.

from the prebiotic functions of guar gum and its hydrolysates.

In conclusion, the results of this study suggested that feeding laying hens 20% GM in a complete diet, with or without Hemicell®, or 15% GM without Hemicell®, was as effective as the conventional FW molting method with respect to the post-molt egg production and egg quality, and potentially more desirable with respect to animal welfare.

CHAPTER VI

IMPROVED RESISTANCE TO *SALMONELLA ENTERITIDIS* OF LAYING HENS INDUCED TO MOLT BY FULL-FEEDING GUAR MEAL DIETS

INTRODUCTION

Artificial molting of late phase laying hens is broadly adopted by the table-egg industry in the US and many other countries to stimulate multiple laying cycles and restore egg quality. Many methods have been used to induce a molt, among which removal of feed for a few days combined with a reduction of light exposure to obtain 25 to 35% body weight reduction, is the most widely used (Ruszler, 1998). The body weight loss is the result of ovary and oviduct regression, depletion of fat and labile protein reserves, the loss of alimentary canal contents, and initial water intake reduction (Ruszler, 1998). This method has many advantages because it is easy to manage, economical, and results in consistent post-molt performance. However, the perceived inhumane appearance of feed removal provokes criticisms from animal right activists, even though naturally occurring molting also leads to anorexia of many avian species (Mrosovsky and Sherry, 1980). In addition, FW decreases the immune function of laying hens making them more susceptible to *Salmonella* infection, indicated by increased intestinal shedding and translocation of *Salmonella* to internal organs such as liver, spleen and ovary (Holt, 1993; Thiagarajan et al., 1994; Holt et al., 1995; Durant et al., 1999). This of course can lead to increased risk of food-borne illness to consumers of

poultry products. Many alternative methods to FW have been studied, which include feeding laying hens high concentrations of zinc, low calcium, low sodium in the diet, or using drugs like nicarbazin, hormone, and others (Ruszler, 1998; Berry, 2003; Webster, 2003; Park et al., 2004). These alternatives are not accepted universally because they are either impractical or costly and because they usually result in poorer post-molt laying performance. Another category of alternative methods, full-feeding laying hens with by-products, such as alfalfa (Kwon et al., 2001), cottonseed meats (Davis et al., 2002), wheat middlings (Biggs et al., 2001; Seo et al., 2001; Biggs et al., 2003) and guar meal (Patel and McGinnis, 1981; Zimmermann et al., 1987) have been studied. Feeding wheat middlings (Seo et al., 2001) or alfalfa diets (Kwon et al., 2001) were reported to have potential as alternative methods for forced molting without increasing the incidence of *S. enteritidis* in eggs and internal organs.

Guar meal (GM) is a by-product of the gum industry which contains fairly high concentrations of gum residue, a polysaccharide of galactomannan (Bakshi et al., 1965; Nagpal et al., 1971). This polysaccharide is expected to have some prebiotic properties which improve immune function of laying hens and also improve resistance to pathogenic bacteria such as *Salmonella* (Bengmark, 1998; Ishihara et al., 2000; Duncan et al., 2002). Partially hydrolyzed guar gum (PHGG), produced by the partial hydrolysis of the guar gum (galactomannan) by β-endo-mannanase, is a neutral polysaccharide consisting of a mannose backbone chain with single galactose side units occurring on approximately two out of every three mannose units. The average molecular weight of PHGG was reported to be about 25,000 Daltons (Greenberg, 2000). Partially hydrolyzed

guar gum is fully fermentable in the large bowel with a high rate of volatile fatty acid formation. Several studies have demonstrated a bifidogenic effect with increased colonization of *Lactobacillus spp*. by PHGG feeding (Okubo et al., 1994; Takahashi et al., 1994a; Takahashi et al., 1995; Tuohy et al., 2001). Ishihara et al. (2000) investigated the effects of PHGG on the prevention of colonization by *S. enteritidis* in pullets and laying hens and found that the incidence of SE in organs (liver, spleen, heart, ovaries) and intestinal track (duodenum, small intestine, cecum, and large intestine) was decreased, and the excretion of SE into feces was increased. The agglutinating antibody titer to SE in serum was also decreased by the administration of PHGG at concentrations as low as 0.025% to young hens. The number of *Bifidobacterium spp*. and *Lactobacillus spp.*, the beneficial probiotic bacteria, in the cecum of young pullets were also increased by the supplementation of 0.025% PHGG. The incidence of SE on the surface of the eggshell, in egg albumin, and in egg yolk was also decreased when the feed of laying hens was supplemented with 0.025% PHGG. These results show that the administration of feed supplemented with PHGG can prevent the colonization of SE in pullets and laying hens and improve the balance of intestinal microflora.

In a previous study, a molting diet containing 20% GM with and without β-mannanase (Hemicell®) had been proved to be as effective as FW for molting laying hens (Chapter V). This study was designed to test the effect of full feeding 20% GM diets with and without enzyme (Hemicell®) on the resistance to *Salmonella* of molted laying hens. Tissue regression of certain organs was also examined.

MATERIALS AND METHODS

Salmonella Enteritidis Inocula

The *S. enteritidis* inocula were prepared as described previously (Ramirez et al., 1997). A primary poultry isolate of *S. enteritidis*, phage type 13A, obtained from the USDA National Veterinary Services Laboratory, was selected for resistance to novobiocin[7] (NO, No. n-1628) and to nalidixic acid [7] (NA, No. n-4382) within our laboratory. In this study, *S. enteritidis* was grown according to the method of Lee and Falkow (1990), allowing for attainment of log-phase growth. Bacterium were washed three times in distilled water by centrifugation (3,000 × *g*) and spectrophotometrically quantified to a stock concentration of approximately 1×10^9 cfu/ML in distilled water, using a standard curve generated from comparison of multiple spread platings and optical densities, and then diluted to challenge concentration. The actual concentration of inocula was approximately 1.65×10^7 cfu/ML as determined by multiple spread platings.

Experimental Design and Molting Procedure

The experimental animal care and treatment in the study was in accordance with the AUP 2003-0256 approved by the Institutional Agricultural Animal Care and Use Committee (IAACUC). A total of 43 Lohmann white late phase laying hens (68-wk-old) at similar BW (Mean ± SD: 1527 ± 114 g) were randomly allocated into 9 cages of a growing battery within an environmentally-controlled room with 5 hens in each of 8 cages and an extra 3 hens in another cage within the same room. The hens were allowed

[7] Sigma Chemical Co., St. Louis, MO.

to acclimate for one wk with free access to a typical corn-soybean based laying hen feed and water while on a 16L:8D light program. At the end of acclimation period, hens were weighed individually. The 8 cages were randomly assigned to 4 treatments consisting of feed withdrawal (denoted as FW control), full-fed diets with standard laying hen diet (denoted as Non-molted control), and full-fed diets combining standard laying hen feed with GM at 80:20 ratio with or without supplementation of 250,000 units/kg β-mannanase (Hemicell®), which were denoted as 20% GM and 20% GM + E. The extra 3 hens were also subjected to FW. The light program was changed to 8L:16D from the 1st d of treatment on. At the 4th day of treatment, all hens except the 3 extras were inoculated with 1 ML *S. enteritidis* (1.65×10^7 cfu/ML) by gavage. The 3 extra hens were not inoculated and served as a negative control group. Eggs laid during the study were collected and recorded daily. Full-fed hens received ad libitum feed and water, all hens received a 8L:16D light program and free access to water. Five days post-inoculation (9th d of treatment), all hens were euthanized by CO_2 asphyxiation, and crops, liver, spleen, ovary, oviduct and ceca were excised aseptically as described below.

Organ Weights and Salmonella Recovery

After clamping across the pre- and post-crop esophagi using surgical Carmalt forceps, the crop was sectioned aseptically with the lumen and contents intact, and collected into individual Whirl-Pac bags. The whole liver, spleen, ovary and oviduct (from infundibulum to shell gland) were sectioned aseptically and collected into individual Whirl-Pac bags separately and weighed. Then 20 ML tetrathionate (TT) broth

base,[8] (No. 0104-17-6) were added into each Whirl-Pac bag. The crop and oviduct samples were then stomached [9] for 60 s. One cecum from each hen was aseptically sectioned and cut into small pieces and collected into 50 ML conic centrifuge tubes with 30 ML TT broth base, and vortexed for 30 s. The other cecum was also aseptically sectioned and approximately 0.5 g cecal content was squeezed into centrifuge tube with 4.5 ML Butterfield's Buffer solution, then vortexed for a few seconds until the cecal content dispersed homogeneously. The solution then was serially diluted to a final dilution of 10^{-4} by sequentially transferring 0.5 ML to another centrifuge tube with 4.5 ML buffer solution. Each dilution (0.1 ML) was plated onto a brilliant green agar (BGA, No. 0285-01-5) [7] plate containing novobiocin 25 μg/ML and nalidixic acid 20 μg/ML (NO-NA-BGA plate) to prohibit growth of *Salmonella* other than the antibiotic-resistant challenge isolate. The plates were then incubated for 24 h at 37°C and the number of *S. enteritidis* cfu per gram of cecal contents was determined. Cecal contents in which *S. enteritidis* was not detected at the 10^{-1} dilution on BGA plates were scored as 0 cfu. The other organ samples with TT broth were stored at 0°C for 108 h prior to incubation for 24 h at 37°C. Following the enrichment phase, each sample was individually streaked onto a NO-NA-BGA plate and then incubated for another 24 h at 37°C and examined for the presence of *S. enteritidis*. The 108 h storage time prior to incubation is a little longer than what is typically reported for this type of procedure.

[8] Difco Laboratories, Detroit, MI.
[9] Tekmar Stomacher 80, Laboratory Blender, Cincinnati, OH.

Statistical Procedure

The days to 0 egg production were not subjected to statistical analysis because of the small sample size and lack of replication. Body weight and body weight reduction (BWR), absolute and relative weight of organs were subjected to ANOVA by the GLM procedure of the SAS System [5] for a complete randomized design. Each hen served as an individual experimental unit. The 3 extra hens that were fasted but not challenged were deemed as replications of the FW control when analyzing these parameters. Data were not collected on feed consumption. The presence of *S. enteritidis* in each organ was analyzed by Fisher's exact test (Uitenbroek, 2000). The *P* value for the same or a stronger association was used in Fisher's test (Garson, 2004). The total *S. enteritidis* positive numbers of organs from each group and from all hens were compared by Pearson's chi-square test (Uitenbroek, 2000). The number of *S. enteritidis* cfu per gram cecal content was first subjected to log transformation then analyzed using the GLM procedure of the SAS System [5] for a complete randomized design. The data from the 3 extra non-challenged fasted hens were not included when analyzing *S. enteritidis* presence and log cfu data. Significance level was set at $P \leq 0.05$.

RESULTS

Body Weight and Laying Activity

The changes of BW and laying activity are shown in Table 6-1. The mean BW of each group was very similar to each other ($P = 0.36$) at the beginning of treatment, and significantly different at the end of 9 d of treatment. Feed withdrawal hens lost 27% of their BW, while hens full fed 20% GM and 20% GM + E lost only 19.1 and 13.8% of

TABLE 6-1. Body weight (BW), body weight reduction (BWR), days to 0 egg production, and absolute and relative organ weight of laying hens molted by various methods[1]

Treatments	Initial BW (g)	Ending BW (g)	BWR (%)	Days to 0 egg[2]	Liver		Spleen		Ovary		Oviduct	
					Wt (g)	%	Wt (g)	%	Wt (g)	%	Wt (g)	%
FW control	1613	1166^{c}	27.01^{a}	5	24.2^{d}	2.08^{c}	1.55	0.13^{a}	10.6^{b}	0.90^{b}	17.7^{b}	1.53^{b}
20% GM	1571	1269^{b}	19.14^{b}	6	31.2^{c}	2.46^{b}	1.97	0.16^{a}	10.4^{b}	0.83^{b}	19.5^{b}	1.55^{b}
20% GM+E	1521	1310^{b}	13.80^{c}	5	36.2^{b}	2.76^{a}	1.96	0.15^{a}	10.5^{b}	0.80^{b}	20.6^{b}	1.57^{b}
Non-molted	1611	1605^{a}	0.07^{d}	--	48.1^{a}	2.99^{a}	1.56	0.10^{b}	45.7^{a}	2.85^{a}	65.2^{a}	4.07^{a}
Pooled MSE	133	110	3.50	--	4.9	0.30	0.45	0.03	4.4	0.28	5.7	0.42

[a-d] Means in the same column lacking of common superscript were significantly different ($P \leq 0.05$).
[1] Experimental laying hens were 69 wk old at the beginning of experiment.
[2] Days needed to cease laying for each treatment were not subjected to statistical analysis.

their BW, respectively, and hens full fed the standard laying feed did not lose any BW. Non-molted hens continued laying during the 9 d study. Both FW hens and full-fed hens with 20% GM or 20% GM + E completely went out of production at the 5th and 6th d of treatment.

Absolute and Relative Organ Weight

Liver. Non-molted hens, full-fed hens with 20% GM or 20% GM + E and FW hens had liver weights of 48, 36, 31 and 24 g, respectively, with significant differences between each other. However, the relative liver weight to body weight for non-molted hens and the 20% GM + E fed hens were not different from each other, and both were higher than the relative liver weight of the FW and 20% GM fed hens (Table 6-1).

Spleen. The differences in the spleen weights of various treatment groups were not significant. However, when the BW of hens was taken into consideration, non-molted hens had significantly lower relative spleen weights than molted hens. This is especially noticeable with respect to the GM fed hens (Table 6-1).

Ovary and Oviduct. Non-molted hens, which kept laying during the study, had significantly higher absolute and relative weights of ovary and oviduct than molted hens. No difference was observed among the 3 groups of molted hens with respect to the absolute and relative weights of ovary and oviduct (Table 6-1).

Salmonella Enteritidis Colonization and Organ Invasion

No *S. enteritidis* was detected from any of the 18 organ samples or cecal contents of the non-challenged negative control hens. Among the 6 organs tested from the challenged hens, cecum was most susceptible to *S. enteritidis* colonization, followed by

oviduct, then liver and ovary (Table 6-2). *Salmonella enteritidis* was less prevalent in crop and spleen. Compared to the non-molted control hens, all molted hens had a higher incidence of *S. enteritidis* in cecum and oviduct. In liver samples, FW hens and 20% GM fed hens had higher incidences of *S. enteritidis* than 20% GM + E and non-molted control hens. Fasted hens also had more *S. enteritidis* present in the crop and ovary than non-molted hens, while no difference between GM fed hens and non-molted hens was detected. When compared to the FW group, 20% GM fed hens had lower *S. enteritidis* positive numbers in the crop, and 20% GM + E fed hens had lower *S. enteritidis* positive numbers in crop, liver, and ovary. More pronounceable differences among treatments were detected when the numbers of *S. enteritidis* present were summed up for all 6 tested organs. All molted hens had higher total *S. enteritidis* positive numbers in the 6 tested organs than non-molted hens. Among molted hens, FW hens had higher numbers than 20% GM fed hens which were higher than 20% GM + E fed hens. With respect to cfu of *S. enteritidis* in cecal contents, FW hens were over 3 logs higher than GM fed hens which were 1.5 to 2 logs higher than non-molted hens.

DISCUSSION

Hens fed 20% GM both with and without β-mannanase (Hemicell®) supplementation completely went out of egg production at almost the same time as the fasted hens, which indicates that feeding GM at high concentrations is equivalently effective for inducing molt as the classic feed withdrawal method. This result confirms the findings of a previous study, in which hens fed 20% GM stopped laying 2 d later than fasted hens (Chapter V). Molted hens lost 200 to 450 g BW after the 9 d molting

TABLE 6-2. Colonization and invasion of *S. enteritidis* to organs and *S. enteritidis* cfu in cecal contents of laying hens induced to molt by various methods[1]

Treatments	Number of hens	*S. enteritidis* positive hens/total hens							Log SE cfu/g cecal content
		Crop	Liver	Spleen	Ovary	Oviduct	Cecum	All organs	
FW control	10	7/10^{a}	9/10^{a}	3/10^{ab}	7/10^{a}	8/10^{a}	10/10^{a}	44/60^{a}	6.717^{a}
20% GM	10	0/10^{b}	6/10^{a}	5/10^{a}	3/10^{ab}	5/10^{a}	9/10^{a}	28/60^{b}	3.457^{b}
20% GM+E	10	0/10^{b}	1/10^{b}	1/10^{ab}	1/10^{b}	5/10^{a}	9/10^{a}	17/60^{c}	2.955^{b}
Non-molted	10	2/10^{b}	0/10^{b}	0/10^{b}	0/10^{b}	0/10^{b}	2/10^{b}	4/60^{d}	1.270^{c}
Non-challenged	3	0/3	0/3	0/3	0/3	0/3	0/3	0/18	ND
Comparison among organs	43	9/43^{z}	16/43^{yz}	9/43^{z}	11/43^{yz}	18/43^{y}	30/43^{x}		MSE=1.529*

[a-d] Means in the same column lacking of common superscript were significantly different ($P \leq 0.05$).
[x-z] Means in the same row lacking of common superscript were significantly different ($P \leq 0.05$).
* Pooled MSE for log SE cfu/g cecal content of 40 experimental hens.
[1] Experimental laying hens were 70 wk old at the time of sampling.

period with a BWR of 27.0, 19.1 and 13.8% for FW, 20% GM and 20% GM + E fed hens, respectively. Higher BW losses of molted hens were observed compared to the previous study but the trend was the same. Non-molted control hens remained at the same BW and continued laying throughout the experiment. The BW loss has been reported to be the result of ovary and oviduct regression, depletion of fat and labile protein reserves, the loss of alimentary canal contents, and initial water intake reduction (Ruszler, 1998). In the present study, molted hens had losses ranging from 92 to 107 g on total weight of oviduct, ovary and liver, and losses ranging from approximately 110 to 340 g due to regression of other tissues, relative to the non-molted hens. We further noticed that all molted groups had almost the same weight of ovary and oviduct which indicates that the difference in BW loss among fasted hens and GM fed hens were due to the differences in the loss of fat, protein, water reserves and alimentary tract regression, and further indicates that the regression of the reproductive system of the GM molted hens were almost the same as those for fasted hens. A total of 25 to 30% BWR has been recommended to obtain optimal post-molt laying performance (Zimmermann et al., 1987; Carey and Brake, 1989), however, no suggestion has been proposed on the extent of the regression of reproductive organs. In a previous study (Chapter V) we observed that the post-molt performance of GM molted hens were similar or even superior to FW hens, whereas in the present study we found that hens molted by different methods lost almost the same weight of ovary and oviduct tissues and less weight of other tissues and organs, therefore, the BW loss other than ovary and oviduct observed in FW hens may not be critical to optimal performance in GM molted hens. Biggs et al. (2003, 2004) also

observed body weight losses of 8 to 18% for hens fed 94% wheat middlings, 94% corn, 71% wheat middlings with 23% corn, 47% wheat middlings with 47% corn, 95% corn gluten or 94% distillers dried grains with solubles for 28 d, but the low BWR did not result in low post-molt egg production compared to FW hens.

In the present study, GM fed hens had higher relative weights of spleen than hens fed our standard laying hen feed. Whether the increase in relative spleen weight was caused by GM feeding or by molting can not be determined, because fasted hens also had higher relative spleen weight than non-molted controls. Yamada et al. (2003) reported that feeding rats 5% dietary guar gum for 3 wk caused a significant increase in liver weight. Guar meal has also been reported to cause abnormality of internal organs (hypertrophy of pancreas, liver and gall bladder, and atrophy of the spleen) when fed at levels of 20% or higher (Nagpal et al., 1971), but no adverse effects on organs (liver, kidney and heart) were noted when GM was fed at 10% in a complete diet (Bakshi et al., 1964; Bakshi, 1966).

For each organ tested, the differences in *S. enteritidis* positive numbers between fasted hens and GM fed hens, as well as between GM fed hens with and without enzyme, were sparsely statistically significant. However, remarkable differences in the pooled *S. enteritidis* positive numbers over the 6 tested organs were detected between each treatment ($P \leq 0.05$). Compared to the fasted control, hens molted by full-fed GM had significantly reduced the *S. enteritidis* organ colonization numbers, and the GM diet with enzyme supplementation had a stronger effect on *S. enteritidis* resistance than the GM diet without enzyme. The evidence of GM's ability to enhance *S. enteritidis*

resistance of molted hens was also supported by the *S. enteritidis* cfu of cecal contents, which was decreased over 3 logs by GM feeding compared to feed withdrawal. The consistency between total *S. enteritidis* positive organ numbers and *S. enteritidis* cfu/g cecal contents indicate that, although the enrichment of *S. enteritidis* in organ samples were delayed for 108 h, the data are still valid supporting a conclusion that induced molt by full fed GM diets improves the resistance to *S. enteritidis* of molted hens. Improved *S. enteritidis* resistance of molted hens by full fed wheat middlings (Seo et al., 2001) and an alfalfa diet (Kwon et al., 2001) were also reported. The results of the study by Durant et al. (1999) suggest that changes in the microenvironment of the crop caused by FW are important regulators of *S. enteritidis* survival and influence the susceptibility of molted hens to *S. enteritidis* infections. In addition, the galactomannan and its enzymatic hydrolysates play a role in the improvement. Ishihara et al. (2000) demonstrated that as low as 0.025% dietary PHGG is effective in preventing SE colonization in internal organs and intestinal track, while also decreasing the presence of SE on the surface of egg shell and in albumin and egg yolk.

In conclusion, the present study demonstrated that full feeding late phase laying hens with 20% GM with and without β-mannanase (Hemicell®) results in an induced molt perhaps preferable to complete feed withdrawal. The laying hens induced to molt by GM feeding have improved resistance to *S. enteritidis* versus hens molted by total feed withdrawal, and supplementation of β-mannanase (Hemicell®) to GM diets may further enhance the *S. enteritidis* resistance of molted laying hens.

CHAPTER VII

CONCLUSION

In the first experiment, guar meal was fed to late phase laying hens at 5, 10 and 15% to determine the upper safe feeding level of guar meal for laying hens. The results showed that, 5% guar meal can be safely fed to late phase laying hens for at least 8 wk without adverse effects on laying performance. Feeding 10% guar meal decreased feed consumption and increased body weight loss. Feeding 15% guar meal to laying hens severely depressed egg production and feed consumption, and showed promise as a molt-inducing diet. The laying performance of hens molted by 15% guar meal feeding was improved compared to those continuously fed the control diet. Egg weight, Haugh units and shell quality were not affected by guar meal feeding, but the luminosity and yellowness of yolk was decreased.

In the 2nd experiment, 2.5 and 5% guar germ fraction and guar meal were fed to laying hen pullets commencing to lay. Through 20 wk of feeding, no adverse effects of feeding up to 5% guar germ or guar meal were observed with respect to egg production, feed consumption, egg weight, egg shell quality and solid egg components. The feed conversion efficiency, Haugh units, and luminosity and redness of egg yolk were decreased, however.

In the 3rd experiment, late phase laying hens were induced to molt by conventional feed withdrawal or full feeding diets with 15 or 20% guar meal with or without β-mannanase (Hemicell®). The results suggested that full-fed guar methods

(except for the 15% guar meal with enzyme), were as effective as the conventional feed withdrawal to induce a molt, with respect to the post-molt laying performance and egg quality.

In the 4th experiment, the resistance to *Salmonella* was compared among laying hens induced to molt by conventional feed withdrawal and by alternative methods of full-feeding 20% guar meal with or without β-mannanase (Hemicell®). The results showed that both alternative molting methods were as effective as the conventional feed withdrawal method. The alternative methods improved the resistance of molted laying hens to infection of *Salmonella*.

In conclusion, results of this research suggest that both guar germ fraction and guar meal can be fed to laying hens at a concentration up to 5% without unfavorable effect on egg production and egg quality, except that some aspects of egg yolk color may be slightly affected. It is doubtful these minor differences in yolk color can be perceived by most table egg consumers, however. Feeding 15 or 20% guar meal to late phase laying hens results in an induced molt which is as complete as the molt induced by conventional feed withdrawal methods. Molt induced by feeding relatively high concentrations of guar meal (20%) improves the resistance to *Salmonella* infection of laying hens, and the improvement may be enhanced by the inclusion of β-mannanase (Hemicell®) into guar meal molting diets.

REFERENCES

Albatshan, H. A., S. E. Scheideler, B. L. Black, J. D. Garlich, and K. E. Anderson. 1994. Duodenal calcium-uptake, femur ash, and eggshell quality decline with age and increase following molt. Poult. Sci. 73:1590-1596.

Ambegaokar, S. D., J. K. Kamath, and V. P. Shinde. 1969. Nutritional studies in protein of 'gawar' (*Cyamopsis tetragonoloba*). J. Nutr. Diet. 6:323-328.

American Association of Cereal Chemists. 2001. The definition of dietary fiber. Cereal Foods World 46:112-126.

Anderson, J. O., and R. E. Warnick. 1964. Value of enzyme supplements in rations containing certain legume seed meals or gums. Poult. Sci. 43:1091-1097.

Bajaj, K. L., M. Rani, and Y. K. Arora. 1978. Polyphenols and lignins of mature guar pods (*Cyamopsis tetragonoloba* L. Taub.). Plant Biochem. J. 5:140-145.

Baker, M., J. Brake, and G. R. McDaniel. 1983. The relationship between body weight loss during an induced molt and postmolt egg production, egg weight, and shell quality in caged layers. Poult. Sci. 62:409-413.

Bakshi, Y. K., C. R. Creger, and J. R. Couch. 1964. Studies on guar meal. Poult. Sci. 43:1302. (Abstr.)

Bakshi, Y. K., J. M. Prescott, C. R. Creger, and J. R. Couch. 1965. Evaluation of guar meal. Poult. Sci. 44:1350. (Abstr.)

Bakshi, Y. K. 1966. Studies on toxicity and processing of guar meal. Ph.D dissertation, Texas A&M University, College Station, TX.

Bar, A., V. Razaphkovsky, E. Wax, and Y. Malka. 2001. Effect of age at molting on postmolting performance. Poult. Sci. 80:874-878.

Bar, A., V. Razaphkovsky, D. Shinder, and E. Vax. 2003. Alternative procedures for molt induction: Practical aspects. Poult. Sci. 82:543-550.

Bengmark, S. 1998. Immunonutrition: Role of biosurfactants, fiber, and probiotic bacteria. Nutrition 14:585-594.

Berman, E., M. Schlicht, V. C. Moser, and R. C. Macphail. 1995. A multidisciplinary approach to toxicological screening. 1. Systemic toxicity. J. Toxicol. Environ. Health 45:127-143.

Berry, W. D., and J. Brake. 1985. Comparison of parameters associated with molt induced by fasting, zinc, and low dietary sodium in caged layers. Poult. Sci. 64:2027-2036.

Berry, W. D., and J. Brake. 1987. Postmolt performance of laying hens molted by high dietary zinc, low dietary-sodium, and fasting - Egg production and eggshell quality. Poult. Sci. 66:218-226.

Berry, W. D., and J. Brake. 1991. Induced molt increases eggshell quality and calbindin-d28k content of eggshell gland and duodenum of aging hens. Poult. Sci. 70:655-657.

Berry, W. D. 2003. The physiology of induced molting. Poult. Sci. 82:971-980.

Biggs, P. E., M. W. Douglas, K. W. Koelkebeck, and C. M. Parsons. 2001. Evaluation of non-feed removal versus feed removal methods for molting programs. Poult. Sci. 80(Suppl.1):91. (Abstr.)

Biggs, P. E., M. W. Douglas, K. W. Koelkebeck, and C. M. Parsons. 2003. Evaluation of non-feed removal methods for molting programs. Poult. Sci. 82:749-753.

Biggs, P. E., M. E. Persia, K. W. Koelkebeck, and C. M. Parsons. 2004. Further evaluation of nonfeed removal methods for molting programs. Poult. Sci. 83:745-752.

Blackburn, N. A., and I. T. Johnson. 1981. The effect of guar gum on the viscosity of the gastrointestinal contents and on glucose uptake from the perfused jejunum in the rat. Br. J. Nutr. 46:239-246.

Bourquin, L. D., E. C. Titgemeyer, and G. C. Fahey. 1996. Fermentation of various dietary fiber sources by human fecal bacteria. Nutr. Res. 16:1119-1131.

Brahma, T. C., S. M. Siddiqui, and C. V. Reddy. 1982. A study on the utilization of toasted guar meal with and without supplemental amino acid in broiler rations. Ind. Vet. J. 59:960-967.

Brake, J., and P. Thaxton. 1979. Physiological changes in caged layers during a forced molt. 2. Gross changes in organs. Poult. Sci. 58:707-716.

Breeding, S. W., J. Brake, J. D. Garlich, and A. L. Johnson. 1992. Molt induced by dietary zinc in a low-calcium diet. Poult. Sci. 71:168-180.

Brouns, F., B. Kettlitz, and E. Arrigoni. 2002. Resistant starch and "the butyrate revolution". Trends. Food Sci. Tech. 13:251-261.

Burke, W. H., and Y. A. Attia. 1994. Molting Single Comb White Leghorns with the use of the Lupron Depot ® formulation of leuprolide acetate. Poult. Sci. 73:1226-1232.

Campos, E. J., and N. C. Baiao. 1979. Effects of methods of forced molting on performance of commercial layers. Poult. Sci. 58:1040-1041. (Abstr.)

Carey, J. B., and J. T. Brake. 1989. Subject: Induced molting of commercial layers. http://www.ces.ncsu.edu/depts/poulsci/. Accessed April 2004.

Carter, J. W., W. E. Hardman, D. W. Heitman, and I. L. Cameron. 1998. Type and amount of individual dietary fibers on: serum lipid profiles, serum glucose concentration and energy intake in rats. Nutr. Res. 18:1743-1756.

Conner, S. R. 2002. Characterization of guar meal for use in poultry rations. Ph.D dissertation, Texas A&M University, College Station, TX.

Couch, J. R., Y. K. Bakshi, T. M. Ferguson, E. B. Smith, and C. R. Creger. 1967a. The effect of processing on the nutritional value of guar meal for broiler chicks. Br. Poult. Sci. 8:243-250.

Couch, J. R., J. A. Lazano, and C. R. Creger. 1967b. Soy protein guar meal and excess calcium in nutrition of commercial layers. Poult. Sci. 46:1248. (Abstr.)

Creger, C. R., and J. T. Scott. 1977. Dietary zinc as an effective resting agent for laying hen. Poult. Sci. 56:1706. (Abstr.)

Curl, C. L., K. R. Price, and G. R. Fenwick. 1986. Isolation and structural elucidation of a triterpenoid saponin from guar, *Cyamopsis tetragonoloba*. Phytochemistry 25:2675-2676.

Dario Frias, A. C., and V. C. Sgarbieri. 1998. Guar gum effects on food intake, blood serum lipids and glucose levels of Wistar rats. Plant Foods Hum. Nutr. 53:15-28.

Davis, A. J., M. M. Lordelo, and N. Dale. 2002. Use of cottonseed meats in molting programs. J. Appl. Poult. Res. 11:175-178.

Demigne, C., C. Morand, M. A. Levrat, C. Besson, C. Moundras, and C. Remesy. 1995. Effect of propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated rat hepatocytes. Br. J. Nutr. 74:209-219.

Dilworth, B. C., and E. J. Day. 1976. Sodium depletion and repletion of White Leghorn hens in cages. Poult. Sci. 55:1593.

Diwan, F. H., I. A. Abdel-Hassan, and S. T. Mohammed. 2000. Effect of saponin on mortality and histopathological changes in mice. East. Mediterr. Health J. 6:345-351.

Douglas, C. R., R. H. Harms, and H. R. Wilson. 1972. Use of extremely low dietary calcium to alter production pattern of laying hens. Poult. Sci. 51:2015-2020.

Duncan, C. J., N. Pugh, D. S. Pasco, and S. A. Ross. 2002. Isolation of a galactomannan that enhances macrophage activation from the edible fungus *Morchella esculenta*. J. Agric. Food Chem. 50:5683-5685.

Durant, J. A., D. E. Corrier, J. A. Byrd, L. H. Stanker, and S. C. Ricke. 1999. Feed deprivation affects crop environment and modulates *Salmonella enteritidis* colonization and invasion of leghorn hens. Appl. Environ. Microbiol. 65:1919-1923.

Fairchild, R. M., P. R. Ellis, A. J. Byrne, S. D. Luzio, and M. A. Mir. 1996. A new breakfast cereal containing guar gum reduces postprandial plasma glucose and insulin concentrations in normal weight human subjects. Br. J. Nutr. 76:63-73.

Favier, M. L., P. E. Bost, C. Guittard, C. Demigne, and C. Remesy. 1997a. The cholesterol-lowering effect of guar gum is not the result of a simple diversion of bile acids toward fecal excretion. Lipids 32:953-959.

Favier, M. L., P. E. Bost, C. Guittard, C. Demigne, and C. Remesy. 1997b. Reciprocal influence of fermentations and bile acid excretion on cholesterol-lowering effect of fermentable carbohydrate. J. Nutr. Biochem. 8:127-132.

Favier, M. L., P. E. Bost, C. Demigne, and C. Remesy. 1998. The cholesterol-lowering effect of guar gum in rats is not accompanied by an interruption of bile acid cycling. Lipids 33:765-771.

Fernandez, F., M. Hinton, and B. Van Gils. 2002. Dietary mannan-oligosaccharides and their effect on chicken caecal microflora in relation to *Salmonella Enteritidis* colonization. Avian Pathol. 31:49-58.

Fontana, E. A., P. L. Ruszler, W. L. Bean, and V. Magar. 1991. The effect of two feed withdrawal and two corticosterone supplementation programs on overall performance, body weight and reproductive organ weights of force-rested layers. Poult. Sci. 70:159. (Abstr.)

Fuller, R. 1989. Probiotics in man and animals. J. Appl. Bacteriol. 66:365-378.

Garlich, J., J. Brake, C. R. Parkhurst, J. P. Thaxton, and G. W. Morgan. 1984. Physiological profile of caged layers during one production year, molt, and postmolt: Egg production, egg shell quality, liver, femur, and blood parameters. Poult. Sci. 63:339-343.

Garson, G. D. 2004. Subject: PA 765 Statnotes: An online textbook - Fisher exact test of significance. http://www2.chass.ncsu.edu/garson/pa765/fisher.htm. Accessed May 2004.

Gatenby, S. J., P. R. Ellis, L. M. Morgan, and P. A. Judd. 1996. Effect of partially depolymerized guar gum on acute metabolic variables in patients with non-insulin-dependent diabetes. Diabet. Med. 13:358-364.

Gibson, G. R., and M. B. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J. Nutr. 125:1401-1412.

Gilbert, A. B., and R. Blair. 1975. Comparison of effects of two low calcium diets on egg production in domestic fowl. Br. Poult. Sci. 16:547-552.

Greenberg, N. A. 2000. The chemical, physical, and physiological properties of partially hydrolyzed guar gum. American Association of Cereal Chemists (AACC) 2000 Annual Meeting, Kansas City, MO.

Grobas, S., G. G. Mateos, and J. Mendez. 1999a. Influence of dietary linoleic acid on production and weight of eggs and egg components in young brown hens. J. Appl. Poult. Res. 8:177-184.

Grobas, S., J. Mendez, C. De Blas, and G. G. Mateos. 1999b. Laying hen productivity as affected by energy, supplemental fat, and linoleic acid concentration of the diet. Poult. Sci. 78:1542-1551.

Groop, P. H., A. Aro, S. Stenman, and L. Groop. 1993. Long-term effects of guar gum in subjects with non-insulin-dependent diabetes-mellitus. Am. J. Clin. Nutr. 58:513-518.

Hansen, R. W., S. M. Byrnes, and A. D. Johnson. 1992. Determination of galactomannan (gum) in guar (*Cyamopsis tetragonolobus*) by high performance liquid chromatography. J. Sci. Food Agric. 59:419-421.

Heitman, D. W., W. E. Hardman, and I. L. Cameron. 1992. Dietary supplementation with pectin and guar gum on 1,2-dimethylhydrazine-induced colon carcinogenesis in rats. Carcinogenesis 13:815-818.

Henningsson, A. M., I. M. Bjorck, and E. M. Nyman. 2002. Combinations of indigestible carbohydrates affect short-chain fatty acid formation in the hindgut of rats. J. Nutr. 132:3098-3104.

Holt, P. S. 1992a. Effects of induced molting on immune responses of hens. Br. Poult. Sci. 33:165-175.

Holt, P. S. 1992b. Effect of induced molting on B cell and CT4 and CT8 T cell numbers in spleens and peripheral blood of White Leghorn hens. Poult. Sci. 71:2027-2034.

Holt, P. S. 1993. Effect of induced molting on the susceptibility of White Leghorn hens to a *Salmonella enteritidis* infection. Avian Dis. 37:412-417.

Holt, P. S. 1995. Horizontal transmission of *Salmonella enteritidis* in molted and unmolted laying chickens. Avian. Dis. 39:239-249.

Holt, P. S., N. P. Macri, and R. E. Porter. 1995. Microbiological analysis of the early *Salmonella enteritidis* infection in molted and unmolted hens. Avian Dis. 39:55-63.

Holt, P. S., B. W. Mitchell, and R. K. Gast. 1998. Airborne horizontal transmission of *Salmonella enteritidis* in molted laying chickens. Avian Dis. 42:45-52.

Holt, P. S. 2003. Molting and *Salmonella* enterica serovar *enteritidis* infection: The problem and some solutions. Poult. Sci. 82:1008-1010.

Hsu, J. C., C. Y. Lin, and P. W. Chiou. 1998. Effects of ambient temperature and methionine supplementation of a low protein diet on the performance of laying hens. Anim. Feed Sci. Tech. 74:289-299.

Hurwitz, S., S. Bornstein, and Y. Lev. 1975. Some responses of laying hens to induced arrest of egg production. Poult. Sci. 54:415-422.

Hurwitz, S., E. Wax, Y. Nisenbaum, and I. Plavnik. 1995. Responses of laying hens to forced molt procedures of variable length with or without light restriction. Poult. Sci. 74:1745-1753.

Hurwitz, S., E. Wax, Y. Nisenbaum, M. Ben-Moshe, and I. Plavnik. 1998. The response of laying hens to induced molt as affected by strain and age. Poult. Sci. 77:22-31.

Ishihara, N., D. C. Chu, S. Akachi, and L. R. Juneja. 2000. Preventive effect of partially hydrolyzed guar gum on infection of *Salmonella enteritidis* in young and laying hens. Poult. Sci. 79:689-697.

Johnson, A. L., and J. Brake. 1992. Zinc-induced molt: Evidence for a direct inhibitory effect on granulosa cell steroidogenesis. Poult. Sci. 71:161-167.

Kaushal, G. P., and I. S. Bhatia. 1982. A study of polyphenols in the seeds and leaves of guar (*Cyamopsis tetragonoloba* L. Taub) feed toxicity studies. J. Sci. Food Agric. 33:461-470.

Keshavarz, K., and S. Nakajima. 1995. The effect of dietary manipulations of energy, protein, and fat during the growing and laying periods on early egg weight and egg components. Poult. Sci. 74:50-61.

Keshavarz, K., and F. W. Quimby. 2002. An investigation of different molting techniques with an emphasis on animal welfare. J. Appl. Poult. Res. 11:54-67.

Kogut, M. H., K. J. Genovese, and L. H. Stanker. 1999. Effect of induced molting on heterophil function in White Leghorn hens. Avian Dis. 43:538-548.

Kuehl, R. O. 1999. Design of Experiments: Statistical Principles of Research Design and Analysis. 2nd ed. Duxbury/Thomson Learning, Pacific Grove, CA.

Kumar, R. 1991. Subject: Anti-nutritional factors, the potential risks of toxicity and methods to alleviate them. http://www.fao.org/DOCREP/003/T0632E/T0632E10.htm. Accessed Feb. 2004.

Kwon, Y. M., L. F. Kubena, C. L. Woodward, J. A. Byrd, R. W. Moore, D. J. Nisbet, and S. C. Ricke. 2001. Use of an alfalfa diet for molting in Leghorn hens to reduce *Salmonella enteritidis* colonization and invasion. Poult. Sci. 80(Suppl. 1):90. (Abstr.)

Lee, C. A., and S. Falkow. 1990. The ability of *Salmonella* to enter mammalian cells is affected by bacterial growth state. Proc. Natl. Acad. Sci. USA 87:4304-4308.

Lee, J. T., C. A. Bailey, and A. L. Cartwright. 2003a. Guar meal germ and hull fractions differently affect growth performance and intestinal viscosity of broiler chickens. Poult. Sci. 82:1589-1595.

Lee, J. T., C. A. Bailey, and A. L. Cartwright. 2003b. Beta-mannanase ameliorates viscosity-associated depression of growth in broiler chickens fed guar germ and hull fractions. Poult. Sci. 82:1925-1931.

Lee, K. 1982. Effects of forced molt period on post-molt performance of Leghorn hens. Poult. Sci. 61:1594-1598.

Lu, Z. X., P. R. Gibson, J. G. Muir, M. Fielding, and K. O'Dea. 2000. Arabinoxylan fiber from a by-product of wheat flour processing behaves physiologically like a soluble, fermentable fiber in the large bowel of rats. J. Nutr. 130:1984-1990.

Maisonnier, S., J. Gomez, and B. Carre. 2001. Nutrient digestibility and intestinal viscosities in broiler chickens fed on wheat diets, as compared to maize diets with added guar gum. Br. Poult. Sci. 42:102-110.

McDaniel, B. A., and D. R. Aske. 2000. Egg prices, feed costs, and the decision to molt. Poult. Sci. 79:1242-1245.

McIntyre, A., G. P. Young, T. Taranto, P. R. Gibson, and P. B. Ward. 1991. Different fibers have different regional effects on luminal contents of rat colon. Gastroenterology 101:1274-1281.

Moriceau, S., C. Besson, M. A. Levrat, C. Moundras, C. Remesy, C. Morand, and C. Demigne. 2000. Cholesterol-lowering effects of guar gum: Changes in bile acid pools and intestinal reabsorption. Lipids 35:437-444.

Moundras, C., S. R. Behr, C. Demigne, A. Mazur, and C. Remesy. 1994. Fermentable polysaccharides that enhance fecal bile acid excretion lower plasma cholesterol and apolipoprotein E-rich HDL in rats. J. Nutr. 124:2179-2188.

Mrosovsky, N., and D. F. Sherry. 1980. Animal anorexias. Science 207:837-842.

Nagata, Y., M. Murase, Y. Kimura, and K. Ebihara. 1996. Effect of guar gum on glucose metabolism in cecectomized rats. J. Nutr. Biochem. 7:303-308.

Nagpal, M. L., O. P. Agrawal, and I. S. Bhatia. 1971. Chemical and biological examination of guar meal (*Cyamopsis tetragonoloba* L.). Ind. J. Anim. Sci. 41:283-293.

Nagra, S. S., B. K. Shingari, and J. S. Ichhponani. 1985. Feeding of guar (*Cyamopsis tetragonoloba*) meal to poultry. 1. Growth of commercial broiler chicks. Ind. J. Poult. Sci. 20:188-193.

Nagra, S. S., and R. S. Virk. 1986. Growth and laying performance of White Leghorn pullets fed toasted guar meal alone or in combination with groundnut and mustard cakes as sources of protein. Ind. J. Poult. Sci. 21:16-20.

Nesbeth, W. G., C. R. Douglas, and R. H. Harms. 1976a. Response of laying hens to a low salt diet. Poult. Sci. 55:2128-2133.

Nesbeth, W. G., C. R. Douglas, and R. H. Harms. 1976b. Potential use of dietary salt deficiency for force resting of laying hens. Poult. Sci. 55:2375-2379.

Nishina, P. M., and R. A. Freedland. 1990. The effects of dietary fiber feeding on cholesterol metabolism in rats. J. Nutr. 120:800-805.

Okubo, T., N. Ishihara, H. Takahashi, T. Fujisawa, M. J. Kim, T. Yamamoto, and T. Mitsuoka. 1994. Effects of partially hydrolyzed guar gum intake on human intestinal microflora and its metabolism. Biosci. Biotechnol. Biochem. 58:1364-1369.

Ou, S. Y., K. C. Kwok, Y. Li, and L. Fu. 2001. In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. J. Agric. Food Chem. 49:1026-1029.

Panda, P. C. 1996. Textbook on Egg and Poultry Technology. Vikas Publishing House, Delhi, India.

Park, S. Y., S. G. Birkhold, L. F. Kubena, D. J. Nisbet, and S. C. Ricke. 2004. Effects of high zinc diets using zinc propionate on molt induction, organs, and postmolt egg production and quality in laying hens. Poult. Sci. 83:24-33.

Patel, M. B., and J. McGinnis. 1981. Induced cessation of egg production (forced molting) by addition of guar meal to the diet. Poult. Sci. 60:1710. (Abstr.)

Patel, M. B., and J. McGinnis. 1985. The effect of autoclaving and enzyme supplementation of guar meal on the performance of chicks and laying hens. Poult. Sci. 64:1148-1156.

Penz, A. M., and L. S. Jensen. 1991. Influence of protein concentration, amino acid supplementation, and daily time of access to high-protein or low-protein diets on egg weight and components in laying hens. Poult. Sci. 70:2460-2466.

Prochaska, J. F., J. B. Carey, and D. J. Shafer. 1996. The effect of L-lysine intake on egg component yield and composition in laying hens. Poult. Sci. 75:1268-1277.

Ramirez, G. A., L. L. Sarlin, D. J. Caldwell, C. R. Yezak, M. E. Hume, D. E. Corrier, J. R. Deloach, and B. M. Hargis. 1997. Effect of feed withdrawal on the incidence of *Salmonella* in the crops and ceca of market age broiler chickens. Poult. Sci. 76:654-656.

Ricke, S. C. 2003. The gastrointestinal tract ecology of *Salmonella enteritidis* colonization in molting hens. Poult. Sci. 82:1003-1007.

Roberson, K. 2004. Subject: Corn dried distillers grains with solubles for laying hens – They're great. http://www.ddgs.umn.edu/articles-poultry/MPF04-DDGS-Roberson.pdf. Accessed May 2004.

Roberson, R. H., and D. W. Francis. 1979. Effect of two molting methods on the performance of Hy-Line and Shaver hens. Poult. Sci. 58:1098. (Abstr.)

Ross, E., and R. B. Herrick. 1981a. Forced rest induced by molt or low-salt diet and subsequent hen performance. Poult. Sci. 60:63-67.

Ross, E., and R. B. Herrick. 1981b. Effect of low-sodium diet on force resting four strains of layers. Poult. Sci. 60:1721. (Abstr.)

Rossi, M., and C. Pompei. 1995. Changes in some egg components and analytical values due to hen age. Poult. Sci. 74:152-160.

Russo, A., T. Wilson, F. Wells, A. Tonkin, M. Horowitz, and K. L. Jones. 2002. Guar gum attenuates the fall in blood pressure after oral glucose in patients with type 2 diabetes. Gastroenterology 122:A454. (Abstr.)

Russo, A., J. E. Stevens, T. Wilson, F. Wells, A. Tonkin, M. Horowitz, and K. L. Jones. 2003. Guar attenuates fall in postprandial blood pressure and slows gastric emptying of oral glucose in type 2 diabetes. Dig. Dis. Sci. 48:1221-1229.

Ruszler, P. L. 1998. Health and husbandry considerations of induced molting. Poult. Sci. 77:1789-1793.

Sathe, B. S., and S. Bose. 1962. Studies on the utilization of industrial and farm byproducts in growing poultry rations. Ind. J. Vet. Sci. 32:74-84.

Saxena, U. C., and K. Pradhan. 1974. Effect of high protein levels on the replacement value of guar meal (*Cyanopsis tetragonoloba*) in layer's ration [Chickens]. Ind. J. Anim. Sci. 44:190-193.

Scott, J. T., and C. R. Creger. 1976. Use of zinc as an effective molting agent in laying hens. Poult. Sci. 55:2089. (Abstr.)

Seo, K. H., P. S. Holt, and R. K. Gast. 2001. Comparison of *Salmonella enteritidis* infection in hens molted via long term feed withdrawal versus full fed wheat middling. J. Food Prot. 64:1917-1921.

Shafer, D. J., J. B. Carey, and J. F. Prochaska. 1996. Effect of dietary methionine intake on egg component yield and composition. Poult. Sci. 75:1080-1085.

Shafer, D. J., J. B. Carey, J. F. Prochaska, and A. R. Sams. 1998. Dietary methionine intake effects on egg component yield, composition, functionality, and texture profile analysis. Poult. Sci. 77:1056-1062.

Shippee, R. L., P. E. Stake, U. Koehn, J. L. Lambert, and R. W. Simmons. 1979. High dietary zinc or magnesium as forced-resting agents for laying hens. Poult. Sci. 58:949-954.

Sierra, M., J. J. Garcia, N. Fernandez, M. J. Diez, A. P. Calle, and A. M. Sahagun. 2001. Effects of ispaghula husk and guar gum on postprandial glucose and insulin concentrations in healthy subjects. Eur. J. Clin. Nutr. 55:235-243.

Spring, P., C. Wenk, K. A. Dawson, and K. E. Newman. 2000. The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of *Salmonella* challenged broiler chicks. Poult. Sci. 79:205-211.

Takahashi, H., N. Wako, T. Okubo, N. Ishihara, J. Yamanaka, and T. Yamamoto. 1994a. Influence of partially hydrolyzed guar gum on constipation in women. J. Nutr. Sci. Vitaminol. 40:251-259.

Takahashi, H., S. I. Yang, Y. Ueda, M. Kim, and T. Yamamoto. 1994b. Influence of intact and partially hydrolyzed guar gum on iron utilization in rats fed on iron-deficient diets. Comp. Biochem. Physiol. A Physiol. 109:75-82.

Takahashi, H., S. Akachi, Y. Ueda, M. Kim, K. Hirano, and T. Yamamoto. 1995. Effect of liquid diets with or without partially hydrolyzed guar gum on intestinal microbial-flora and function of rats. Nutr. Res. 15:527-536.

Thakur, R. S., and K. Pradhan. 1975a. A note on inclusion of guar meal (*Cyamopsis tetragonoloba* L.) in broiler rations. Ind. J. Anim. Sci. 45:98-102.

Thakur, R. S., and K. Pradhan. 1975b. Guar meal (*Cyamopsis tetragonoloba* L.) inclusion in broiler rations. Effect on carcass yield and meat composition. Ind. J. Anim. Sci. 45:880-884.

Thiagarajan, D., A. M. Saeed, and E. K. Asem. 1994. Mechanism of transovarian transmission of *Salmonella enteritidis* in laying hens. Poult. Sci. 73:89-98.

Tulung, B., C. Remesy, and C. Demigne. 1987. Specific effects of guar gum or gum-arabic on adaptation of cecal digestion to high-fiber diets in the rat. J. Nutr. 117:1556-1561.

Tungland, B. C., and D. Meyer. 2002. Nondigestible oligo- and polysaccharides (dietary fiber): Their physiology and role in human health and food. Compr. Rev. Food Sci. Food Saf. 1:73-92.

Tuohy, K. M., S. Kolida, A. M. Lustenberger, and G. R. Gibson. 2001. The prebiotic effects of biscuits containing partially hydrolyzed guar gum and fructo-oligosaccharides: A human volunteer study. Br. J. Nutr. 86:341-348.

Uitenbroek, D. G. 2000. Subject: SISA-Fisher's exact. http://home.clara.net/sisa/fisher.htm. Accessed May 2004.

USDA. 2000. Subject: Egg-grading manual. Agriculture Handbook Number 75. http://www.ams.usda.gov/poultry/pdfs/EggGrading%20manual.pdf. Accessed Feb. 2004.

VanEtten, C. H., R. W. Miller, I. A. Wolff, and Q. Jones. 1961. Amino acid composition of twenty seven selected seed meals. J. Agric. Food Chem. 9:79-82.

Velazquez, M., C. Davies, R. Marett, J. L. Slavin, and J. M. Feirtag. 2000. Effect of oligosaccharides and fibre substitutes on short-chain fatty acid production by human fecal microflora. Anaerobe 6:87-92.

Verma, S. V. S., and J. M. McNab. 1982. Guar meal in diets for broiler chickens. Br. Poult. Sci. 23:95-105.

Verma, S. V. S., and J. M. McNab. 1984a. Chemical, biochemical and microbiological examination of guar meal. Ind. J. Poult. Sci. 19:165-170.

Verma, S. V. S., and J. M. McNab. 1984b. Performance of hens fed guar meal containing diets with or without supplemental cholesterol. Ind. J. Poult. Sci. 19:245-250.

Vermaut, S., K. De Coninck, O. Onagbesan, G. Flo, M. Cokelaere, and E. Decuypere. 1998. A jojoba-rich diet as a new forced molting method in poultry. J. Appl. Poult. Res. 7:239-246.

Vohra, P., and F. H. Kratzer. 1964. Growth inhibitory effect of certain polysaccharides for chickens. Poult. Sci. 43:1164-1170.

Walzem, R. L., C. Simon, T. Morishita, L. Lowenstine, and R. J. Hansen. 1993. Fatty liver hemorrhagic syndrome in hens overfed a purified diet: Selected enzyme activities and liver histology in relation to liver hemorrhage and reproductive performance. Poult. Sci. 72:1479-1491.

Wasan, H. S., and R. A. Goodlad. 1996. Fibre-supplemented foods may damage your health. Lancet 348:319-320.

Webster, A. B. 2003. Physiology and behavior of the hen during induced molt. Poult. Sci. 82:992-1002.

Whitehead, C. C., and D. W. Shannon. 1974. Control of egg production using a low-sodium diet. Br. Poult. Sci. 15:429-434.

Whitehead, C. C., A. S. Bowman, and H. D. Griffin. 1991. The effects of dietary fat and bird age on the weights of eggs and egg components in the laying hen. Br. Poult. Sci. 32:565-574.

Williams, J. A., C. S. Lai, H. Corwin, Y. Y. Ma, K. C. Maki, K. A. Garleb, and B. W. Wolf. 2004. Inclusion of guar gum and alginate into a crispy bar improves postprandial glycemia in humans. J. Nutr. 134:886-889.

Wood, F. E., and S. J. Stoll. 1991. The effect of dietary guar gum and cellulose on mineral excretion and status in young male Fischer-344 rats. Nutr. Res. 11:621-632.

Wright, R. S., J. W. Anderson, and S. R. Bridges. 1990. Propionate inhibits hepatocyte lipid synthesis. Proc. Soc. Exp. Biol. Med. 195:26-29.

Yamada, K., Y. Tokunaga, A. Ikeda, K. Ohkura, S. Mamiya, S. Kaku, M. Sugano, and H. Tachibana. 1999. Dietary effect of guar gum and its partially hydrolyzed product on the lipid metabolism and immune function of Sprague-Dawley rats. Biosci. Biotechnol. Biochem. 63:2163-2167.

Yamada, K., Y. Tokunaga, A. Ikeda, K. Ohkura, S. Kaku-Ohkura, S. Mamiya, B. O. Lim, and H. Tachibana. 2003. Effect of dietary fiber on the lipid metabolism and immune function of aged Sprague-Dawley rats. Biosci. Biotechnol. Biochem. 67:429-433.

Yamamoto, Y., I. Sogawa, A. Nishina, S. Saeki, N. Ichikawa, and S. Iibata. 2000. Improved hypolipidemic effects of xanthan gum - galactomannan mixtures in rats. Biosci. Biotechnol. Biochem. 64:2165-2171.

Yamatoya, K., K. Kuwano, and J. Suzuki. 1997. Effects of hydrolyzed guar gum on cholesterol and glucose in humans. Food Hydrocolloids 11:239-242.

Yannakopoulos, A. L., A. S. Tservenigousi, and P. Nikokyris. 1994. Egg composition as influenced by time of oviposition, egg weight, and age of hens. Arch. Geflugelkd. 58:206-213.

Zimmermann, N. G., D. K. Andrews, and J. McGinnis. 1987. Comparison of several induced molting methods on subsequent performance of Single Comb White Leghorn hens. Poult. Sci. 66:408-417.

APPENDIX I

Nutrient matrix of guar by-products used in these studies[1]

	Guar germ fraction	Guar meal
Moisture (%)	8.80	7.00
Crude protein (%)	43.7	38.3
ME (kcal/kg)	2,140	2,033
Calcium (%)	0.16	0.16
Av P (%)	0.16	0.16
Methionine (%)	0.53	0.45
Lysine (%)	2.00	1.64
Arginine (%)	6.00	4.90
Threonine (%)	1.20	1.04
Tryptophan (%)	0.50	0.43

[1]Nutrient values represent a compilation of assayed guar by-products determined by Conner (2002), Office of the Texas State Chemist, Texas A&M Protein Chemistry Laboratory and the Degussa-Huls Corporation.

APPENDIX I

Continued

User : SUPER — Texas A & M University — Date : 6/24/2004
Ingredient Report — Time : 15:00
As Fed — Page : 1
Plant : TAMU - TAMU Research Farm

Ingredient/ Nutrient Code	Description		Price for Plant TAMU
2211	Guar Germ		10.00
1	Weight	Lbs	1.0000
2	Dry Matter	Pct	91.20
3	Moisture~	Pct	8.80
4	Crude Protein	Pct	43.70
9	Ether Extract	Pct	3.50
10	Crude Fiber	Pct	6.50
12	Ash	Pct	4.80
13	Calcium	Pct	0.16
14	Total Phosphorus	Pct	0.50
15	Available Phos	Pct	0.16
17	Ca/TPhos	Pct/Pct	0.32
18	Ca/AvPhos	Pct/Pct	1.00
20	Poultry ME/kg	Kcal/kg	2,140
22	Poultry ME/lb~	Kcal/lb	973
23	Poultry Mcal/lb~	Mcal/lb	0.973
35	Non Structural CHO~	Pct	56.30
40	Methionine	Pct	0.53
41	Cystine	Pct	0.58
42	Met + Cys~	Pct	1.11
43	Lysine	Pct	2.00
44	Arginine	Pct	6.00
45	Threonine	Pct	1.20
46	Tryptophan	Pct	0.50
47	Glycine	Pct	2.32
48	Serine	Pct	2.12
49	Histidine	Pct	1.19
50	Isoleucine	Pct	1.31
51	Leucine	Pct	2.45
52	Valine	Pct	1.64
53	Phenylalanine	Pct	1.73
54	Tyrosine	Pct	1.60
55	Phe + Tyr~	Pct	3.33
104	NA+K-CL~	Meq/kg	167.67
105	(Na + K)-(Cl-S)~	Meq/100g	3.02
107	Sodium	Pct	0.02
108	Potassium	Pct	0.62
110	Sulfur	Pct	0.22

APPENDIX I

Continued

User : SUPER — Texas A & M University — Date : 6/24/2004
Ingredient Report — Time : 15:00
As Fed — Page : 2
Plant : TAMU - TAMU Research Farm

Ingredient/ Nutrient Code	Description		Price for Plant TAMU
2213	Guar Meal		10.00
1	Weight	Lbs	1.0000
2	Dry Matter	Pct	93.00
3	Moisture~	Pct	7.00
4	Crude Protein	Pct	38.30
9	Ether Extract	Pct	3.50
10	Crude Fiber	Pct	6.50
12	Ash	Pct	4.80
13	Calcium	Pct	0.16
14	Total Phosphorus	Pct	0.50
15	Available Phos	Pct	0.16
17	Ca/TPhos	Pct/Pct	0.32
18	Ca/AvPhos	Pct/Pct	1.00
20	Poultry ME/kg	Kcal/kg	2,033
22	Poultry ME/lb~	Kcal/lb	924
23	Poultry Mcal/lb~	Mcal/lb	0.924
35	Non Structural CHO~	Pct	61.70
40	Methionine	Pct	0.45
41	Cystine	Pct	0.50
42	Met + Cys~	Pct	0.95
43	Lysine	Pct	1.64
44	Arginine	Pct	4.90
45	Threonine	Pct	1.04
46	Tryptophan	Pct	0.43
47	Glycine	Pct	2.01
48	Serine	Pct	1.85
49	Histidine	Pct	0.98
50	Isoleucine	Pct	1.10
51	Leucine	Pct	2.07
52	Valine	Pct	1.38
53	Phenylalanine	Pct	1.45
54	Tyrosine	Pct	1.38
55	Phe + Tyr~	Pct	2.83
104	NA+K-CL~	Meq/kg	167.67
105	(Na + K)-(Cl-S)~	Meq/100g	3.02
107	Sodium	Pct	0.02
108	Potassium	Pct	0.62
110	Sulfur	Pct	0.22

APPENDIX II

Egg interior and exterior shell quality of laying hens fed diets containing 0, 5, 10 or 15% guar meal (Chapter III) [1]

Wk	Guar meal concentration (%)			
	0	5.0	10.0	15.0→0 [2]
	---------- Egg weight (g)----------			
0	61.2 ± 3.7	61.5 ± 4.6	62.7 ± 4.4	64.7 ± 6.8
1	63.3 ± 4.2	61.1 ± 4.5	60.8 ± 4.8	59.9 ± 5.4
2	60.9 ± 4.9	58.3 ± 6.1	59.5 ± 6.2	No data
3	60.6 ± 8.1	58.1 ± 5.8	59.5 ± 5.9	58.4 ± 5.7
4	59.3 ± 7.3	57.0 ± 5.3	59.0 ± 6.6	No data
5	58.7 ± 4.0	59.3 ± 5.6	58.5 ± 6.1	60.1 ± 3.7
6	56.2 ± 5.0	57.7 ± 5.9	55.8 ± 4.4	59.7 ± 4.4
7	58.7 ± 7.8	56.6 ± 4.8	56.7 ± 2.7	61.4 ± 5.3
8	57.3 ± 6.1	57.8 ± 3.2	60.3 ± 6.3	58.7 ± 4.4
	---------- Haugh units (g)----------			
0	74.0 ± 10.7	75.0 ± 9.7	70.4 ± 15.0	78.1 ± 11.1
1	75.4 ± 12.1	81.3 ± 10.7	70.1 ± 13.8	73.6 ± 13.8
2	73.2 ± 16.2 [b]	86.7 ± 5.8 [a]	77.8 ± 18.9 [ab]	No data
3	72.2 ± 13.8	79.4 ± 15.7	75.1 ± 15.4	82.5 ± 7.9
4	78.7 ± 14.5	77.4 ± 13.3	75.8 ± 13.7	No da ta
5	80.3 ± 8.2	84.1 ± 11.5	76.1 ± 11.2	86.5 ± 7 .4
6	76.4 ± 11.7	80.7 ± 15.8	74.8 ± 20.5	86.0 ± 7.7
7	78.9 ± 13.3	82.3 ± 8.4	71.9 ± 16.2	79.2 ± 11.6
8	76.5 ± 9.5	80.3 ± 10.2	78.6 ± 15.1	83.5 ± 9.6
	---------- Shell breaking force (kg)----------			
0	2.46 ± 0.57	2.55 ± 0.58	2.18 ± 0.83	2.16 ± 0.62
1	2.76 ± 0.75[a]	2.25 ± 0.88[ab]	2.33 ± 0.71[a]	1.59 ± 0.56[b]
2	2.65 ± 0.92	2.51 ± 0.96	3.01 ± 1.05	No data
3	2.00 ± 1.02	2.86 ± 0.96	2.63 ± 0.64	2.28 ± 1.17
4	2.59 ± 0.74	2.48 ± 0.53	2.47 ± 0.88	No data
5	2.32 ± 1.20	2.63 ± 1.06	2.80 ± 0.82	2.77 ± 0.53

APPENDIX II

Continued

Wk	Guar meal concentration (%)			
	0	5.0	10.0	15.0→0 [2]
	---------- Shell breaking force (kg)----------			
6	2.52 ± 0.60	2.68 ± 0.72	3.17 ± 0.70	2.65 ± 0.67
7	2.30 ± 0.92	2.77 ± 0.77	2.12 ± 1.03	3.10 ± 0.81
8	1.99 ± 0.80[b]	2.07 ± 0.79[b]	2.61 ± 0.69[ab]	2.95 ± 0.81[a]
	---------- Shell thickness (mm)----------			
0	0.327 ± 0.026	0.324 ± 0.026	0.312 ± 0.030	0.304 ± 0.026
1	0.339 ± 0.023[a]	0.329 ± 0.024[ab]	0.314 ± 0.026[bc]	0.303 ± 0.029[c]
2	0.333 ± 0.027	0.331 ± 0.033	0.309 ± 0.023	No data
3	0.328 ± 0.032	0.341 ± 0.021	0.329 ± 0.043	0.296 ± 0.028
4	0.324 ± 0.025	0.316 ± 0.034	0.315 ± 0.024	No data
5	0.316 ± 0.021	0.329 ± 0.029	0.318 ± 0.022	0.314 ± 0.044
6	0.320 ± 0.038	0.340 ± 0.022	0.321 ± 0.029	0.333 ± 0.032
7	0.321 ± 0.020	0.314 ± 0.045	0.309 ± 0.032	0.329 ± 0.019
8	0.310 ± 0.031[b]	0.309 ± 0.028[b]	0.317 ± 0.028[ab]	0.339 ± 0.020[a]
	---------- Luminosity (L*) of yolk ----------			
0	56.55 ± 1.66	55.47 ± 1.32	56.43 ± 1.52	56.00 ± 1.89
4	58.72 ± 2.76[a]	56.81 ± 2.52[b]	56.62 ± 1.91[b]	54.29 ± 2.91[c]
	---------- Redness (a*) of yolk ----------			
0	-4.50 ± 0.58	-4.20 ± 0.53	-4.40 ± 0.46	-4.14 ± 0.56
4	-4.49 ± 0.58[ab]	-3.94 ± 0.57[a]	-4.73 ± 0.68[b]	-4.00 ± 1.03[a]
	---------- Yellowness (b*) of yolk ----------			
0	41.48 ± 3.14	41.88 ± 1.94	42.48 ± 2.48	42.61 ± 2.84
4	44.85 ± 2.94[a]	43.13 ± 2.55[ab]	41.57 ± 3.02[b]	38.88 ± 3.41[b]

[a-c] Means within a row lacking a common superscript are significantly different ($P < 0.05$).
[1] Experimental laying hens were 76 wk old at the beginning of the experiment.
[2] The 15% guar meal treatment was only fed for 4 wk at which time the 15% diet was replaced with the 0% guar meal diet (control).

VITA

Cheng Zhang

Permanent Address:

Yangbian cun #8, Shibi, Ninghua, Fujian Province, P.R. China

Education:

1989 – 1993, Beijing Agricultural University, B.S., Animal Science

1997 – 2000, M.S., China Agricultural University, Animal Nutrition and Feed Science

2000 – 2004, Ph.D., Texas A&M University, Poultry Science

Publications:

Zhang, C., Y. M. Guo, and W. Li. 2002. Effects of isomalto-oligosaccharide supplementation on the growing performance and gastro-intestinal physiological and biochemical parameters in broiler chicks. *Chinese Journal of Animal Science*, 38 (5): 7-9.

Zhang, C., and Y. M. Guo. 2000. Effects of isomalto-oligosaccharide on the performance and gastro-intestinal physiological and biochemical parameters of broilers. in *Proceedings of the 1st Conference of Chinese Cereals and Oils Association (CCOA). Shijiazhuang, Hebei prov. China. Page* 383-387.

Guo, Y. M., and C. Zhang. 1998. The optimal allowance of iodine for broiler chicks. *China Feed*, 5:35.