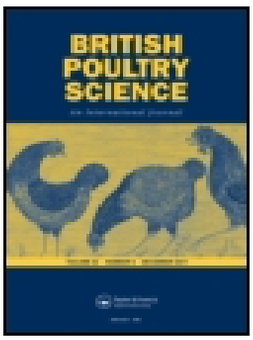

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Comparison of Single and Multi-Strain Probiotics Effects on Broiler Breeder Performance, Egg Production, Egg Quality and Hatchability

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Comparison of Single and Multi-Strain Probiotics Effects on Broiler Breeder Performance, Egg Production, Egg Quality and Hatchability

Abstract

1. This study was conducted to investigate the effect of multi-strain probiotic (containing *Lactobacillus acidophilus* 2.5×10^7 cfu/g, *Lactobacillus casei* 2.5×10^7 cfu/g, *Bifidobacterium thermophilum* 2.5×10^7 cfu/g and *Enterococcus faecium* 2.5×10^7 cfu/g) and single-strain probiotic (*Pediococcus acidilactici* 1×10^{10} cfu/g) on broiler breeder performance and gastrointestinal health.

2. A completely randomised trial was conducted using 300 broiler breeder hens (Ross 308) aged51 weeks old which were randomly allocated to one of five dietary treatments with six replicates per treatment in a 10 week trial. Treatments included 1) the basal diet a negative control, 2) basal diet supplemented with 0.1 g/kg multi-strain probiotic (MS), 3) basal diet supplemented with 0.1 g/kg single-strain probiotic (SS), 4) basal diet supplemented with 0.1 g/kg of both of probiotics (MS+SS) and 5) positive control basal diet supplemented with 0.5 g/kg oxytetracycline antibiotic (OX).

3. Body weight, egg production, yolk weight, egg shell thickness and weight, Haugh unit, fertility and hatchability were determined. Results showed that dietary treatments had no significant effect on total hen house or total hatching egg production, egg weight, yolk colour index, shell weight, mortality, body weight, fertility, hatchability, oviduct and stroma weight or number of large and small yellow follicles ($P>0.05$). None of the jejunum morphological parameters, apparent ileal digestibility of protein and ileal

Accepted for publication 21 April 2018

Lactobacillus population were influenced by supplemental probiotics (P>0.05), although ileum *Escherichia coli* count was reduced by inclusion of dietary probiotics (P<0.05).

4. It was concluded that although both probiotic treatments reduced coliforms, they did not improve broiler breeder performance or gastrointestinal tract (GIT) function.

Key words: Broiler breeder, probiotic, performance, digestibility, jejunum morphology.

Introduction

Commensal bacteria have been conventionally used in poultry feeding trials over past 30 years with the advancement of the competitive exclusion (CE) concept (Nurmi and Rantala, 1973). There are many types of probiotics around and their efficacy depends on their multi or single-strain usage. Probiotics are defined as live microbial supplements which affects the host animal by modifying its intestinal microbial balance and responses depend on the kind and population of bacterial load in the probiotics. Probiotic selection criteria included satisfactory growth and stability, performance in co-cultivation with a range of common pathogens, antibiotic resistance and virulence factors (Klose *et al.*, 2006). Probiotics produce beneficial effects by maintaining favourable microbial populations in the gastrointestinal tract, as well as improving feed intake and digestion (Nahashon *et al.*, 1994), changing microbial metabolism by increasing digestive enzyme and diminishing bacterial enzyme activity (Jin, *et al.*, 1997), improving nutrient digestibility (Park *et al.*, 2016, Zaghari *et al.*, 2015, Zaghari *et al.*, 2017), modifying intestinal flora (Forte *et al.*, 2016). These effects can lead to increased egg shell weigh and thickness (Panda *et al.*, 2008) and deactivation of toxins

Accepted for publication 21 April 2018

and stimulation of the immune system (Nahashon *et al.*, 1992). Specific studies on layers and breeders have indicated that supplementation of probiotics improved egg production, feed conversion and egg quality (Abdulrahim *et al.*, 1996, Guclu, 2011, Mohan *et al.*, 1995, Sultan and Abdul-Rahman, 2011), however, other feeding trials have shown no positive effects on layer and breeder poultry performance (Balevi *et al.*, 2001, Panda, *et al.*, 2008). Such variation in the effects of probiotics has been ascribed to the difference in strains (Oyarzabal and Conner, 1995), form of bacteria (Kalbande *et al.*, 1992), concentration in the diet (Jin, *et al.*, 1997) and viability in the gastrointestinal tract (Goodling *et al.*, 1987).

Probiotics composed of one or several strains of bacteria including *Lactobacillus, Bifidobacterium, Clostridium, Streptococcus, Bacillus and Pediococcus spp.* (Ritzi *et al.*, 2014, Wang and Gu, 2010, Zhang and Kim, 2014). Most experiments have concentrated on the use of single or mixed bacterial probiotics in poultry. Supplementation with *Pediococcus acidilactici* improved egg weight and egg shell quality (Mikulski *et al.*, 2012) and reduced number of broken eggs in laying hens (Mikulski, *et al.*, 2012). Multi-strain probiotics have been reported to lower feed conversion ratio and numbers of damaged eggs (Balevi, *et al.*, 2001). It has been reported that multi-strain probiotics enhance performance more than single strain products (Balevi, *et al.*, 2001, Gardiner *et al.*, 2004, Timmerman *et al.*, 2004). Recently, a new single and multi-species probiotic product has been developed as a competitive exclusion product for poultry in Iran (single-strain and multi-strain probiotics, denoted SS MS, respectively). Little information is available about the role of these probiotics in broiler breeder performance. Therefore, the present experiment investigated a

Accepted for publication 21 April 2018

comparison of functionality of these SS and MS probiotics on the broiler breeder performance (production, fertility and hatchability), egg quality and intestinal morphology.

Material and methods

Experimental Design and diets

This experiment was conducted at the experimental farm of the Department of Animal Science, University of Tehran. The trial was based on a completely randomised design, using three hundred Ross 308 broiler breeder hens and twenty roosters (for artificial insemination) allocated to one of five dietary treatments giving six pen replicates (10 birds in each pen). Breeder hens were housed in pens with 3 m^2 floor spaces (1.5×2 m), while roosters were housed in a separate pen with 4m^2 floor space. The trial was conducted in an open-sided breeder house under natural environmental conditions from March to July. The temperature in the layer house was set in the range of 18°C to 24°C. The trial lasted for 10 weeks, starting when breeders were 51 weeks old. A regime of 15.5 h light was provided and all hens and roosters were kept under uniform management conditions throughout the experimental period. Each pen was illuminated with one 90-watt incandescent light bulb. Daily feed allocations were adjusted weekly to maintain body weight gain (BWG) as recommended by the Ross 308 Parent Stock Management Manual. Egg production (total hen day and hen house egg production) and body weight gain of the hens were recorded at the end of each week from 51-61 weeks of age and egg quality parameters were analysed at the end of each week from 57-60 weeks of age. The corn-soybean meal based basal diet (mash form) was formulated to

Accepted for publication 21 April 2018

meet or exceed the National Research Council's recommendations (National Research Council, 1994). The dietary treatments included 1) basal diet negative control, 2) basal diet supplemented with 0.1 g/kg multi-strain probiotic (MS), 3) basal diet supplemented with 0.1 g/kg single-strain probiotic (SS), 4) basal diet supplemented with 0.1 g/kg of both of probiotics (MS+SS) and 5) positive control basal diet supplemented with 0.5 g/kg oxytetracycline antibiotic (OX). The probiotics were supplemented in diets according to manufacturer's recommendation. The MS probiotic is a multi-strain probiotic comprising four bacteria strains including *Lactobacillus acidophilus* 2.5×10^7 cfu/g, *Lactobacillus casei* 2.5×10^7 cfu/kg, *Bifidobacterium thermophilum* 2.5×10^7 cfu/g, *Enterococcus faecium* 2.5×10^7 cfu/g. The SS probiotic contained *Pediococcus acidilactici* 1×10^{10} cfu/g. Administration level of probiotics was recommendation by the manufacturer. Daily feed allocated to broiler breeder ranged from 155-159 g/day/bird during the experiment. In order to check mixing condition and probiotic activity and growth in the feed, the populations of bacteria in the feed samples of each diet were measured as described by (Lei *et al.*, 2009).

Response criteria

Ejaculates from 15 males were pooled and diluted to 2×10^9/ml viable spermatozoa with poultry semen extender. All hens were artificially inseminated once in the afternoon between 3 to 4 pm, on two consecutive days (d 0 and 1) with 0.5 ml extended semen (1×10^8 spermatozoa) at 50 weeks of age. Artificial insemination was carried out within 30 min after semen collection. Inseminations were standardised to prevent problems with sperm quality, number of viable spermatozoa, time of insemination and age,

Accepted for publication 21 April 2018

percentage and duration of fertility (Beaumont *et al.*, 1992, Brillard and Antoine, 1990, Wishart, 1985).

Egg production and hatchability

Hen-day egg production (HDEP), hen-house egg production (HHEP) and settable egg production were calculated at the end of each week and end of the experiment as described below:

$$\text{HDEP} = \frac{\text{Total number of eggs produced during the week}}{\text{Total number of hen} - \text{day in the same period}} \times 100$$

$$\text{HHEP} = \frac{\text{Total number of eggs laid during the weeks}}{\text{Total number of hens housed at the beginning of laying period}}$$

Fertile egg numbers and fertility percentage were calculated for each treatment. By the age of 59 weeks, eggs were gathered and set daily from the day after the final insemination (d 2). All eggs were candled on 7th day after incubation and those with unclear (probable live) embryos were removed and opened for visual admission as unfertile or initial dead. Eggs were assigned 'fertile' if early embryonic death was diagnosed. The fertile eggs, dead embryonic eggs and clear eggs (assumed infertile) were recorded separately. The fertility percentage was calculated as:

number of fertile eggs×100)/number of eggs set.

Early embryonic mortality was calculated as:

number of early mortality×100/number of fertile eggs

Egg quality

The quality traits of collected eggs during 57-60 weeks of age including yolk and white percentage, yolk colour, Haugh unit, shell weight, shell thickness in middle, narrow and width parts of eggs were determined by digital micrometer. Briefly, 12 eggs from each

Accepted for publication 21 April 2018

replicate during each week were weighed individually then broken in a glass plate to measure the albumen height (Haugh units) using a micrometer. The yolk colour was measured and scored according to the Roche yolk colour fan (ORKA Egg Analyzer®). To determine Haugh unit, two eggs from each replicate were used and calculated using the formula:

$$HU = \text{Log}\,(\text{Albumen height} + 7.57 - (1.7 \times W^{0.37})) \times 100$$

Before yolk weight was assessed, each yolk was rolled on a blotting paper towel to eliminate adhering albumen. To determine shell weight, albumen was removed from egg shell, the membrane was cleaned, and then the eggshell dried at room temperature and recorded as a percentage of the whole egg.

Protein digestibility and microbial population count

Five hens from each pen at the age of 61 weeks were randomly selected for the measurement of ileal microflora populations and ileal apparent digestibility of crude protein. During this period, to attain uniformity in feed intake and digesta content, the feed troughs were removed for 30 min, then replaced for 2 h before the birds were taken out for the measurements. After euthanasia by an intravenous injection of pentobarbitone, each bird was immediately dissected and the ileum position (defined as extending from Meckel's diverticulum to the ileo-caecal junction) was located, the distal 50 mm of the ileum were tied off and excised. This segment was bisected transversely and its contents were gently squeezed out into a plastic cup. Digesta pH was measured by a pH meter device (AD132, Romania). Then the samples were freeze-dried, ground through 1-mm mesh, and immediately prepared for the analysis of crude protein. Nitrogen content was measured by Kjeldahl method and chromic oxide was analysed

using a flame atomic absorption spectrophotometer (Model 2380, Perkin Elmer, USA) after wet digestion with concentrated nitric and perchloric acid (AOAC, 1990). All samples were assayed in duplicate.

For bacterial enumeration, samples of ileal digesta from each bird; frozen at -80º C; were thawed and removed from storage bags. Ileal digesta contents were then aseptically emptied in a new sterile bag and were immediately diluted 10-fold (i.e., 10% wt/vol) with sterile ice-cold anoxic phosphate buffer saline (PBS; 0.1 M; pH 7.0) and subsequently homogenised for 3 min in a stomacher (Bagmixer 100 Minimix, Interscience, France). Each homogenate digesta was serially diluted from 10^{-1} to 10^{-7}. Dilutions were subsequently plated on duplicate selective agar media for enumeration of target bacterial groups. In particular, coliforms, *Lactobacillus*. and *Salmonella spp*. were enumerated using MacConkey, Rogosa and Brilliant Green agar respectively according to Tuohy *et al.* (2002). Plates were incubated at 39º C for 24-72 h aerobically (MacConkey and Brilliant Green agar) or 48-120 h anaerobically (Rogosa agar) and colonies were counted. Results were expressed as $\log_{10}$ colony-forming units per gram of ileum digesta.

Reproductive organs trait and intestinal morphology

At the end of the experiment, two hens from each pen were killed and stroma and oviduct weight and large and small yellow follicle numbers were recorded. Likewise, immediately after killing, small intestines were removed and jejunum samples were fixed in 10% phosphate-buffered formalin for a minimum of 48 h and 4 µm sections were prepared. The sections were stained with standard haematoxylin-eosin solution and villus height (VH), villus width (VW), crypt depth (CD) and lamina propria (LP)

Accepted for publication 21 April 2018

thickness were measured at 100X magnification by light microscopy (CH30, Olympus, Japan) using a calibrated ocular micrometer. Ten microscopic fields were measured per bird (AVMA, 2007).

Feather and faecal score

To measure the feather and faecal indices, five hens from each pen (30 hens from each treatment) were selected and, after measuring the feather and faecal indices, five samples of each pen were pooled and analysed. Feathers were scored in the cage according to the five point feather score scale (Webster and Hurntk, 1990) where 1 = smooth and complete plumage; 2 = ruffled, no naked spots; 3 = naked spots up to 5 cm at the widest part; 4 = naked spots greater than 5 cm wide; and 5 = naked spots with injury to skin. Faecal scores are a qualitative estimation of the deviation of the appearance of the droppings from normal. A score of 0 indicated normal droppings, whereas a score of 4 indicated maximum departure of the faeces from normal (diarrhoea). Scores of 1, 2, and 3 represented intermediate gradations (Morehouse and Baron, 1970).

Statistical analysis

All measured criteria on the effect of dietary probiotics on broiler breeder performance and reproductive characteristics were analysed by one way ANOVA using GLM procedure of SAS software (SAS, 2001) with diet as the main effects. Duncan's multiple range tests was used to compare means ($P<0.05$). Because the feather and faecal indices were categorical, they were pooled (mean of five per pen) and then analysed. A repeated measurements analysis was used to compare treatment groups for performance during

Accepted for publication 21 April 2018

the trial (age), as well as their response patterns with age. Diversity between treatments were considered using Tukey comparisons.

Results

There were no significant differences between SS and MS probiotics on broiler breeder body weight, hen-day and hen-house egg production and hen-day settable eggs while time (weeks) of production significantly affected these traits (Table 2).

TABLE 2 NEAR HERE

The results showed no significant differences due to supplemented probiotic treatments on egg weight, Haugh unit, yolk percentage and yolk colour, shell weight, shell thickness in upper, middle and narrow parts of settable eggs from 57-60 weeks of age (Table 3).

TABLE 3 NEAR HERE

On the other hand, as hens aged, egg weight and Haugh units significantly increased but shell thickness in all areas was decreased. Clear benefits of supplementation with probiotics in total and settable egg production, hatchability and fertility could not be identified in this experiment (Table 4).

TABLE 4 NEAR HERE

Accepted for publication 21 April 2018

Carcass traits and ileum villi morphometry did not significantly differ between dietary treatments (Table 5 and 6). Dietary probiotics did not affect oviduct and stroma weights, large and small yellow follicles number, villus height, villus width, crypt depth, villus height to crypt depth index or *Lamina propria* thickness of broiler breeder hens (P>0.05).

TABLES 5 AND 6 NEAR HERE

The differences between MS and SS probiotic effects on broiler breeder ileal microbial population, digesta pH and faecal index are shown in Tables 7 and 8. Compared to the unsupplemented, negative control group, there was a significant reduction of *Escherichia coli* in the ileum of breeder hens fed diet containing either the antibiotic or probiotics. Protein apparent ileal digestibility and *Lactobacillus spp.* counts were not affected by dietary treatments. No *Salmonella spp*. was detected in the ileum of breeder hens (data not shown). From this data, any benefit of probiotics in the elevation of lactic acid bacteria could not be identified. Addition of probiotics or antibiotic increased faecal index of broiler breeders (P<0.05) while no decrease in feather score and ileal pH were detected (P > 0.05).

Discussion

The objective of this trial was to compare the effect of single-strain (SS) and multi-strain (MS) probiotics on broiler breeder performance and identify the more efficient product. In addition to performance parameters, the determination of protein apparent

digestibility, hatchability and reproductive organ development and ileal microflora composition were examined in this study. However, most of the detected and calculated parameters of this study were not affected by probiotics, except for a reduction in coliforms in the broiler breeder's small intestine.

Egg production (total laid eggs during 59 weeks of age) of broiler breeders in the negative control, MS, SS, combined probiotics and antibiotic fed groups were 408.83, 356, 372.5, 413.8 and 382, respectively, and was not statistically different. These results were similar to others, who reported that commercial probiotic (multi-strain) supplementation has no effect on laying hen egg production (Balevi, *et al.*, 2001) and supplementation with 100 mg *Lactobacillus sporogenes* per kg had no effect on white leghorn layer breeders performance (Panda, *et al.*, 2008). Conversely, the findings of the current study did not support previous findings, which concluded that egg production increased with probiotic supplementation in laying hens (Khan *et al.*, 2011, Panda, *et al.*, 2008). Other trials have shown that novel probiotics increased broiler chicken body weight (Khan *et al.*, 2007, Olnood *et al.*, 2015, Timmerman, *et al.*, 2004) and laying hen performance (Tang *et al.*, 2015).

As far as commercial products are concerned, probiotic dose should be based on efficacy seen in human and animal studies and the cfu/g product is an important consideration. Although information on minimal concentrations is still insufficient, it has been generally confirmed that probiotics should have a minimum concentration of 10^6 CFU/ml (or gram) and that animals efficacy for most probiotics should be demonstrated with a daily intake of 10^8 to 10^9 microorganisms (Patterson and Burkholder, 2003, Toma and Pokrotnieks, 2006). It is generally accepted that a probiotic

Accepted for publication 21 April 2018

should have several billion microorganisms to increase the possibility of sufficient gut colonization. For example, typical doses of *Lactobacilli spp*. used in studies ranged from 1-20 billion cfu per day (Williams, 2010) while in this current experiment, the administration level of probiotics (as recommendation) was lower than that the minimum level of common effective dose. In a previous experiment using a five bacterial strain probiotic product, efficacy in improving broiler growth and FCR was demonstrated with doses of 10^9 cfu/kg diet, resulting in an average daily intake of 2×10^8 microorganisms per bird (Mountzouris *et al.*, 2007). However, no consistent conclusions could be drawn regarding the effect of increasing probiotic administration level on growth performance (Mountzouris *et al.*, 2010). Essentially, probiotics increased broiler and laying hen performance in most experiments except in the case of broiler breeders. In the current experiment, feed intake of broiler breeders ranged from 155 to 159 g/day/bird and birds consumed 1.7×10^7 cfu/bird, while a total of 10^8 to 10^9 probiotic microorganisms should be consumed daily for benefits to be seen.

Probable reasons for these result are: 1) levels of probiotics were 1×10^8 cfu/g while others used higher doses(1×10^{13}) and the minimum effective dose is 1×10^8/g, 2) concentrations of the beneficial bacteria (*Lactobacillus* or *Bifidobacterium spp.*) in the small intestine of broilers was lower than the negative (unsupplemented) control group which means that proliferation of bacteria in small intestine did not occur and 3) health status of the breeder hens may have been too high, because no performance improvements were seen with the antibiotic OX treatment.

is the data revealed that broiler breeders may respond to probiotic bacteria in different manner, probably due to the feeding method (restricted or *ad-libitum*), frequency of

Accepted for publication 21 April 2018

feeding (once or many times per day) and age of birds (young or old). Since broiler breeders were restricted to only one feed a day and feed transition from their gastrointestinal tract may differ from layers, probiotic bacteria colonisation in GIT might have been influenced and increased the likelihood of reduction of any competitive exclusion.

Based on these results, egg weight and quality of broiler breeders did not differ between probiotic and antibiotic treatments. In contrast, it has been reported that probiotics increased laying hen breeders egg weight between 29-62 weeks of age (Peebles *et al.*, 2000) and hatchability of eggs (Narushin and Romanov, 2002). A mixture of 12 *Lactobacillus spp.* cultures elevated egg weight in laying hens (Ramasamy *et al.*, 2009) and tended to increase hatchability of eggs, while in the present experiment, average egg weight from hens fed SS diet was heavier than that those fed diets containing both of the probiotics but this was not significant and there were no hatchability improvements. However, many authors have suggested that the positive effect of probiotics depends on other factors, such as adhesion and replication of bacteria in small intestine (Forte, *et al.*, 2016), age of birds, microbial species, liveability, single or multi strain, feed composition, usage amount and method of delivery (Mahdavi *et al.*, 2005, Mikulski, *et al.*, 2012, Zhou *et al.*, 2010).

In the case of egg shell quality, other researchers have revealed that probiotic supplementation improved egg shell by enhancing calcium concentration in serum (Panda, *et al.*, 2008), and phosphorous (Mutuş *et al.*, 2006) and calcium retention (Mikulski, *et al.*, 2012, Mutuş, *et al.*, 2006). It was suggested that this may be related to the replication of lactic acid bacteria which facilitated the ionization of minerals

Accepted for publication 21 April 2018

(Mikulski, *et al.*, 2012) and reduced lumen pH (Forte, *et al.*, 2016, Sobczak and Kozłowski, 2015). Based on the respective pH and ileal *Lactobacillus spp.* data, it appeared that egg shell thickness should not differ between dietary treatments in the current study.

Reduction of hatchability may contribute to increased liquid content of eggs and Haugh unit/or albumen height and egg content consistency (Narushin and Romanov, 2002). Probiotic supplementation can increase bioavailability of several minerals (iron, copper, zinc and manganese) and gross energy of the diet. This may enhance Haugh units (Balevi, *et al.*, 2001). A possible explanation for lack of hatchability response may because using MS or SS probiotic had no noticeable effect on Haugh unit, egg yolk and white content and yolk colour.

Carotenoids plays an important role in antioxidant capacity and immune function in growing embryos and deposition in egg yolk (Tang, *et al.*, 2015). Due to the equal amount of corn (as a source of carotenoids) used in the diets, yolk colour index was not significantly different between treatments.

Supplementation of broiler breeders with either SS or MS probiotic did not increase ileal villus height or height to crypt depth ratio. Hence, epithelial cell turnover was not affected due to feeding SS or MS. In most published experiments, administration of beneficial bacteria and their replication in the GIT, influenced intestinal morphology, although longer villi and greater cell proliferation occurred only after increasing the probiotic bacteria populations in the gut It *was* recently reported that a probiotic containing *Lactobacilli, Bifidobacterium thermophilum spp.* and *Enterococcus faecium* bacteria increased jejunal villus height and decreased crypt depth compared to

Accepted for publication 21 April 2018

salinomycin and a negative control (Chichlowski *et al.*, 2007). Also *Lactobacillus, Bifidobacterium and Pediococcus spp.* may produce short-chain fatty acid which increase jejunal villus height and epithelial cell proliferation that leads to improved absorption (Forte, *et al.*, 2016, Sobczak and Kozłowski, 2015). Shorter and thinner villi were caused by toxins produced by detrimental bacteria like coliforms (Awad *et al.*, 2006). It is understood that greater villus height is an indicator of activity and function of intestinal villi (Shamoto and Yamauchi, 2000), and villus function is activated after feeding of dietary probiotic.

It has been speculated that the efficacy of a probiotic to promote broiler breeder performance could be due to the fine tuning of the complex gut ecosystem, resulting in improved digestive function, intestinal environment, and hen health. However, the current trials showed that inclusion of single- and multi-strain probiotics had no effect on protein apparent digestibility and gut *Lactobacillus spp.* Populations, although supplementation decreased gut *Escherichia coli* counts. Only a few studies have examined nutrient digestibility in poultry fed probiotics and, contrary to the current results, it was shown that, depending on inclusion level, probiotic intake resulted in an improved ileal apparent digestibility coefficients of nitrogen and fat in broilers (Apata, 2008), crude protein (Mountzouris, *et al.*, 2010), energy and most amino acids in 21-d and 42-d-old broilers (Li *et al.*, 2008). Intake of live microorganisms modulate the gut environment and enhance the gut barrier function via the fortification of the beneficial members of the intestinal microflora and the competitive exclusion of pathogens (Farnell *et al.*, 2006, Higgins *et al.*, 2008, Mountzouris, *et al.*, 2007). Nevertheless, this beneficial protective probiotic function may have a nutrient and energy cost for the host

Accepted for publication 21 April 2018

because live microbes have their own requirements for their growth and proliferation. However, in the current study, fortification of the beneficial bacteria did not occur and this could explain the fact that the probiotic treatments (MS or SS) did not show significant differences in apparent ileal digestibility of protein compared with the negative control group.

A consistent effect of the probiotic treatments to suppress the levels of coliforms was evidenced, however. The SS and MS and antibiotic treatments in these older broiler breeders resulted in significantly lower ileal coliform concentrations compared to the control group. Other studies have demonstrated the potential of probiotics to fortify the intestinal microflora of broiler chickens to suppress potentially pathogenic bacteria (Higgins, *et al.*, 2008, Koenen *et al.*, 2004).

In conclusion, none of the probiotics had a significant effect on performance, gut morphology, egg quality, ileal *Lactobacillus spp.* composition and apparent ileal protein digestibility. Although both of the probiotics reduced the *Escherichia coli* number in the ileum of broiler breeder, neither can be promoted in broiler breeder nutrition. It is proposed that optimal probiotic inclusion levels for growth performance in broiler breeder diets should be explicitly examined in context with feed ingredients and the levels of essential amino acids. A higher inclusion level (e.g. $>10^9$ cfu/kg) may be required for the beneficial modulation of the ileal and caecal microflora composition, determined by microbial culture at a genus level. From a practical point of view, this study highlighted the need for a proper adjustment of probiotic inclusion levels in the broiler breeder diet to achieve the desired beneficial outcome.

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Accepted for publication 21 April 2018

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Table 1- Ingredients and calculated nutrient content of basal diet

Ingredients	Percent	Nutrients	
Corn	377.4	AME (Kcal/Kg)	2900
wheat	326.2	CP (%)	15.00
soybean meal	170	Lys (dig) %	0.60
Soybean Oil	10	Meth (dig) %	0.28
D-L Methionine	0.8	Meth + Cys (Dig) %	0.53
L-Lysin-Chl	0.4	Thr (Dig) %	0.48
Carbonate Calcium	74.2	Trp (Dig) %	0.15
Di-Calcium Phosphate	7.6	Arg (Dig) %	0.82
Salt	3.2	Ile (Dig) %	0.54
Sodium Bicarbonate	2	Val (Dig) %	0.62
Breeder Supplement1	5.2	Ca %	3.20
Vitamin E supplement	1	P %	0.35
Vitamin B	1	Na %	0.23
Antioxidant	1	Cl %	0.23
Sodium Bentonite	10	DCAB MEq/Kg	200
Probiotic, antibiotic or sand	10	Linoleic acid	1.46
Total	1000	Fiber %	4.8

1- Each kilogram of broiler breeder diet contained the following: vitamin A, 11,000 IU; vitamin D3,3,500 IU; vitamin K3, 5 mg; vitamin E, 60 IU; vitamin B1, 0.25 mg; vitamin B2, 12 mg; vitamin B5, 15 mg; vitamin B6, 4 mg; vitamin B9, 2 mg; vitamin B12, 0.03 mg; choline chloride, 1,000 mg; iron, 50 mg; Zn, 100 mg; Mn, 120 mg; Cu, 10 mg; Se, 0.3 mg; antioxidant, 1,000 mg.

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Table 2. Effect of dietary treatments on broiler breeder performance from 51-61 week of age▲					
		Parameters			
Treatment♦	Week of age	BW (kg)	HD %	HH %	Set-HD %
Control		3.98	54.92	23.09	54.20
MS		4.00	49.84	20.46	49.45
SS		4.05	52.95	21.84	52.52
MS + SS		4.10	57.44	23.14	57.32
OX		4.12	50.53	20.65	49.23
SEM		0.083	3.098	1.529	3.021
	51	4.03^{edc}	60.26^{a}	4.21^{k}	59.14^{a}
	52	3.99^{ed}	55.63^{ab}	8.07^{j}	54.80^{abcd}
	53	3.92^{e}	52.94^{bc}	11.71^{i}	52.50^{bcde}
	54	3.93^{e}	49.16^{cd}	15.05^{h}	48.67^{e}
	55	3.94^{e}	47.83^{d}	18.28^{g}	47.39^{e}
	56	4.02^{ed}	50.85^{bcd}	21.71^{f}	50.24^{cde}
	57	4.04^{edc}	50.21^{cd}	25.07^{e}	49.71^{de}
	58	4.09^{bcd}	51.53^{bcd}	28.54^{d}	50.88^{cde}
	59	4.15^{abc}	52.50^{bcd}	32.07^{c}	51.94^{cde}
	60	4.21^{ab}	55.53^{ab}	35.80^{b}	55.33^{abc}
	61	4.24^{a}	57.85^{a}	39.67^{a}	57.19^{ab}
	SEM	0.041	1.624	0.599	1.341
Sources	*P-Value*				
Treatment	0.41	0.61	0.637	0.71	0.611
Time	0.01	0.01	0.001	0.001	0.001
Treatment × Time	0.99	1.00	0.97	0.99	1.00

^{abc} Means within the same line with no common differ significantly (P< 0.05).

♦Control= basal diet, MS= basal diet plus multi-strain probiotic, SS= basal diet plus single-strain probiotic, MS+SS= basal diet plus multi-strain and single-strain probiotic and OX= basal diet plus oxytetracycline antibiotic.

♦ BW= body weight, HD-Set= settable hen day egg production (%), HD= hen-day egg production (%), HH=hen-house egg production.

▲The values are means of the 6 pens.

Table 3. Effect of dietary treatments on broiler breeder egg quality from 57-60 weeks of age▲

		Parameters•							
Treatment♦	Time	EW(g)	HU	YC	SW(g)	TM(mm)	TN(mm)	TW(mm)	Yolk%
Control		69.06	79.35	7.67	6.41	42.60	43.71	42.52	31.46
MS		69.33	76.19	7.62	6.58	43.73	44.69	43.94	31.69
SS		70.87	73.77	7.77	6.47	43.48	44.00	51.20	32.14
MS + SS		70.19	76.37	7.72	6.67	43.46	44.71	43.53	31.05
OX		70.04	73.46	7.75	6.44	43.54	43.61	43.12	32.45
SEM		1.82	1.52	0.07	0.17	0.79	1.03	4.71	0.37
	57	69.23^{b}	71.62^{b}	7.68^{a}	6.47^{b}	45.83^{a}	47.45^{a}	52.22^{a}	31.81^{a}
	58	68.87^{b}	78.55^{a}	7.35^{b}	6.88^{a}	45.48^{a}	46.30^{a}	45.35^{ab}	31.05^{b}
	59	69.23^{b}	76.45^{ab}	7.90^{a}	6.25^{c}	41.80^{b}	41.96^{b}	41.55^{b}	31.81^{a}
	60	72.27^{a}	76.70^{ab}	7.90^{a}	6.46^{b}	40.33^{c}	40.87^{b}	40.33^{b}	31.48^{ab}
	SEM	0.82	1.89	0.09	0.07	0.48	0.52	3.37	0.36
Source		*P-Value*							
Treatment		0.71	0.26	0.42	0.47	0.43	0.53	0.87	0.54
Time		0.01	0.06	0.01	0.01	0.01	0.01	0.06	0.01
Treatment× Time		0.97	0.45	0.11	0.22	0.50	0.61	0.31	0.93

^{abc} Means within the same line with no common differ significantly (P< 0.05).

♦Control= basal diet, MS= basal diet plus multi-strain probiotic, SS= basal diet plus single-strain probiotic, MS+SS= basal diet plus multi-strain and single-strain probiotic and OX= basal diet plus oxytetracycline antibiotic.

• EW= egg weight, HU= Haugh unit, YC= yolk colour, SW= shell weight, TM= shell thickness of middle part, TN= shell thickness of narrow part, TW= shell thickness of width part.

▲The values are means of the 6 pens.

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Table 4. Effect of dietary treatments on broiler breeder fertility and hatchability (59 wk)▲

		Treatments♦					
Parameter (%)	Control	MS	SS	MS + SS	OX	SEM	*P-Value*
Total laid eggs	408.8	356.0	372.5	413.8	382.0	21.0	0.28
Total hen house	41.6	36.9	39.5	42.10	38.2	2.3	0.49
Total hen day eggs	42.9	38.9	41.1	45.3	39.9	2.0	0.21
Total settable eggs	403.5	353.2	369.3	413.0	372.3	21.4	0.28
Settable hen house eggs	41.1	36.6	39.2	42.0	37.2	2.3	0.44
Settable hen day eggs	42.3	38.6	40.7	45.2	38.9	2.0	0.16
Hatchability (%)	67.2	82.5	74.3	70.9	79.8	6.4	0.44
Fertility (%)	71.5	71.7	75.5	67.3	71.6	6.6	0.93

[abc] Means within the same line with no common differ significantly (P<0.05).

♦Control= basal diet, MS= basal diet plus multi-strain probiotic, SS= basal diet plus single-strain probiotic, MS+SS= basal diet plus multi-strain and single-strain probiotic and OX= basal diet plus oxytetracycline antibiotic.

▲The values are means of the 6 pens.

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Table 5. Effect of dietary treatments on broiler breeder carcass parameters (62 wk.)▲

	Treatments♦						
Parameter•	Control	MS	SS	MS + SS	OX	SEM	*P-Value*
Oviduct weight (gr)	68.91	64.87	56.88	70.83	75.03	5.07	0.32
Stroma weight (gr)	10.10	11.75	7.91	9.30	11.03	1.03	0.17
LYF number	5.66	5.25	5.00	5.40	5.83	0.38	0.59
SYF number	21.00	25.25	21.00	16.83	23.00	3.49	0.61

♦Control= basal diet, MS= basal diet plus multi-strain probiotic, SS= basal diet plus single-strain probiotic, MS+SS= basal diet plus multi-strain and single-strain probiotic and OX= basal diet plus oxytetracycline antibiotic.

• LYF= Large yellow follicle and SYF= small yellow follicle.

▲The values are means of the 6 pens.

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Table 6. Effect of dietary treatments on broiler breeder ileal morphology (µm)▲

	Treatments♦						
Parameter•	Control	MS	SS	MS + SS	OX	SEM	*P-Value*
VH (µm)	1139.0	1032.0	1127.7	1091.7	1034.3	60.31	0.59
VW (µm)	189.3	217.0	171.33	216.7	216.3	22.99	0.52
CD (µm)	112.3	116.7	100.7	121.7	111.1	11.13	0.74
VH:CD	10.1	8.8	11.2	9.0	9.3	1.03	0.34
LPT (µm)	59	71.66	73.66	82.33	77	5.41	0.07

♦Control= basal diet, MS= basal diet plus multi-strain probiotic, SS= basal diet plus single-strain probiotic, MS+SS= basal diet plus multi-strain and single-strain probiotic and OX= basal diet plus oxytetracycline antibiotic.

•VH= villus height, VW= villus width, CD= crypt depth, and LPT= lamina propria thickness.

•The values are means of the 6 pens.

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Table 7. Effect of dietary treatments on caeca pH, bacterial counts (log cfu/g) and faecal index▲

	Treatments♦						
Parameter	Control	MS	SS	MS + SS	OX	SEM	*P-Value*
Lactobacillus	14.40	12.27	12.39	13.18	11.92	0.69	0.12
Escherichia coli	8.93[a]	6.12[d]	8.04[b]	6.75[c]	5.78[e]	0.045	0.001
Ileal pH	6.93	7.10	6.82	6.92	6.82	0.163	0.75
Fecal index	1.50[C]	1.87[bc]	2.04[abc]	2.08[ab]	2.46[a]	0.18	0.016
Protein digestibility%	66.86	68.59	64.69	63.86	66.97	2.32	0.62
Feather index	3.86	3.69	4.04	3.92	3.97	0.087	0.07

[abc] Means within the same line with no common differ significantly (P<0.05).

♦Control= basal diet, MS= basal diet plus multi-strain probiotic, SS= basal diet plus single-strain probiotic, MS+SS= basal diet plus multi-strain and single-strain probiotic and OX= basal diet plus oxytetracycline antibiotic.

▲The values are means of the 6 pens.

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