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**A natural approach to prevent
reproductive tract problems in
free-range hens**

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Executive Summary

In Australia, free-range and organic production system has expanded due to consumer demand for quality eggs and improved hen welfare. Following the completion of the project CRC 05-13 (Pilot trial – Mortality in free-range flocks) one of the recommendations was to provide advice to producers on how to prevent and combat bacterial infections which cause reproductive tract lesions in free-range laying hens. Apart from improvement of the hygienic conditions and an increase in the level of biosecurity, the use of probiotics was recommended as a natural solution for free-range producers.

Hens held under free-range conditions are exposed to various microbial agents that can influence the types of commensal bacteria present in their intestinal and reproductive systems and causing a wide range of disease problems. One key problem facing the egg industry is that there are virtually no medications for use against infections of the reproductive tract. Australia has adopted a policy of restricting antibiotic use in food-producing animals and has strict registration procedures for veterinary antibiotics. Therefore, the majority of free-range egg producers sooner or later will seriously look at other alternatives to control health and performance of hens. This raises the question of whether these alternatives have a sound effect. In recent years, there has been considerable interest in finding or developing alternatives to antibiotics, and probiotics have often been proposed as a useful alternative.

The objective of this research project was to explore the ability of two selected commercial probiotics for laying hens applied in drinking water for 4 weeks (from 18 to 22 weeks of age) in preventing or reducing the occurrence of reproductive tract pathologies in laying birds, and improving their general health and performance.

The experiments performed in this study demonstrated that treatment with probiotics for 4 weeks (from 18 to 22 weeks of age) in the drinking water significantly reduced the occurrence of reproductive tract pathologies, reduced cumulative mortality and increased performance (egg production, egg weight and BW) of laying hens, during and in the subsequent period (i.e. for a further 20 weeks) post-treatments. Free-range birds treated with probiotics achieved their level of production at peak of lay while maintaining their BW and egg weights at standard ranges. Un-treated birds did not perform at this level. Furthermore, the results of this study provided some initial evidence that the manipulation of bacterial communities by prophylactic and therapeutic administration of effective and competitive beneficial cultures could be a useful approach to control and prevent reproductive tract infections in adult hens. More research and field trials are needed to establish the efficacy of probiotics and confirm subtle changes in the levels of beneficial bacteria in the intestinal and reproductive tracts of hens. Overall, the data from this trial suggested that the probiotics may be able to increase the resistance of laying hens to reproductive infections and improve their liveability. However, further studies are needed to understand how immune response is elicited and how this contributes to the prevention of reproductive pathologies.

This study recommends increased producer awareness should be increased of alternatives to antibiotics such as natural products (e.g. probiotics) that can be used to combat pathogenic bacteria and prevent reproductive tract infections in free-range laying flocks. Moreover, free range producers need to be educated that, to ensure continuous health and egg production, laying flocks should be regularly monitored for causes of decreased egg production and increased mortality. An early detection of reproductive tract lesions will help to employ appropriate strategies to decrease their impact on hen health and egg production. The suggestion to use probiotics before the onset of lay for 4 weeks will help in reducing reproductive tract pathologies and improving general health and welfare of hens operated in free-range systems. Moreover, egg production can be increased resulting in economic benefits to egg producers.

Table of Contents

EXECUTIVE SUMMARY	III
TABLE OF CONTENTS	IV
TABLES	V
FIGURES	VI
INTRODUCTION	1
ALTERNATIVE AND ORGANIC SYSTEMS FOR LAYING HENS IN AUSTRALIA	2
MORTALITY CAUSES IN FREE-RANGE HOUSING SYSTEMS	2
REPRODUCTIVE TRACT AND REPRODUCTIVE TRACT DISORDERS IN LAYING HENS	2
ANTIBIOTIC ALTERNATIVES IN LAYING HEN PRODUCTION	4
<i>Probiotics</i>	4
<i>Prebiotics</i>	5
<i>Plant extracts (or phytogenics)</i>	5
<i>Organic acids</i>	5
<i>Bacteriophages</i>	5
<i>Antimicrobial peptides</i>	6
<i>Cytokines</i>	6
PROBIOTICS: A NATURAL APPROACH TO PREVENT REPRODUCTIVE TRACT INFECTIONS	6
OBJECTIVES	7
METHODOLOGY	8
ANIMALS AND HOUSING	8
PROBIOTICS	8
EXPERIMENTAL DESIGN	9
POST-MORTEM EXAMINATION	9
MICROBIOLOGICAL TESTS	11
PERFORMANCE RECORDS	11
STATISTICS	11
RESULTS	12
BACTERIAL EVALUATION OF THE CLOACA OF HENS	12
EVALUATION OF REPRODUCTIVE TRACT (PATHOLOGICAL AND BACTERIAL FINDINGS)	12
CUMMULATIVE MORTALITY	15
BODY WEIGHT	15
EGG PRODUCTION AND EGG WEIGHTS	16
FEED CONSUMPTION AND FCR	17
DISCUSSION	18
PREVENTION OF REPRODUCTIVE TRACT PROBLEMS AND IMPROVEMENT OF HEN LIVEABILITY	18
IMPROVEMENT OF HEN PERFORMANCE	19
IMPLICATIONS	20
RECOMMENDATIONS	21
ACKNOWLEDGMENTS	21
REFERENCES	22
PLAIN ENGLISH SUMMARY	28

Tables

TABLE 1: AN OVERVIEW OF TREATMENT, SAMPLING, AND MONITORING OF CONTROL AND PROBIOTIC-TREATED BIRDS.	9
TABLE 2: RECOGNITION OF REPRODUCTIVE TRACT PATHOLOGIES IN LAYING HENS	10
TABLE 3: TYPE AND THE FREQUENCY (%) OF BACTERIA ISOLATED FROM CLOACA	13
TABLE 4: PATHOLOGICAL AND BACTERIAL FINDINGS OF OVIDUCTS	14

Figures

FIGURE 1: LAYING HEN REPRODUCTIVE SYSTEM. 3

FIGURE 2: EXAMPLES OF REPRODUCTIVE TRACT PATHOLOGIES IN LAYING HENS 10

FIGURE 3: CUMMULATIVE MORTALITY (%) IN CONTROL AND PROBIOTIC-TREATED HENS FROM START TO PEAK OF LAY 15

FIGURE 4: AVERAGE BW IN CONTROL AND PROBIOTIC-TREATED HENS FROM START TO PEAK OF LAY 16

FIGURE 5: AVERAGE HDP (%) IN CONTROL AND PROBIOTIC-TREATED HENS FROM START TO PEAK OF LAY. 16

FIGURE 6: AVERAGE EGG WEIGHTS IN CONTROL AND PROBIOTIC-TREATED HENS FROM START TO PEAK OF LAY 17

Introduction

At present, the poultry egg and meat industries have gained a lot of ground, being viewed as providers of a healthy alternative to red meat and other protein sources. If this trend is to be maintained, solutions must be found to prevent disease in chickens, which often are weakened by various environmental stressors (Shini et al., 2009). During the last 2 decades, production systems for laying hens have totally changed, and an increasing proportion of eggs are being produced by alternative housing systems. However, there is not enough scientific evidence that alternative laying hen production can be highly successful, in terms of health, welfare and production performance. It should be noted that alternative housing systems require careful planning and management to overcome potential risks to production and the health of hens (Tauson, 2005).

Within the last decade in Australia, free-range and organic farming has expanded due to consumer demands for quality eggs and improved hen welfare. Based on Australian Bureau of Agricultural and Resource Economics (ABARE) and Australian Bureau of Statistics (ABS) figures, Australian chicken egg production in 2005-06 was around 195 million dozen eggs produced from more than 13 million laying hens. Approximately 20% of the eggs were produced in barn and organic and free-range farms. Currently there are 1.69 million free range hens in Australia (11% of total laying hens in Australia) and free range eggs are worth 23% of the value of the Australian egg industry, which is more than \$71 million a year (http://www.poultryhub.org/index.php/Alternative_poultry_production_systems). However, margins are slim due to production costs and mortality, which can be high in free-range conditions. An epidemiological study conducted recently with free-range producers found that the most common causes of death in free-range hens were reproductive tract lesions and cannibalism (Nagle and Shini, 2008). From this study, it was recommended that an improvement of hygienic conditions and biosecurity, combined with the use of antimicrobials and antihelmintics would optimise the liveability and welfare of free-range layers.

Australia has adopted a policy of restricting antibiotic use in food-producing animals and has strict registration procedures for veterinary antibiotics (Unicomb et al., 2006). In 2006, the EU banned the feeding of all antibiotics and related drugs to livestock for growth promotion purposes (LayWel, 2006). Concerns over a possible link between antibiotic-resistant bacterial infections in humans and similar antibiotic-resistant infections in chickens have led the Food and Drug Administration (FDA) to consider a ban of certain antimicrobials used in poultry. Therefore, the majority of poultry producers sooner or later will seriously look at other alternatives to control health and performance of their birds. This raises the question of whether these alternatives have a sound effect.

Many bacteria that are commonly found in the intestines of healthy food producing animals are harmful to humans and may cause food-borne diseases. In free-range operations hens live in an open environment and eggs can become contaminated at different stages from a number of different sources. Hence eggs from free-range systems are typically more contaminated than those from cage systems (De Reu et al., 2008). Until such problems are addressed, outbreaks of food-borne diseases will continue to occur with increased frequency.

In summary, free-range and organic egg producers are very interested in using alternatives to antibiotics to prevent and control bacterial infections in laying hens. However they need reliable evidence about the efficacy and applicability of these alternatives from randomized controlled trials. In recent years, there has been considerable interest in finding or developing alternatives to antibiotics, and probiotics have often been proposed as a useful alternative.

Alternative and organic systems for laying hens in Australia

In Australia there are three main alternative operations for laying hens. These are free range, organic free range and barn egg production systems, supplying consumers with a variety of eggs' standards, tastes and prices. More importantly, consumers' concerns about hen welfare and environmental sustainability are also addressed by these differing production systems.

Free-range housing is widely perceived as the most welfare-friendly system of egg production. The main advantages of the free range system are that hens can nest, dust bathe and perch. Free range production systems provide birds with the ability to range or move around freely in both in-door and out-door spaces, therefore they are exposed to vegetation, and both natural and artificial light.

According to the national standards, only free range hens can be used to produce certified organic eggs and must be grown without the use of artificial colours and synthetic chemicals. Birds are fed certified organic feed grown from soil that is certified as organic and does not contain pesticides or inorganic fertilisers. Under the regulations, no antibiotic medication is to be used for treatment of organic poultry (http://www.poultryhub.org/index.php/Alternative_poultry_production_systems).

Mortality causes in free-range housing systems

The free range and organic egg production systems in which the hens are offered access to outdoor facilities appear to result in a range of diseases several of which are not seen in the cage system. The literature suggests that worldwide there is an increased frequency of disease incidence in non-cage layer operations which results in an increased mortality (Eigaard et al., 2003; Petermann, 2003; Rodenburg et al., 2005; Vits et al., 2005; Shini et al., 2006; Whay et al., 2007; Moesta et al., 2008; Fossum et al., 2009). Mortality in free range flocks can be caused by numerous factors including feather pecking and/or cannibalism, infectious and parasitic diseases and predators.

Data from extensive surveys in Europe and Australia show that reproductive infections such as salpingitis and peritonitis are more common in layers in non-cage/litter-based (Tauson et al., 1999) and free range systems (Nagle and Shini, 2008; Fossum et al., 2009; Neubauer, 2009). These studies have provided a strong link between bacteria and reproductive disorders in free range hens. A recent research project in Australia, funded by the Poultry CRC evaluated causes of mortality in commercial free-range layer flocks and concluded that problems associated with reproductive tract such as egg peritonitis, prolapse/protrusion, cannibalism and vent pecking were seen as important causes of mortality nationwide (Nagle and Shini, 2008). Moreover, the epidemiological survey carried out during this study showed that reproduction tract lesions such as oophoritis, salpingitis, egg peritonitis and salpingoperitonitis were frequently encountered necropsy findings, presumably causing death of laying hens. Microbiological testing demonstrated that, in many cases gram positive and gram negative cocci, and gram negative rods were isolated from ovary and oviduct samples of hens with reproductive tract problems.

However, there are few infection prevention and control tools available to poultry producers to help them to reduce reproductive tract diseases in laying hen and subsequently improve egg production and/or hen liveability.

Reproductive tract and reproductive tract disorders in laying hens

The reproductive tract of the hen differs significantly from that in mammals. Birds have different anatomical features that perform different functions in specialized segments of the tract (Fig. 1) (Johnson, 2000). The main anatomical structures and their functions are ovary, oviduct, vagina and cloaca.

In the chicken only the left ovary is functional and contains immature and mature follicles. The oviduct is also present only on the left side and is defined in special divisions participating in different steps of egg formation. The first part of the oviduct is infundibulum (an expanded upper end)

that catches the released egg yolk. The infundibulum also makes the first of the overlying egg coats, the chalazae. The second division of the oviduct is magnum where different layers of the albumin or egg white are formed. The isthmus is the third part of the oviduct and produces the soft shell membranes (the tough outer membrane located just beneath the egg shell). The last part of oviduct is the uterus (also referred to as the "shell gland") which manufactures the calcareous shell in which the egg is laid. Most of the transit time from ovulation until the egg is laid is spent in the uterus. The vagina is a muscular tube through which the egg is expelled to the cloaca and then outside. The cloaca is the common external opening from which the contents of the urinary tract (urates), the intestinal tract (faeces) and the reproductive tract (eggs) exit the hen.

The reproductive tract of hens starts to produce eggs at 4-5 months of age (i.e. 18 to 20 weeks of age). However, the reproductive system is not functioning completely normally at the onset and hens at this age produce small egg sizes, and high percentages of eggs with twin yolks (<http://ag.ansc.purdue.edu/nielsen/www245/lecnotes/avianrepro.html>). A commercial hen is capable of producing an egg every 25 hours and approximately 300 eggs per year (http://www.poultryhub.org/index.php/Chicken_layer_industry).

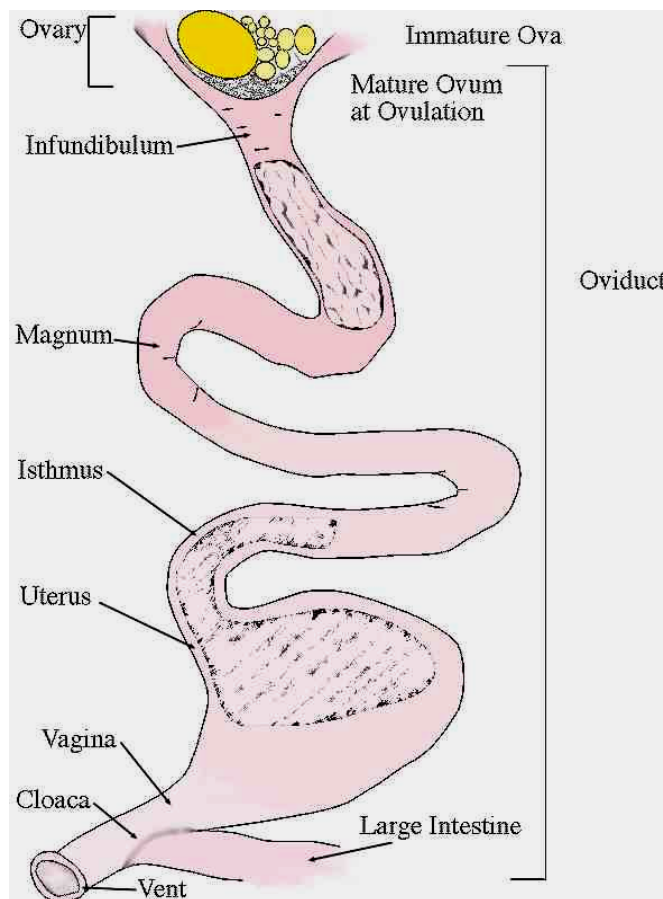


Fig 1. Laying hen reproductive systems.
 © This image is taken from the [Purdue Avian Sciences](http://www.purdue.edu/avian) web page.

As the oviduct is the site for egg formation, defence against pathogenic agents in this organ is essential not only for the health of birds but also for the production of safe eggs. Successful defence relies on local and systemic arms of both innate and acquired immunity. If this defence is suppressed the bacteria will colonise the oviduct and cause inflammation and infection of the tract. It has been shown that a variety of bacteria such as coliforms, *Salmonella* spp. and *Pasteurella* spp. may infect birds of

any age and cause local infections of the ovary and oviduct in adult chickens (Jones and Owen, 1981; Riddell, 1996; Timothy et al., 2008). Most bacteria commonly associated with reproductive tract infections originate from ascending infections from colonized cloacal tissues (faecal contamination) and/or descending infections e.g. systemic infections, transovarian transmission following colonization of the intestinal tract (Snoeyenbos et al., 1969; Shivaprasad et al., 1990). An outbreak of colibacillosis associated with reproductive tract infection, salpingitis and peritonitis was described in a layer breeder flock by Jordan et al. (2005) who suggested the possibility of the infection coming more likely via the airsacs in the case of peritonitis, whereas in salpingitis and salpingoperitonitis the infection was thought to come more likely via the oviduct.

Common reproductive tract problems encountered in egg laying birds are: egg binding, dystocia, prolapsed oviduct, egg yolk peritonitis, chronic egg laying, oviduct impaction, oophoritis, salpingitis, metritis, ectopic eggs, cystic hyperplasia of the oviduct, neoplasia, and cloacal pathologies (Romagnano, 1996). These reproductive disorders result from a complex combination of bacterial, hormonal, physiologic, and behavioural actions reacting to photoperiods, food availability and availability of nest sites. To date, the pathogenesis of reproductive tract infection in hens has not received the full attention it merits in relation to its importance in reducing egg production and transmitting bacterial infections within the poultry population and from poultry to man. A proper identification and subsequent prevention or treatment of these disorders needs immediate attention, especially in free-range and organic farms, mainly due to economic and ethical issues associated with egg production in these systems. A promising, and at present a very common strategy, is the use of probiotics, which have been shown to reduce enteric diseases, improve the immunity and enhance the performance of broiler chicks and laying hens (Patterson and Burkholder, 2003).

Antibiotic alternatives in laying hen production

In recent years there has been considerable interest in finding or developing alternatives to antibiotics that are used for disease prevention and growth promotion. Many types have been examined: bacterial cultures, oligosaccharides and yeast, ethereal oils, taste and aromatic compounds, plant extracts, yucca products, clay minerals, organic acids/salts, and fermented mash. In general, all these products have produced variable results in pig and poultry production, best results being obtained with the probiotics, organic acids and fermented mash (<http://www.thepigsite.com/articles/3/feed-and-nutrition/291/>).

Probiotics

Probiotics are defined as “live microbial feed supplements which beneficially affect the host animal by improving intestinal microbial balance” (Fuller, 1989). It has been shown that probiotics can change the bacterial community structure in the avian gastro-intestinal tract (GIT) (Netherwood et al., 1999). Many different strains and mixtures of bacteria have been used in laying hens in attempts to prevent pathogens from colonizing the gut, improve egg laying and feed conversion and increase immune responses and resistance to diseases.

The use of lactobacilli (non-spore-forming gram-positive bacteria) as probiotics for laying hens has been suggested as an option to reduce *Salmonella* Enteritidis infection (Garriga et al. 1998; Gusils et al. 1999). The data have demonstrated that *Lactobacillus* isolates from laying hens inhibit *Salmonella* (e.g. *S. Enteritidis*) growth *in vivo* most probably through competition for attachment to the gastrointestinal epithelial cells (Jin et al., 1996) and production of lactic acid (Van Coillie et al., 2007). Moreover, the presence of lactobacilli in the vagina and cloaca of laying hens has been seen an important step in maintaining the microbial ecosystem that prevents the growth and invasion of pathogens, such as *Salmonella* (Miyamoto et al. 1998, Van Coillie et al., 2007). Chang and Chen (2000) found that *Lactobacillus* had a marked inhibitory effect on *Campylobacter*. In general, single products have not been very effective. Feeding poultry with mixture of multiple strains of *Lactobacillus* spp. and other bacteria (e.g. *Enterococcus faecium*, *Bifidobacterium bifidum*, *Bacillus subtilis*) and/or (*Candida* spp) and prebiotics (carbohydrates) has been shown to be highly effective (Nisbet et al., 1993 a, b; 1996; Simon et al., 2001; Patterson and Burkholder, 2003; Nava et al., 2005; O’Bryan et al., 2008).

Commercially produced probiotic products are usually species-specific, with products intended for use in chickens comprised of bacterial species that have been isolated from the GIT of chickens. Past research has shown that administering probiotics can provide the same protection as a naturally developed commensal GIT microflora (Nurmi and Rantala, 1973; Pascual et al., 1999; Kubena et al., 2001; LaRagione et al., 2001).

Prebiotics

Prebiotics are “non-digestible feed supplements which beneficially affect the host by selectively stimulating the growth and/or activity of one or limited number of bacteria in the colon (Gibson and Roberfroid, 1995) and thus improving the host health. Fructooligosaccharides (FOS), inulin, mannose-oligosaccharides (MOS) and arabinogalactans are considered as the standard prebiotics used for improved gut function. They favour the growth of normal bacterial flora and inhibit the growth of pathogenic organisms. They are not digested in the human or animal small intestine but are selectively fermented in the colon by bifidobacteria to short chain fatty acids and lactic acid, resulting in a decreased pH in the intestine, an environment that is unfavourable to pathogenic bacteria such as *Escherichia coli* and *Clostridium perfringens* (Patterson and Burkholder, 2003). MOS in fact are thought to act by binding and removing pathogens from the intestinal tract and stimulating the immune system (Spring et al., 2000).

FOS and MOS are two of the most studied prebiotics in poultry. FOS can be found naturally in some cereal crops and onions (Bailey et al., 1991). MOS is obtained from the cell wall of the yeast *Saccharomyces cerevisiae*. Laying hens showed improvements in egg production, feed consumption and feed conversion rate, when 2000 mg/kg FOS was added to the diets (Li et al., 2007). Sims et al. (2004) showed that MOS supplementation might be beneficial for turkey producers. At 6 wk of age, but not 18 wk of age, the turkeys in the MOS treatment group had significantly less *Clostridium perfringens* in their large intestines than the controls.

Plant extracts (or phytochemicals)

Various plant extracts have been studied for their antimicrobial abilities (Kamel, 2000; Burt and Reinders, 2003). Examples include oregano, thyme, rosemary, cinnamon, clove and anise oils (Lambert et al, 2001; Friedman et al., 2002).

Organic acids

The use of organic acids in poultry is aimed at replacing antibiotic growth promoters but it can also be targeted at more specific uses, like the prevention of necrotic enteritis, and the reduction of *Salmonella* and *Campylobacter* shedding (Thompson and Hinton, 1997; Van Immerseel et al., 2004). The key basic principle on the mode of action of organic acids on bacteria is that non-dissociated (non-ionized, more lipophilic) organic acids can penetrate the bacteria cell wall and disrupt the normal physiology of certain types of bacteria and kill them (Lambert and Stratford, 1999).

Bacteriophages

Bacteriophages (often called phages) are viruses that infect and replicate in bacteria, leading to the destruction of the host and release of great quantities of virus that will re-infect other bacteria (Carlton, 1999). Bacteriophages are safe having no activity against animal or plant cells, and appear to have evolved with bacteria as they are ubiquitous in nature. There is significant research on the use of bacteriophages to control foodborne pathogens, such as *Salmonella*, *Listeria monocytogenes*, *E. coli* O157:H7 and *Campylobacter* in agricultural products (Huff et al., 2005). Huff et al. (2002) demonstrated that when a bacteriophage was mixed with *E. coli* prior to challenging the birds, a total protection from colibacillosis could be achieved. When the bacteriophage was administered as an aerosol spray prior to challenging the bird with *E. coli*, colibacillosis could be prevented for up to 3 days (Huff et al., 2003a, b). In addition, severe colibacillosis was treated by the administration of

bacteriophages as an intramuscular injection (Huff et al., 2003b). Multiple injections of bacteriophage provided greater therapeutic value than a single injection (Huff et al., 2003a).

Antimicrobial peptides

Antimicrobial peptides (AMPs) are small effector molecules of the innate immune system that are not confined to bacteria, but appear to occur in all living species. Their structure usually contains elements that facilitate the interaction with negatively charged membranes, and their mode of action involves the cell membranes of target organisms (Hancock and Rozek, 2002). There is evidence for the ability of chickens to produce such antimicrobial peptides (Joerger, 2003). These peptides play important biological roles in the defence against various pathogens, such as adjustment of host inflammatory response and chemotactic function to recruit other leukocytes (Sugiarto and Yu, 2004).

Cytokines

Cytokines are proteins that control immune responses following infection or vaccination and represent excellent, naturally-occurring therapeutics and vaccine adjuvants (Lowenthal et al., 1999). The use of cytokines in poultry has become more feasible with the discovery of a number of avian cytokine genes. One of the most characterised chicken cytokines is interferon gamma (ChIFN- γ), which has been used as a vaccine adjuvant and a growth promoter (Lowenthal et al., 1997; Hilton et al., 2002).

Probiotics: a natural approach to prevent reproductive tract infections

Studies in humans have shown that the use of probiotics containing *Lactobacillus* spp. restores commensal vaginal flora and are recommended to treat or prevent bacterial urogenital infections (Barrons and Tassone, 2008). However, in order for oral probiotic supplementation to benefit the reproduction tract, the bacteria must be able to colonize the intestinal and reproductive tracts. The rationale for the use of probiotics in genitourinary tract infections is based on the gastrointestinal and genitourinary regulatory role played by the commensal microflora and the need for restoration of this microbial ecosystem after disturbances. Oral formulations of lactobacilli for genitourinary infections have been demonstrated to be capable of maintaining their structural integrity during passage through the gut and delivery to the rectal area for colonization of the vaginal tract. The normalisation of the tract was observed after 14 days of oral administration with probiotic (Borchert et al., 2008)

In the natural environment, the intestinal tract of the chicken is colonised by a broad spectrum of microorganisms from an early age. These resident microbes have a profound effect on some of the physiological processes in the gut and other body systems such as the respiratory and reproductive systems. Under normal circumstances there is some balance between beneficial and pathogenic bacteria in the gastrointestinal tract. This is influenced by symbiotic and competitive interactions and relationships. However, there are a number of conditions during rearing and laying periods that decrease the resistance of birds to diseases making them more susceptible to various pathogens especially enteropathogenic microbes such as *E. coli*, *Salmonella* spp., *Clostridium perfringens* and *Campylobacter* spp. Other predisposing factors such as mucosal damage (of gut or reproductive tract) and immunosuppression caused by stress can contribute to reproductive system diseases in laying chickens.

There appear to have been no previous attempts of using probiotics to aid in controlling reproductive tract infections in laying hens. Egg producers who are interested in the use of probiotics to combat infections of reproductive tract without antibiotics require evidence of efficacy and applicability of appropriate commercial products. The key question is whether using the probiotics before or at onset of lay supports the colonisation of reproductive tract with beneficial bacteria, prevents reproductive tract problems and improves egg production during the laying period.

Objectives

The objective of this study was to investigate the effects of two commercially available probiotics on the prevention of reproductive tract problems in free range laying hens. Additionally, the effect of probiotics on general hen health and performance of hens was evaluated. It was hypothesized that each of the probiotic treatments would result in improved hen health and egg production compared with hens not exposed to probiotics.

To achieve these objectives different approaches have been followed:

- Both microbiological testing and gross examination of hens were used to identify reproductive tract problems, and relate any changes to the treatments;
- Bacteriological examination of the cloaca was carried out to monitor broad treatment-associated changes of the microbial population of the intestinal tract (i.e. stimulation of intestinal bacterial colonisation with beneficial bacteria and/or the exclusion/suppression of pathogenic bacteria from the GIT) following probiotic treatments;
- Bacteriological examination of the oviduct was carried out to observe broad treatment-associated changes of the microbial microflora of the reproductive tract, and/or to any associate normal or non-normal (i.e. clinically manifested reproductive tract infections) condition with the colonisation of the oviduct with specific bacteria;
- The evaluation of health and performance parameters was used to assess the potential role of probiotics in maintaining general health and improving metabolism and egg production of hens.

Methodology

Animals and housing

Six hundred thirty, 17-wk old HY-SEX Brown layers were sourced from a free range farm that had previously shown problems with reproductive tract pathologies and decreased egg production. During rearing birds were vaccinated for infectious bronchitis (IB), Marek's disease, Newcastle disease (ND), fowl pox, *Mycoplasma gallisepticum* (MG), Egg Drop Syndrome (EDS), fowl cholera, infectious coryza, *Mycoplasma synoviae* and avian encephalomyelitis and had regular worming. Birds were transferred to the UQ Gatton free-range facility and randomly divided into 3 groups. Each group of 210 chickens contained 3 replicates with 70 birds each. Birds were housed in freshly cleaned free-range sheds. The bird management (feed and feeding regime, lighting, and indoor and outdoor conditions) were similar to those used on the source farm and in accordance with the "Australian Model Codes of Practice for the Welfare of Animals: Domestic Poultry, 4th ed. (2002).

Probiotics

Two commercial probiotic products available for use in poultry in Australia were employed in this study.

Probiotic 1. Biomin® Poultry5Star (Biomin, GmbH, Austria), is a multi-strain probiotic product and contains a source of live viable naturally occurring microorganisms isolated from the crop (*Lactobacillus reuteri*), jejunum (*Enterococcus faecium*), ileum (*Bifidobacterium animalis*), and caecum (*Pediococcus acidilactici* and *Lactobacillus salivarius*) of healthy adult chickens. The product has a total bacterial count, expressed as colony-forming units (CFU), of 2×10^{12} CFU/kg of product. The fructooligosaccharides used in Biomin® Poultry5Star are derived from a natural plant source and are selected for their ability to stimulate the growth of beneficial bacteria such as *Bifidobacteria* and *Lactobacilli* in the intestine. Following recommendations of the manufacturers, the probiotic product was administered in the drinking water at a level to supply 10^8 bacteria/hen/day or 20 g/1000 hens/day for 4 weeks (from 18 to 22 weeks of age).

Probiotic 2. Protexin® (International Animal Health Products P/L, Australia) is a highly concentrated pre-mix containing seven strains of bacteria and two yeasts (*Lactobacillus plantarum* 1.89×10^{10} cfu/kg, *Lactobacillus delbrueckii* subsp. *bulgaricus* 3.09×10^{10} cfu/kg, *Lactobacillus acidophilus* 3.09×10^{10} cfu/kg, *Lactobacillus rhamnosus* 3.09×10^{10} cfu/kg, *Bifidobacterium bifidum* 3.00×10^{10} cfu/kg, *Streptococcus salivarius* subsp. *thermophilus* 6.15×10^{10} cfu/kg, *Enterococcus faecium* 8.85×10^{10} cfu/kg, *Aspergillus oryza* 7.98×10^9 cfu/kg, *Candida pintolopesii* 7.98×10^9 cfu/kg). All the microorganisms in Protexin are naturally occurring and have been isolated from a wide range of feed, plant, animal, bird and human sources. Protexin is reported to be safe, non-toxic and residual free. The product was also administered for 4 weeks at a dose recommended by manufacturer (1g/L in the drinking water).

Experimental design

All groups (2 treatments and 1 control) received the same diet, a corn-based organic diet (containing an average of 11.6 MJ/kg ME, 19 % crude protein, 4.3 % fiber, 3.82 % Ca, and 0.83 P). The probiotics were in a powder form and were added in the drinking water on a daily basis. Probiotic 1, Biomin® Poultry5Star, was administered in the drinking water at a level to supply 10^8 bacteria/hen/day or 20 g/1000 hens/day (following recommendations of the manufacturer) for 4 weeks (from 18 to 22 weeks of age). Probiotic 2, Protexin® was also administered for 4 weeks at a dose recommended by manufacturer (1g/L in the drinking water). The bacterial status of the intestinal and reproductive tract in hens was screened by using sterile swabs. For treatment and sampling details see Table 1. All procedures conducted in this study were approved by the Animal Ethics Committee of the University of Queensland, under Ethics Approval Number: SVS/248/09/POULTRY CRC.

Table 1. An overview of treatment, sampling, and monitoring of control and probiotic-treated birds

Age (wks)	Treatment	Samples collected	Other parameters	N of samples	Comments
17	630 laying chickens were allocated to 3 groups with 3 replicates per each group/treatment (control, probiotic 1, probiotic 2).		BW ¹ , health status	20% of hens were weighed (or 14 hens/replicate)	Hens were given 1 week to adapt to the housing conditions
18	Treatment with probiotic started	Cloacal swabs were collected before treatment	BW, health status, egg production, egg weight, FC ²	6 cloacal swabs/replicate or 18 for each treatment	Dead chicken were necropsied during the whole experimental period
22	Treatment with probiotics completed	Cloacal & oviduct swabs	BW, health status, egg production, egg weight, FC	6 cloacal swabs/replicate; 3 oviduct swabs/replicate	Chicken were euthanized & oviduct swabs were taken (3/replicate)
26	None	Cloacal & oviduct swabs	BW, health status, egg production, egg weight, FC	6 cloacal swabs/replicate; 3 oviduct swabs/replicate	
30	None	Cloacal & oviduct swabs	BW, health status, egg production, egg weight, FC		
34	None	Cloacal & oviduct swabs	BW, health status, egg production, egg weight, FC	6 cloacal swabs/replicate; 3 oviduct swabs/replicate	
36	None	None	BW, health status, egg production, egg weight, FC		

¹Body weight = BW

²FC = feed consumption

Post-mortem examination

All birds that died during the whole experimental period (from 18 to 38 wks of age) and birds that were euthanized at each sampling point (at 22, 26, 30 and 34 wks of age) were subjected to a post-mortem examination to identify the cause of death or evaluate the reproduction tract, respectively. The necropsy included the body weight of the bird and an examination of the overall condition, as well as external and internal observations. The tentative diagnosis was based on the presence of crucial clinical macroscopic lesions in organs. Microbiological samples from oviduct were collected only from freshly euthanized and necropsied birds. The aim was to find a relationship between pathology findings and possible bacterial agents. For the most effective recognition of macroscopic changes occurring during reproductive tract problems in laying hens we used a reference table showing key features present/absent in specific pathology (Table 2). Figure 2 (A and B) represent some of the pathological reproductive lesions that were found during post-mortem of clinically normal birds euthanized during sampling.

Table 2. Recognition of reproductive tract pathologies in laying hens

Reproductive tract pathology	Gross examination			
	Abdominal cavity	Ovary	Oviduct	Cloaca & vent
Acute peritonitis	Inflamed, amorphous white exudates and hyperaemia of the mesenterial membrane	Hyperaemic	Normal	Normal
Acute oophoritis	Haemorrhagic and/or amorphous exudates attached to ovary	Hyperaemic or haemorrhagic, surrounded by exudates	Hyperaemic, sometimes with filled with mucid exudates	Normal
Acute salpingitis	Hyperaemic mesenterial membrane, exudates surrounding oviduct	Normal or hyperaemic	Inflamed, hyperaemic, containing white exudates	Sometimes, prolapsed with exudates
Salpingo-peritonitis	Haemorrhagic mesenterial membrane, fibrinous/caseous exudates surrounding oviduct	Normal or hyperaemic, sometimes deformed and shrunken	Inflamed, haemorrhagic, dilated with exudates	Prolapsed with exudates
Saplingo-oophoritis	Haemorrhagic or fibrinous mesenterial membrane	Haemorrhagic or broken follicles	Fibrin or yolk material	Prolapsed with white or yellow exudates
Chronic peritonitis	Caseous exudates attached to ovary and oviduct, abdominal swelling	Deformed or atrophied follicles	Blocked or atrophied	Sometimes, prolapsed with exudates
Chronic oophoritis	Fibrinous and/or caseous deformed & old yolks	Atrophied ovary/follicles	Blocked or atrophied	Prolapsed with pale cream-coloured exudates
Chronic salpingitis	Thickened, fibrinous and/or caseous mesenterial membrane	Atrophied ovary/follicles	Dilated with thick cream-coloured exudates	Prolapsed with pale cream-coloured exudates

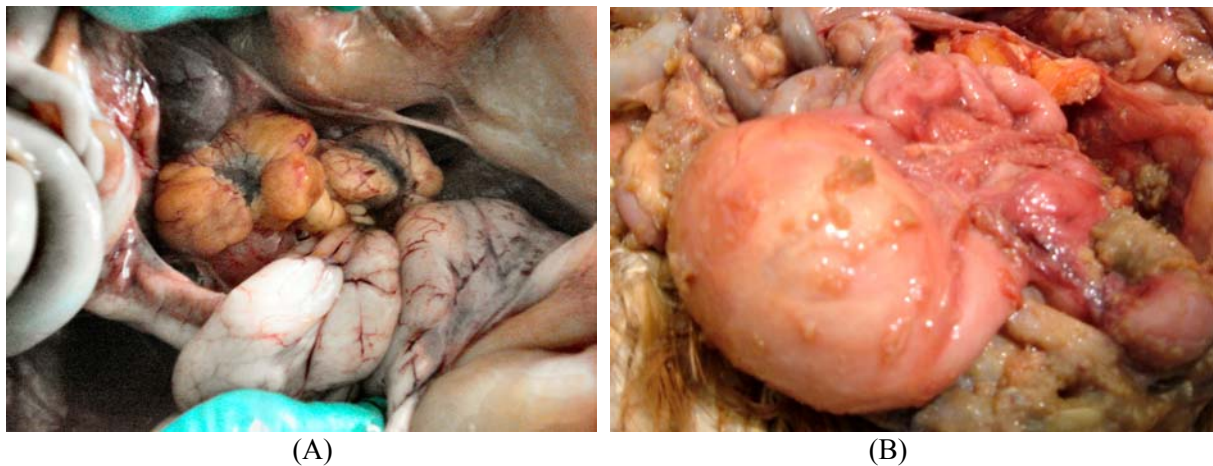


Fig. 2. Examples of reproductive tract pathologies in laying hens
 (A) Chronic oophoritis; (B) Chronic salpingo-peritonitis
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Microbiological tests

Microbiological samples were taken from birds euthanized at each sampling point (see Table 1). Samples from cloaca and oviduct (magnum) were collected aseptically and streaked onto sheep blood agar, MacConkey agar and Xylose-Lysine Decarboxylase (XLD) agar. The plates were incubated aerobically at 37°C overnight. The following day, bacteria considered significant were single colony picked onto a fresh sheep blood agar plate. All primary sheep blood plates were re-incubated for a further 24 hours and re-examined. Normal flora bacteria (coliforms, *Pseudomonas* spp., *Bacillus* spp.) were identified purely on colony morphology. Conventional phenotypic tests were used to identify *Escherichia coli* (only if present as pure culture with no other coliforms evident on the MacConkey agar plate) and *Gallibacterium anatis* biovar *haemolytica*.

Performance records

Body weight (BW) of hens was recorded on a monthly basis (before treatments started and after treatments until peak of lay, i.e. 18, 22, 26, 30, 34, 38 weeks of age). Fourteen hens per replicate were weighed individually at each time and the average was calculated.

Feed intake was recorded daily per each replicate (i.e. 70 birds), and the average was calculated per bird. The feed conversion ratio (FCR) was also calculated weekly for the whole duration of the experiment.

Egg production was calculated as hen day production (HDP %). Egg production was recorded daily for each replicate and HDP is expressed on a weekly basis from 18 to 38 weeks of age.

Egg weight was recorded on a monthly basis at 18, 22, 26, 30, 34, 38 weeks of age. Fifty percent of eggs (or 30-35 eggs) per replicate were individually weighed at each time and the average was calculated.

Statistics

All analyses were performed using the GLM procedure of SAS (SAS Institute, 1996). To test for treatment effect at each sampling point, recorded values were subjected to one-way ANOVA. Data on performance parameters (BW, HDP and egg weight) were based on a replicate basis. The group size used (number of birds per replicate) was sufficient to obtain reliable results. With 70 birds per replicate, an effect of the probiotic that may have occurred at a rate of 5% could have been detected around 97% of the time. If the effect would have been beyond 10%, a 100% detection was guaranteed to an accuracy of $\pm 1\%$. Statistical significant effects were further analysed, and means were compared using Duncan's multiple range test. Statistical significance was determined at $P \leq 0.05$.

To evaluate whether significant differences existed for pathological findings, an unpaired t-test was used comparing two means (control and probiotic 1 or 2 treated) and determine the p-value. A 99% confidence interval for the true difference between the means was set, and in this case the values were considered significant at $P < 0.01$.

Results

Bacterial evaluation of the cloaca

Table 3 presents data on the type of bacteria and the frequency (%) isolated from cloacal swabs before and after treatments with probiotics. There were no major changes in the colonisation of the intestinal (i.e. cloacal) microflora between probiotic-treated and control hens before the treatment started and at each sampling point (after 4 weeks of treatment with probiotics, and after 4, 8 and 16 weeks post-treatment with probiotics). There was a change in the type and frequency of the bacterial colonisation of cloaca in all groups (2 treatments and control) during all sampling period, presumably related to age of hens. Coliforms were present in all samples analysed, while other bacteria were present or absent (in all three treatment groups) as the trial progressed. An interesting observation was that at 22 and 26 wks of age all treatment groups demonstrated a marked presence in the cloaca of coryneforms (with a frequency from 56 to 100% of birds). At 34 wks of age the presence of coryneforms decreased again with a frequency similar to 18 wks of age. Coryneforms are a group of Gram positive rod-shaped bacteria (a common genus being *Corynebacterium*) that are often isolated from litter and poultry droppings (Chinivasagam *et al.* 2009). All isolates of *Gallibacterium anatis* were the haemolytic form of this species.

Evaluation of reproductive tract (pathological and bacterial findings)

A total of 98 birds (out of approximately 630 birds) were euthanized and the reproductive tract was examined (data presented in Table 4). At 22 wks of age, or 4 weeks post-treatment with probiotics, treated hens showed a significantly lower occurrence of the reproduction tract pathologies when compared to control hens (22% vs. 44% of birds necropsied). At 38 wks of age the incidence of reproductive tract pathologies in control hens persisted (33%), while probiotic-treated hens were normal except for one bird (11%). Moreover, at 38 weeks of age most of the hens necropsied showed infestation with parasites (*A. galli* and/or cestodes), but surprisingly with a lower frequency in probiotic-treated hens (control vs. Probiotic 1 and 2, 66% vs. 33%).

In general there was a low contamination of the oviduct with aerobic bacteria (Table 4), and a correlation between clinical symptoms of reproductive pathologies and specific bacteria could not be established.

The necropsy examination of chickens that died during the experimental period (34 in total) showed that 18 birds died in the control group. Specific details were that two were cannibalised, seven died from acute/chronic reproductive pathologies, and four had a combination of pericarditis, perihepatitis and airsacculitis (poliserositis). In five birds the cause of death could not be confirmed. In the probiotic 1-treated group only seven died during the experimental period (one cannibalised, two from acute/chronic reproductive pathologies, while in four of birds the cause of death was not established). In the probiotic 2-treated chickens nine birds died in total (four exhibiting signs of acute/chronic reproductive pathologies, two from predators, one cannibalised, and two due to high burdens with intestinal parasites and potential associated complications).

Table 3 Type and the frequency¹ (%) of bacteria isolated from cloaca

Treatments	Before treatments started ³ (at 18 wks of age)	4 wks after treatments started (at 22 wks of age)	4 wks post-treatments (at 26 wks of age)	16 wks post-treatments (at 38 wks of age)
Control²				
Coliforms	100	100	100	100
Coryneforms	33.3	55.5	77.7	33.3
<i>Gallibacterium anatis</i>	55.5	66.6	77.7	100
α Streptococci	44.4	55.5	44.4	55.5
<i>Proteus</i> spp.	11.1	11.1	11.1	-
Micrococci	22.2	11.1	-	55.5
<i>Bacillus</i> spp.	11.1	22.2	-	22.2
β Streptococci	11.1	-	-	22.2
Probiotic 1²				
Coliforms	100	100	100	100
Coryneforms	-	100	77.7	22.2
<i>Gallibacterium anatis</i>	44.4	66.6	88.8	66.6
α Streptococci	44.4	33.3	55.5	66.6
<i>Bacillus</i> spp.	-	-	44.4	11.1
<i>Proteus</i> spp.	11.1	-	22.2	11.1
Probiotic 2²				
Coliforms	100	100	100	100
Coryneforms	22.2	77.7	88.8	55.5
<i>Gallibacterium anatis</i>	-	100	88.8	88.8
<i>Proteus</i> spp.	11.1	-	-	44.4
α Streptococci	77.7	44.4	22.2	55.5
Micrococci	11.1	-	-	22.2
<i>Staphylococcus</i> spp.	11.1	-	-	-
<i>Bacillus</i> spp.	22.2	-	-	-
<i>Pseudomonas</i> spp.	-	-	11.1	-

¹Percentage of samples containing different types of bacteria

²Number of samples per treatment = 9

³Probiotic treatments were carried out for 4 weeks (from 18 to 22 weeks of age)

Table 4. Pathological and bacterial findings of oviducts

Treatments	Bird	4 wks after treatments started (at 22 wks of age)	4 wks post-treatments (at 26 wks of age)	16 wks post-treatments (at 38 wks of age)
Control				
	1	Normal ¹	Acute salpingo-oophoritis	Normal, parasites (<i>A. galli</i>)
	2	Salpingo-oophoritis	Caseous peritonitis	Normal
	3	Normal	Normal	Normal; parasites (<i>A. galli</i>)
	4	Fibrinous yolk peritonitis	Normal	Normal, parasites (<i>A. galli</i> and tapeworms/cestodes)
	5	Salpingitis and prolapse	Salpingo-oophoritis	Chronic caseous salpingo-oophoritis
	6	Normal	Normal	Normal
	7	Normal	Normal	Fibrinous peritonitis & perihepatitis; parasites (<i>A. galli</i>)
	8	Ovary immature, growing follicles	Atrophy ovary-oviduct	Oophoritis, parasites (<i>A. galli</i> /cestodes); Micrococci ²
	9	Normal	Oophoritis	Normal, parasite (<i>A. galli</i> & cestodes); Micrococci ²
Probiotic 1				
	1	Salpingo-oophoritis/prolapsed; α Streptococci ²	Normal, <i>G. anatis</i> ²	Normal, parasites (<i>A. galli</i>)
	2	Ovary immature, growing follicles	Normal	Normal
	3	Normal	Normal	Normal
	4	Normal	Normal	Salpingitis; α Streptococci ²
	5	Normal	Normal	Normal, parasites (<i>A. galli</i>)
	6	Normal	Normal	Normal
	7	Normal	Normal	Normal
	8	Normal	Normal	Normal, Micrococci ²
	9	Normal	Normal, Micrococci ²	Normal, parasites (<i>A. galli</i> & cestodes)
Probiotic 2				
	1	Normal	Normal, Micrococci ²	Normal
	2	Normal	Normal	Normal; parasites (<i>A. galli</i>)
	3	Normal	Normal	Normal; parasites (<i>A. galli</i>)
	4	Normal	Normal	Normal
	5	Normal	Normal	Chronic salpingitis
	6	Normal	Normal	Normal
	7	Acute salpingitis & oophoritis, <i>G. anatis</i> ²	Atrophy of ovary-oviduct	Normal, Coryneform ²
	8	Blocked eggs, <i>Bacillus</i> spp. & Micrococci ²	Normal	Normal
	9	Normal	Normal	Normal, parasites (<i>A. galli</i>)

¹Normal = reproduction tract appears normal; ²Bacteria isolated from oviduct swabs

Cumulative mortality

The percentage of cumulative mortality in control and probiotic-treated hens in the period from 18 to 38 weeks of age is presented in Fig. 3. Mortality in commercial layers is typically in the range of 0.5 to 1.0% per month, but may exceed this level for various reasons in individual flocks. Starting at the age 26 wks cumulative mortality in control hens was significantly higher ($P<0.01$) than in both probiotic-treated groups. It should be noted that the mortality rates seen in all treatment groups were within the limits recommended by breeder. The lowest mortality rate was found in probiotic 2 treated-hens, while both probiotic-treated groups had a lower ($P<0.01$) incidence of reproductive tract lesions compared to controls.

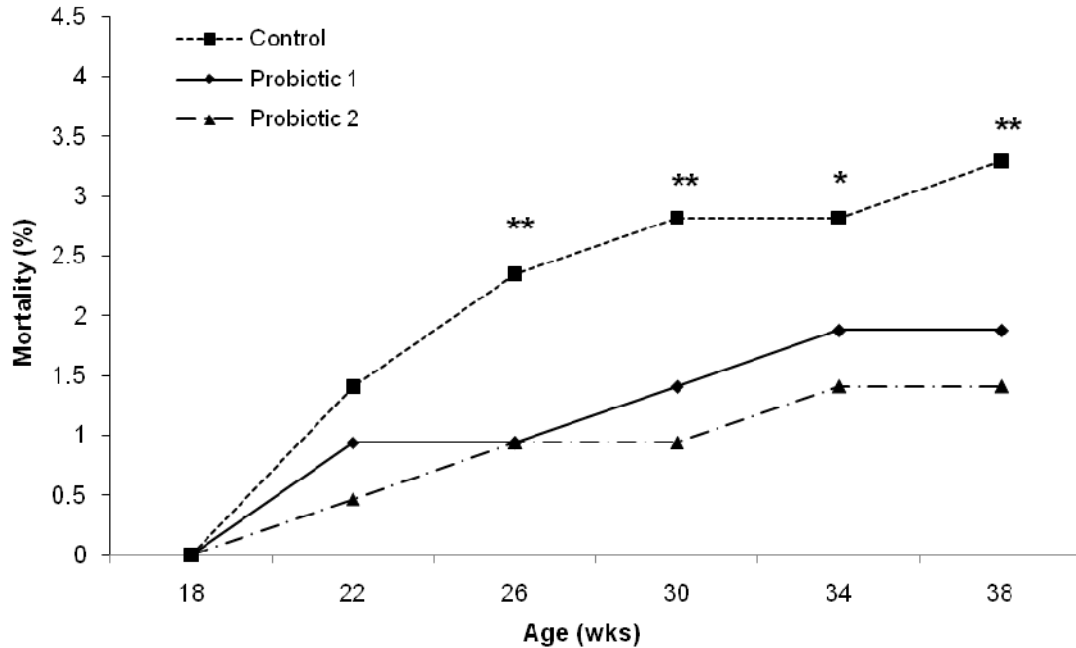


Fig. 3. Cumulative mortality (%) in control and probiotic-treated hens from start to peak of lay
* = $P<0.05$
** = $P<0.01$

Body weight

Hen weight increased as the flock aged (Fig. 4). At 18 weeks of age there were no significant differences in body weight (BW) between all experimental groups. At all other points of measurement probiotic-treated hens were heavier ($P<0.01$) than non-treated (control) hens. Furthermore, at 30, 34, and 38 weeks of age the BW of the probiotic 2-treated hens was significantly higher ($P<0.05$) than that of probiotic-1 treated hens.

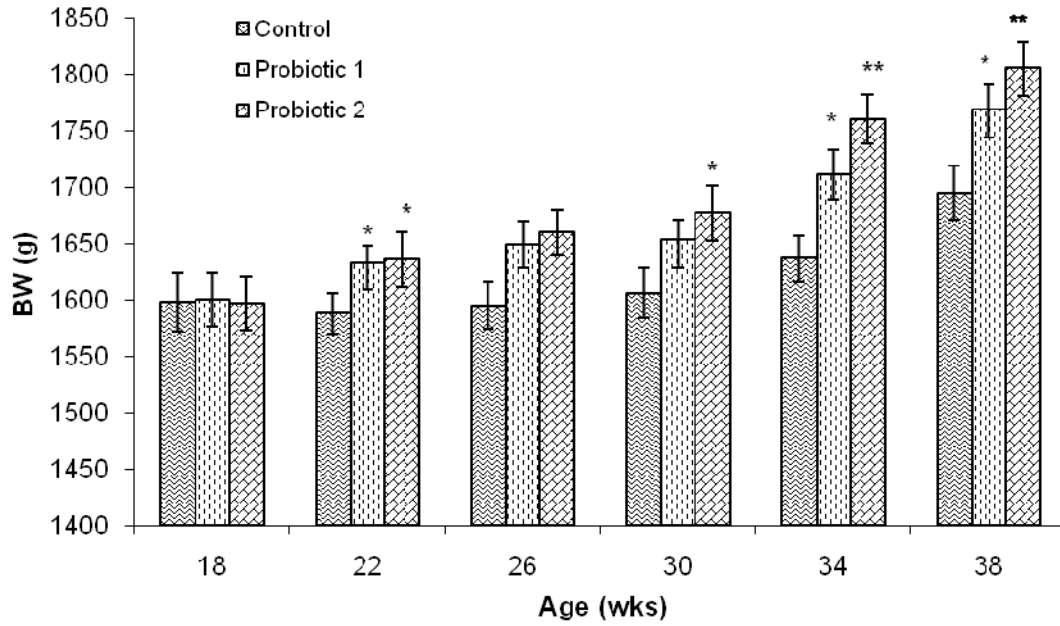


Fig. 4. Average BW in control and probiotic-treated hens from start to peak of laying
 * = P<0.05
 ** = P<0.01

Egg production and egg weights

From 18 to 23 weeks of age the percentage hen day production (HDP) increased at a similar rate in all experimental groups. There were two significant (P<0.01) drops in HDP (starting at week 23-24 and 34-35), which appear to correlate with increased mortality and occurrence of pathological changes in the reproductive tract. Overall birds in the probiotic-treated groups performed better than control group.

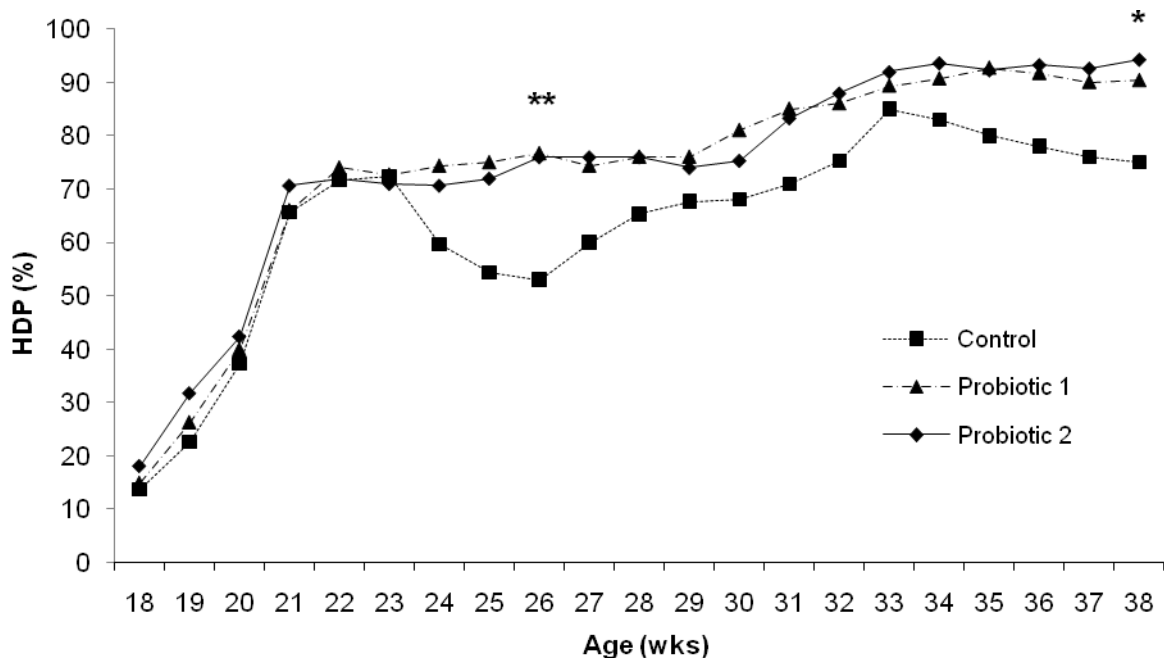


Fig. 5. Average HDP (%) in control and probiotic-treated hens from start to peak of lay
 * = P<0.05
 ** = P<0.01

Average egg weights from onset until peak of lay (i.e. from 18 to 38 wks of age) are presented in Fig 5. The egg weights increased as the flock aged. However, at 34, and 38 wks of age the average egg weights of both probiotic treated groups were significantly higher ($P<0.05$) than that of control birds. As well, the group treated with probiotic 2 had a significantly higher egg weight at 26 weeks of age ($P<0.5$).

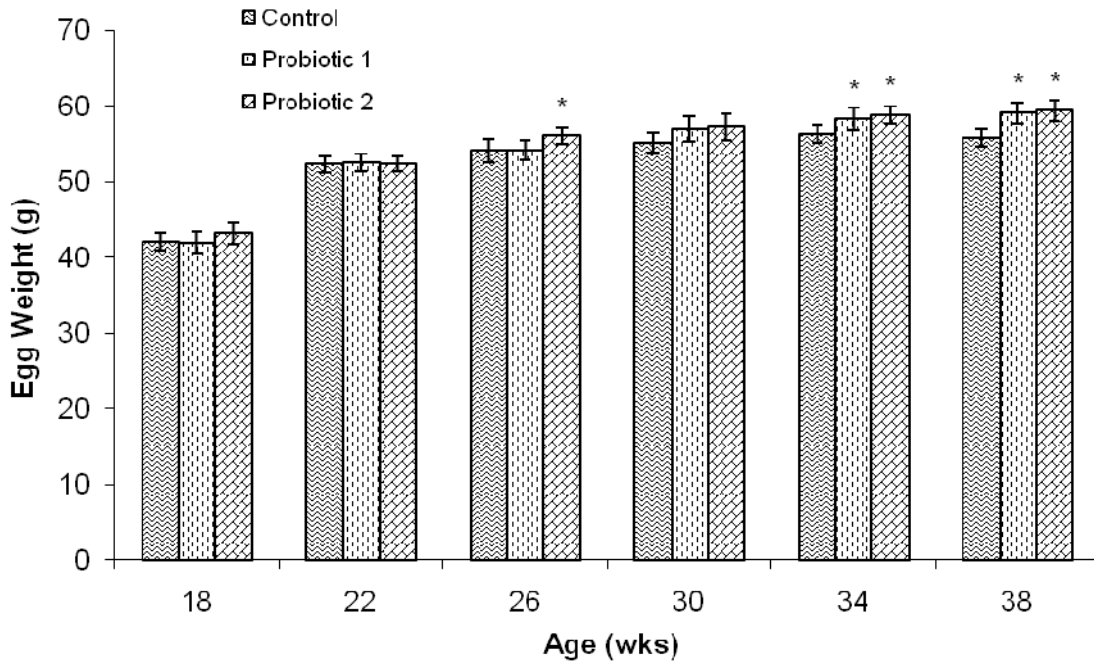


Fig. 6. Average egg weights in control and probiotic-treated hens from start to peak of laying
 * = $P<0.05$
 ** = $P<0.01$

Feed consumption and FCR

There were no significant differences ($P>0.05$) between control and probiotic 1 and 2-treatment replicates for FC and FCR.

Discussion

The objective of this project was to explore the effects of two selected commercial probiotics administered in the drinking water for 4 weeks (from 18 to 22 weeks of age) to prevent or reduce the occurrence of reproductive tract pathologies in laying birds and improve their general health and performance. The experiments performed in this study demonstrated that treatment with probiotics significantly improved the reproductive tract health, reduced mortality and increased performance (egg production, egg weight and BW) of laying hens, during the treatment period and in the subsequent period (i.e. for a further 20 weeks post-treatments). Bacterial evaluation of the intestinal and reproductive tract did not demonstrate particular changes of the microbial populations. The microbiological analysis was limited in nature. It is possible that a more extensive examination, including the use of molecular profiling methods may have detected changes. Nevertheless, an enhancement of the resistance of laying birds to reproductive tract diseases together with an improvement of overall performance was demonstrated in this study. To the best of our knowledge this is the first attempt using probiotics to improve bacterial colonisation of the intestinal and reproductive tract, and prevent reproductive tract problems in laying hens.

Prevention of reproductive tract problems and improvement of hen liveability

Pathologies of laying hen reproduction such as oophoritis, salpingitis, peritonitis, salpingo-peritonitis and metritis are frequently encountered at onset and during the laying period, subsequently causing reduced egg production and hen welfare. There are many factors that can initiate such pathologies. However, mortalities in most of cases are caused by other complications such as acute and chronic peritonitis. In some cases hens may look healthy but they have stopped egg production. Bacterial infections are a major contributory factor that should be taken in consideration if the frequency of reproductive lesions increases in a flock, in particular under free-range housing conditions. Various bacteria have been frequently reported to cause primary or secondary reproductive tract infections in free-range birds (Shini et al., 2008; Neubauer et al., 2009). Although the route of infection is not clearly known, contamination of vent, cloaca and oviduct with faecal material has been seen as an important source of such infections (Keller et al., 1995).

Previously, many investigators have frequently isolated pathogenic bacteria (e.g. *Mycoplasma gallisepticum*, *E. coli*, *Salmonella* spp., *Pasteurella multocida*, *Staphylococcus aureus*) from lesions in the peritoneum and reproduction tract of laying chickens (Gross and Siegel, 1959; Jones and Owen, 1981; Riddell, 1996; Trampel et al., 2007). Mirlle et al. (1991) examined 496 hens with reproductive tract lesions and isolated *Gallibacterium* in pure culture from 23% of the diseased organs. Haemolytic *G. anatis* was associated with infection in birds kept in alternative husbandry systems and suffering from reproductive disorders (Neubauer et al., 2009). The current study showed no correlation between reproductive pathologies and bacterial contamination of the oviduct. Moreover, we were unable to reveal any association between normal structural and functional reproductive tract and colonisation of GIT and/or reproductive tract with beneficial bacteria in control and probiotic-treated birds.

From this study it was demonstrated that probiotic-treated hens showed an improved of local and systemic immunity (of the oviduct and ovary) and increased general resistance to the diseases resulting in an increased liveability and well-being of birds. Control hens demonstrated higher incidence of reproductive pathologies than probiotic-treated birds. An additional risk to free-range flocks is increased parasitic incidence. Heavy worm burdens can predispose birds to develop secondary bacterial infections of GI and reproductive tracts. In some cases *A. galli* eggs may act as mechanical vectors of other bacterial infections (Chadfield et al., 2001). In this study, at 38 wks of age birds from control group were found to be highly infested with round- and band-worms. The mechanism, by which probiotics enhanced mucosal immune response in the reproductive tract of treated birds and reduced reproductive pathologies is unclear, and was not investigated in this study. However, it could be that cellular and molecular events in the local environment (i.e. oviduct) improved mucosal defences against pathogens and preserved homeostasis in mucosal tissues of reproductive system.

Improvement of hen performance

In this study, hen performance (egg production and egg weight, and BW) were increased in all treatment groups as the flock aged. However, significant differences were found between probiotic-treated and control birds. Both probiotics seem to significantly improve the performance of birds, with probiotic 2 showing a greater effect on all production parameters.

Previous investigators have shown that addition of probiotics to feeds of poultry (broilers and laying hens) has beneficial effects on growth performance and egg production. In laying hens, Gallazzi et al. (2008) indicated that egg production and FCR were significantly improved when hens were treated with probiotic strain *Lactobacillus acidophilus* D2/CSL. Similar results were found by Li et al (2005) when 500 mg/kg *Bacillus subtilis* culture was added to the diets. It has been proposed that these effects are achieved by different mechanisms including competitive exclusion of pathogens (Morishita et al., 1997, Nisbet, 1998) and improved digestion and absorption of nutrients (Thomke and Elwinger, 1998). This is of particular interest particularly in stress conditions when the balance of beneficial bacteria is disturbed. *Lactobacilli* and bifidobacterial species seem to be sensitive to stress (Patterson and Burkholder, 2003).

In conclusion, experiments conducted in this project indicated that reproductive system lesions in laying hens, that often cause drops in egg production and sudden deaths of birds, can be reduced if hens are treated with a commercial probiotic before or during the onset of lay for 4 weeks. The general health and performance status of hens may be also improved, resulting in significant economic gains to egg producers and better health and welfare of birds in free range systems.

Implications

The intestinal microflora of chickens has an important role not only in digestion and absorption but also in the protection of the host from pathogens. The addition of probiotics to the drinking water was found to reduce the occurrence of reproductive tract pathologies and improve performance in laying hens (egg production/egg weight and BW). In terms of implications for the study and practice it can be generalised that:

1. The probiotic use did not show any significant effect on microbial colonisation of the intestinal and reproductive tracts of hens. However, a reduction of reproductive tract pathologies and improved hen health and liveability was achieved.
 - a. The resources available to perform this work limited the microbiological studies to a screen for significant pathogens. The methods used in the study would not detect subtle changes in the levels of beneficial bacteria. Hence, it is possible that changes in “beneficial” bacteria colonisation levels did occur but were not detected. Further studies using more extensive culture and molecular methods are required.
 - b. The concept of supporting the hen’s normal flora with live microorganisms conferring a beneficial health effect is a natural medical strategy. Many other investigators have previously noted that an alteration of microbial population in adult chickens is often not successful. The treatment of hens started when they were 18 weeks. An earlier treatment with beneficial bacteria and a subsequent use before the onset of lay could have been more constructive.
 - c. While, application of a probiotic product direct into cloaca could have been more useful, this technique is not practical under commercial conditions.
 - d. The wide ranges of conditions under which laying hens are kept in free-range affect the types of commensal bacteria present in the intestinal tracts. For this reason, a simple culture with few types of bacteria is less likely to be successful in preventing infections, whereas a defined culture with a broad range of bacteria is potentially able to mitigate or slow against bacteria of special concerns likely to be encountered in layer production operations such as free range. However, considering the strain specificity of probiotic it is to be thought that employment of probiotic products that are member of the intestinal microbiota of the host could produce a better effect.
2. The results from this study provide some initial evidence that the manipulation of bacterial communities by prophylactic administration of effective and competitive beneficial cultures could be a useful approach to control and prevent reproductive tract infections in adult hens. More research and field trials are needed to establish the efficacy of probiotics and make their use more convenient for producers.
3. It is possible that a new probiotic composition with strains from laying hens kept in free-range conditions would result in a more effective competitive exclusion of pathogenic bacteria. Additional screening for beneficial bacteria colonising reproductive tract of laying hens may help to design a consistently successful defined probiotic that will be able to exclude specific bacteria present in reproductive pathologies.
4. The preliminary data from this project suggest that the probiotics may be able to increase the resistance to reproductive diseases and improve liveability of hens most probably by an enhancement of innate immune response at a local and systemic level of the reproductive tract. Further studies could help to understand how local and systemic immune response is elicited and how this contributes to the prevention of reproductive pathologies.

5. Overall probiotic use enhanced bird performance. Free-range birds treated with probiotics achieved their level of production at peak of lay while maintaining BW and egg weights at standard levels. Un-treated birds did not perform at this level.

Recommendations

One key problem facing the poultry industry is that there are virtually no medications for use against infections causing reproduction tract problems in laying hen. However, other approach can be employed to prevent or reduce reproductive tract diseases. Specific recommendations arising from this work are as follows:

- Producers need to be aware that successful control and prevention of bacterial infections in free-range layer operations requires specific inputs. Commonly recommended measures such as improvements of hygienic conditions and biosecurity standards are essential tools to keep the flock healthy and demonstrate compliance with production standards for poultry.
- Producers should be provided with information about alternatives to antibiotics such as natural products (probiotics) that can be used to combat pathogenic bacteria and prevent reproductive tract infections in free-range laying flocks.
- Producers should be given advice on how to utilize commercial probiotics to control and prevent reproduction tract problems, reduce mortality and improve profitability of free-range laying hens.
- Free range producers need to be educated that to ensure continuous health and egg production, laying flocks should be regularly monitored for causes of decreased egg production and increased mortality. An early detection of reproductive tract lesions will help to employ appropriate strategies to decrease their consequences on hen health and egg production. The use of probiotics before or during onset of lay for 4 weeks is a specific option that should be considered. This will reduce reproductive tract pathologies and improve general health and welfare of hens housed in free-range systems. Moreover, egg production will be increased resulting in economic benefits to egg producers. Food safety will also be enhanced.

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Plain English Compendium Summary

Project Title:	A natural approach to prevent reproductive tract problems in free-range hens
Project No.:	09-02
Researcher:	Shaniko Shini and Pat Blackall
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Objectives	<ul style="list-style-type: none"> • To investigate the effects of two commercially available probiotics on the prevention of reproductive tract problems in free-range laying hens. • To test the effect of probiotics on general health and performance of hens.
Background	<p>Hens operated under free-range conditions are exposed to various microbial agents that can influence the types of bacteria present in their intestinal and reproductive systems thus cause a wide range of disease problems. One key problem facing the egg industry is that there are virtually no medications for use against infections of the reproductive tract problems. In addition, there are worldwide attempts to ban antibiotic use in poultry. Therefore free-range egg producers sooner or later will seriously look at other alternatives to control health and performance of hens. This raises the question whether these alternatives have a sound effect. In recent years, there has been considerable interest in finding alternatives to antibiotics, and probiotics have more often been proposed as a useful alternative.</p>
Research	<p>A trial with 18 weeks old HY-SEX Brown layers and two probiotic products available for use in laying hens in Australia was conducted. Six hundred thirty laying chickens were divided in three group treatments (probiotic 1, probiotic 2, and control or not-treated) with three replicates each. Probiotics were added in the drinking water on a daily basis at a dose recommended by manufacturers for 4 weeks (from 18 to 22 weeks of age). Post-mortem examination of birds euthanized at each sampling point (at 22, 26, 30 and 34 weeks of age) and birds that died during the experimentation period were used to identify reproductive tract problems, and relate any changes to the treatments. The examination of the bacterial profile of the cloacae and oviduct was carried out to monitor treatment-associated changes of the microbial population of the intestinal and reproductive tracts. The evaluation of health and performance parameters was used to assess the potential role of probiotics in maintaining good health and increasing egg production in laying hens.</p>
Outcomes	<p>The addition of probiotics to the drinking water was found to reduce the occurrence of reproductive tract pathologies and improve performance of laying hens (egg production, egg weight and body weight). The recommendations arising from this work are: A) regular monitoring of flocks for early detection of reproductive tract lesions and B) use of natural alternatives to antibiotics such as probiotics to help prevent reproductive tract infections and increase the production of quality eggs from hens operated in free-range systems.</p>
Implications	<p>This project identified some initial evidence that the manipulation of bacterial communities by administration of probiotics in drinking water could be used as an approach to control and prevent reproductive tract infections in adult hens. More research is needed to establish the effects of probiotics and identify subtle changes stimulated in the levels of beneficial bacteria in the intestinal and reproductive tract.</p>
Publications	Under preparation.