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Comparison of single- and multi-strain probiotics effects on broiler breeder performance, egg production, egg quality and hatchability

M. Aalaei^a, A. Khatibjoo^b, M. Zaghari^c, K. Taherpour^b, M. Akbari Gharaei^b and M. Soltani^d

^aDepartment of Animal Science, University of Ilam, Ilam, IRAN; ^bPoultry Nutrition, Department of Animal Science, University of Ilam, Ilam, IRAN;

^cDepartment of Animal Science, University of Tehran, Tehran, IRAN; ^dDepartment of Biotechnology, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran

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) aged 51 weeks old which were randomly allocated to 1 of 5 dietary treatments with 6 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, eggshell 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 *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.

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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 and their efficacy depends on their multi or single-strain usage. Probiotics are defined as live microbial supplements which affect 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, 2017), modifying intestinal flora (Forte et al. 2016). These effects can lead to increased eggshell weight and thickness (Panda et al. 2008) and deactivation of toxins 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 eggshell 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 CE 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 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 300 Ross 308 broiler breeder hens and 20 roosters (for artificial insemination) allocated to 1 of 5 dietary treatments giving 6 pen replicates (10 birds in each pen). Breeder hens were housed in pens with 3 m² floor spaces (1.5 × 2 m), while roosters were housed in a separate pen with 4 m² 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 BWG of the hens were recorded at the end of each week from 51 to 61 weeks of age and egg quality parameters were analysed at the end of each week from 57 to 60 weeks of age. The corn-soybean meal based basal diet (mash form) was formulated (Table 1) to 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 oxy-tetracycline antibiotic (OX). The probiotics were supplemented in diets according to manufacturer's recommendation. The MS probiotic is a multi-strain probiotic comprising 4 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 Takgene Company (Pharmaceutical Company in Tehran, Iran). Daily feed allocated to broiler breeder ranged from 155–159 g/d/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, 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:

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

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

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 d 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:

$$\text{number of fertile eggs} \times 100 / \text{number of eggs set.}$$

Early embryonic mortality was calculated as:

$$\text{number of early mortality} \times 100 / \text{number of fertile eggs.}$$

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 micro-metre. Briefly, 12 eggs from each replicate during each week were weighed individually then broken in a glass plate to measure the albumen height (Haugh units) using a micro-metre. 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:

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

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 -80°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; 0.1M; 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 39°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) 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, 5 hens from each pen (30 hens from each treatment) were selected and, after measuring the feather and faecal indices, 5 samples of each pen were pooled and analysed. Feathers were scored in the cage according to the 5-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 5 per pen) and then analysed. A repeated measurements analysis was used to compare treatment groups for performance during the trial (age), as well as their response patterns with age. Diversity between treatments was 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).

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 to 60 weeks of age (Table 3).

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).

Carcass traits and ileum villi morphometry did not significantly differ between dietary treatments (Tables 5 and 6). Dietary probiotics did not affect oviduct and stroma weights,

Table 1. Ingredients and calculated nutrient content of basal diet.

Ingredients	Per cent	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 Supplement ¹	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	Fibre %	4.8

¹Each 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.

Table 2. Effect of dietary treatments on broiler breeder performance from 51 to 61 week of age².

Treatment ¹	Week of age	Parameters			
		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 ^{ad}
	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 ^a	52.50 ^{bcd}	32.07 ^c	51.94 ^{cde}
	60	4.21 ^{ab}	55.53 ^{ab}	35.80 ^b	55.33 ^a
	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

^{a-k}Means within the same line with no common superscript letter differ significantly ($P < 0.05$).

¹Control = 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, OX = basal diet plus oxytetracycline antibiotic, BW = body weight, HD-Set = settable hen day egg production (%), HD = hen-day egg production (%) and HH = hen-house egg production.

²The values are means of the 6 pens.

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 ($P > 0.05$).

The differences between MS and SS probiotic effects on broiler breeder ileal microbial population, digesta pH and faecal index are shown in (Table 7). 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; Olmood 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 micro-organisms (Patterson and Burkholder 2003; Toma and

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

Treatment ¹	Time	Parameters ²							Yolk %
		EW (g)	HU	YC	SW (g)	TM (mm)	TN (mm)	TW (mm)	
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

^{a-c}Means within the same line with no common superscript letter differ significantly ($P < 0.05$).

¹Control = 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.

²EW = 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.

³The values are means of the 6 pens.

Table 4. Effect of dietary treatments on broiler breeder fertility and hatchability (59 wk).²

Parameter (%)	Control	Treatments ¹				SEM	P-Value
		MS	SS	MS + SS	OX		
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

¹Control = 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.

²The values are means of the 6 pens.

Table 5. Effect of dietary treatments on broiler breeder carcass parameters (62 wk).³

Parameter ²	Control	Treatments ¹				SEM	P-Value
		MS	SS	MS + SS	OX		
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

¹Control = 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.

²LYF = Large yellow follicle and SYF = small yellow follicle.

³The values are means of the 6 pens.

Table 6. Effect of dietary treatments on broiler breeder ileal morphology (µm).³

Parameter ²	Control	Treatments ¹				SEM	P-Value
		MS	SS	MS + SS	OX		
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

¹Control = 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.

²VH = villus height, VW = villus width, CD = crypt depth, and LPT = lamina propria thickness.

³The values are means of the 6 pens.

Table 7. Effect of dietary treatments on caeca pH, bacterial counts (log cfu/g) and faecal index.²

Parameter	Control	Treatments ¹				SEM	P-Value
		MS	SS	MS + SS	OX		
<i>Lactobacillus</i>	14.40	12.27	12.39	13.18	11.92	0.69	0.12
<i>Escherichia coli</i>	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
Faecal index	1.50 ^c	1.87 ^{bc}	2.04 ^a	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

^{a-e}Means within the same line with no common superscript letter differ significantly ($P < 0.05$).

¹Control = 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.

²The values are means of the 6 pens.

Pokrotnieks 2006). It is generally accepted that a probiotic should have several billion microorganisms to increase the possibility of sufficient gut colonisation. 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 5 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/d/bird and birds consumed 1.7×10^7 cfu/bird, while a total of 10^8 – 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.

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 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 CE.

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 and 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 eggshell quality, other researchers have revealed that probiotic supplementation improved eggshell 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 ionisation of minerals (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 eggshell 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 be because using MS or SS probiotic had no noticeable effect on Haugh unit, egg yolk and white content and yolk colour.

Carotenoids play 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 salinomycin and a negative control (Chichłowski 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 modulates the gut environment and enhance the gut barrier function via the fortification of the beneficial members of the intestinal microflora and the CE 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 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.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

A. Khatibjoo  <http://orcid.org/0000-0002-9214-8146>

References

- ABDULRAHIM, S., M. HADDADIN, E. HASHLAMOUN, AND R. ROBINSON. 1996. "The Influence of *Lactobacillus Acidophilus* and Bacitracin on Layer Performance of Chickens and Cholesterol Content of Plasma and Egg Yolk." *British Poultry Science* 37: 341–346. doi:10.1080/00071669608417865.
- AOAC. 1990. *Association of Official Analytical Chemists. Official methods of analysis*. 15th ed. Arlington (VA): Association of Official Analytical Chemists.
- APATA, D. 2008. "Growth Performance, Nutrient Digestibility and Immune Response of Broiler Chicks Fed Diets Supplemented With A Culture of *Lactobacillus Bulgaricus*." *Journal of the Science of Food and Agriculture* 88: 1253–1258. doi:10.1002/(ISSN)1097-0010.
- AVMA (2007) AVMA Guidelines on Euthanasia (Formerly Report of the AVMA Panel on Euthanasia). Accessed October 2009.
- AWAD, W. A., J. BOHM, E. RAZZAZI-FAZELI, K. GHAREEB, AND J. ZENTEK. 2006. "Effect of Addition of A Probiotic Microorganism to Broiler Diets Contaminated With Deoxynivalenol on Performance and Histological Alterations of Intestinal Villi of Broiler Chickens." *Poultry Science* 85: 974–979. doi:10.1093/ps/85.6.974.
- BALEVI, T., U. UCAN, B. COŞUN, V. KURTOĞU, AND I. CETİNGÜL. 2001. "Effect of Dietary Probiotic on Performance and Humoral Immune Response in Layer Hens." *British Poultry Science* 42: 456–461. doi:10.1080/00071660120073133.
- BAUMONT, C., J. BRILLARD, N. MILLET, AND M. DE REVIERS. 1992. "Comparison of Various Characteristics of Duration of Fertility in Hens." *British Poultry Science* 33: 649–661. doi:10.1080/00071669208417503.
- BRILLARD, J., AND H. ANTOINE. 1990. "Storage of Sperm in the Uterovaginal Junction and Its Incidence on The Numbers of Spermatozoa Present in the Perivitelline Layer of Hens' Eggs." *British Poultry Science* 31: 635–644. doi:10.1080/00071669008417294.
- CHICHLOWSKI, M., J. CROOM, B. W. MCBRIDE, L. DANIEL, G. DAVIS, AND M. D. KOCL. 2007. "Direct-Fed Microbial Primalac and Salinomycin Modulate Whole-Body and Intestinal Oxygen Consumption and Intestinal Mucosal Cytokine Production in the Broiler Chick." *Poultry Science* 86: 1100–1106. doi:10.1093/ps/86.6.1100.
- FARNELL, M., A. DONOGHUE, F. S. DE LOS SANTOS, P. BLORE, B. HARGIS, G. TELLEZ, AND D. DONOGHUE. 2006. "Upregulation of Oxidative Burst and Degranulation in Chicken Heterophils Stimulated with Probiotic Bacteria." *Poultry Science* 85: 1900–1906. doi:10.1093/ps/85.11.1900.
- FORTE, C., G. ACUTI, E. MANUALI, P. C. PROIETTI, S. PAVONE, M. TRABALZA-MARINUCCI, L. MOSCATI, A. ONOFRI, C. LORENZETTI, AND M. FRANCOSINI. 2016. "Effects of Two Different Probiotics on Microflora, Morphology, and Morphometry of Gut in Organic Laying Hens." *Poultry Science* 95: 164. doi:10.3382/ps/pew164.
- GARDINER, G. E., P. G. CASEY, G. CASEY, P. B. LYNCH, P. G. LAWLOR, C. HILL, G. F. FITZGERALD, C. STANTON, AND R. P. ROSS. 2004. "Relative Ability of Orally Administered *Lactobacillus* Murinus to Predominate and Persist in the Porcine Gastrointestinal Tract." *Applied and Environmental Microbiology* 70: 1895–1906.
- GOODLING, A., G. CERNIGLIA, AND J. HEBERT. 1987. "Production Performance of White Leghorn Layers Fed *Lactobacillus* Fermentation Products." *Poultry Science* 66: 480–486. doi:10.3382/ps.0660480.
- GUCLU, B. K. 2011. "Effects of Probiotic and Prebiotic (Mannanoligosaccharide) Supplementation on Performance, Egg Quality and Hatchability in Quail Breeders." *Ankara Üniv Veterinary Fak Derg* 58: 27–32. doi:10.1501/Vetfak_0000002445.
- HIGGINS, S., J. HIGGINS, A. WOLFENDEN, S. HENDERSON, A. TORRES-RODRIGUEZ, G. TELLEZ, AND B. HARGIS. 2008. "Evaluation of a *Lactobacillus*-Based Probiotic Culture for the Reduction of *Salmonella* Enteritidis in Neonatal Broiler Chicks." *Poultry Science* 87: 27–31. doi:10.3382/ps.2007-00210.
- JIN, L., Y. HO, N. ABDULLAH, AND S. JALALUDIN. 1997. "Probiotics in Poultry: Modes of Action." *World's Poultry Science Journal* 53: 351–368. doi:10.1079/WPS19970028.
- KALBANDE, V., M. GAFFAR, AND S. DESHMUKH. 1992. "Effect of Probiotic and Nitrofurin on Performance of Growing Commercial Pullets." *Indian Journal of Poultry Science* 27: 116–117.
- KHAN, M., D. RAULT, H. RICHEL, H. LEPIDI, AND B. LA SCOLA. 2007. "Growth-Promoting Effects of Single-Dose Intragastrically Administered Probiotics in Chickens." *British Poultry Science* 48: 732–735. doi:10.1080/00071660701716222.
- KHAN, S. H., M. ATIF, N. MUKHTAR, A. REHMAN, AND G. FAREED. 2011. "Effects of Supplementation of Multi-Enzyme and Multi-Species Probiotic on Production Performance, Egg Quality, Cholesterol Level and Immune System in Laying Hens." *Journal of Applied Animal Research* 39: 386–398. doi:10.1080/09712119.2011.621538.
- KLOSE, V., M. MOHNL, R. PLAIL, G. SCHATZMAYR, AND A. P. LOIBNER. 2006. "Development of a Competitive Exclusion Product for Poultry Meeting the Regulatory Requirements for Registration in the European Union." *Molecular Nutrition & Food Research* 50: 563–571. doi:10.1002/mnfr.200500166.
- KOENEN, M., J. KRAMER, R. VAN DER HULST, L. HERES, S. JEURISSEN, AND W. BOERSMA. 2004. "Immunomodulation by Probiotic *Lactobacilli* in Layer- and Meat-Type Chickens." *British Poultry Science* 45: 355–366. doi:10.1080/00071660410001730851.
- LEI, Y., K.-Y. ZHANG, X.-M. DING, S.-P. BAI, AND J. S. CHOI. 2009. "Effect of Probiotics on Growth Performance, Development of Small Intestinal Tract and Microbial Populations in Broilers." *Journal of Agricultural Science and Technology* 3: 24–31.
- LI, L. L., Z. P. HOU, T. J. LI, G. Y. WU, R. L. HUANG, Z. R. TANG, C. B. YANG, J. GONG, H. YU, AND X. F. KONG. 2008. "Effects of Dietary Probiotic Supplementation on Ileal Digestibility of Nutrients and Growth Performance in 1-To 42-Day-Old Broilers." *Journal of the Science of Food and Agriculture* 88: 35–42. doi:10.1002/jsfa.2910.
- MAHDAVI, A., H. RAHMANI, AND J. POURREZA. 2005. "Effect of Probiotic Supplements on Egg Quality and Laying Hen's Performance." *International Journal Poultry Sciences* 4: 488–492. doi:10.3923/ijps.2005.488.492.
- MIKULSKI, D., J. JANKOWSKI, J. NACZMANSKI, M. MIKULSKA, AND V. DEMEY. 2012. "Effects of Dietary Probiotic (*Pediococcus Acidilactici*) Supplementation on Performance, Nutrient Digestibility, Egg Traits, Egg Yolk Cholesterol, and Fatty Acid Profile in Laying Hens." *Poultry Science* 91: 2691–2700. doi:10.3382/ps.2012-02370.
- MOHAN, B., R. KADIRVEL, M. BHASKARAN, AND A. NATARAJAN. 1995. "Effect of Probiotic Supplementation on Serum/Yolk Cholesterol and on Egg Shell Thickness in Layers." *British Poultry Science* 36: 799–803. doi:10.1080/00071669508417824.
- MOREHOUSE, N. F., AND R. R. BARON. 1970. "Coccidiosis: Evaluation of Coccidiostats by Mortality, Weight Gains, and Faecal Scores." *Experimental Parasitology* 28: 25–29.
- MOUNTZOURIS, K., P. TSIRTOSIKOS, E. KALAMARA, S. NITSCH, G. SCHATZMAYR, AND K. FEGEROS. 2007. "Evaluation of the Efficacy of a Probiotic Containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* Strains in Promoting Broiler Performance and Modulating Cecal Microflora Composition and Metabolic Activities." *Poultry Science* 86: 309–317. doi:10.1093/ps/86.2.309.

- MOUNTZOURIS, K. C., P. TSITSIKOS, I. PALAMIDI, A. ARVANITI, M. MOHNL, G. SCHATZMAYR, AND K. FEGEROS. 2010. "Effects of Probiotic Inclusion Levels in Broiler Nutrition on Growth Performance, Nutrient Digestibility, Plasma Immunoglobulins, and Cecal Microflora Composition." *Poultry Science* 89: 58–67. doi:10.3382/ps.2009-00308.
- MUTUŞ, R., N. KOCABAĞLI, M. ALP, N. ACAR, M. EREN, AND Ş. GEZEN. 2006. "The Effect of Dietary Probiotic Supplementation on Tibial Bone Characteristics and Strength in Broilers." *Poultry Science* 85: 1621–1625. doi:10.1093/ps/85.9.1621.
- NAHASHON, S., H. NAKAUE, AND L. MIROSH. 1992. "Effect of Direct-Fed Microbials on Nutrient Retention and Production Parameters of Laying Pullets." *Poultry Science* 71: 111. doi:10.3382/ps.0710302.
- NAHASHON, S., H. NAKAUE, AND L. MIROSH. 1994. "Production Variables and Nutrient Retention in Single Comb White Leghorn Laying Pullets Fed Diets Supplemented with Direct-Fed Microbials." *Poultry Science* 73: 1699–1711. doi:10.3382/ps.0731699.
- NARUSHIN, V., AND M. N. ROMANOV. 2002. "Egg Physical Characteristics and Hatchability." *Worlds Poultry Science Journal-Wyton* 58: 297–304. doi:10.1079/WPS20020023.
- NATIONAL RESEARCH COUNCIL. 1994. *Nutrient Requirements of Poultry*. 9th rev. ed. Washington, DC, USA: National Academy Press.
- NURMI, E., AND M. RANTALA. 1973. "New Aspects of Salmonella Infection in Broiler Production." *Nature* 241: 210–211.
- OLNOOD, C. G., S. S. BESKI, M. CHOCT, AND P. A. IJI. 2015. "Novel Probiotics: Their Effects on Growth Performance, Gut Development, Microbial Community and Activity of Broiler Chickens." *Animal Nutrition* 1: 184–191. doi:10.1016/j.aninu.2015.07.003.
- OYARZABAL, O. A., AND D. E. CONNER. 1995. "In Vitro Fructooligosaccharide Utilization and Inhibition of Salmonella Spp. by Selected Bacteria." *Poultry Science* 74: 1418–1425. doi:10.3382/ps.0741418.
- PANDA, A. K., S. S. RAMA RAO, M. V. RAJU, AND S. S. SHARMA. 2008. "Effect of Probiotic (Lactobacillus Sporogenes) Feeding on Egg Production and Quality, Yolk Cholesterol and Humoral Immune Response of White Leghorn Layer Breeders." *Journal of the Science of Food and Agriculture* 88: 43–47. doi:10.1002/(ISSN)1097-0010.
- PARK, J., J. JEONG, S. LEE, AND I. KIM. 2016. "Effect of Dietary Supplementation with a Probiotic (Enterococcus Faecium) on Production Performance, Excreta Microflora, Ammonia Emission, and Nutrient Utilization in Isa Brown Laying Hens." *Poultry Science* 95: 2829–2835. doi:10.3382/ps/pew241.
- PATTERSON, J., AND K. BURKHOLDER. 2003. "Application of Prebiotics and Probiotics in Poultry Production." *Poultry Science* 82: 627–631. doi:10.1093/ps/82.4.627.
- PEEBLES, E., C. ZUMWALT, S. DOYLE, P. D. GERARD, M. LATOUR, C. BOYLE, AND T. SMITH. 2000. "Effects of Breeder Age and Dietary Fat Source and Level on Broiler Hatching Egg Characteristics." *Poultry Science* 79: 698–704. doi:10.1093/ps/79.5.698.
- RAMASAMY, K., N. ABDULLAH, S. JALALUDIN, M. WONG, AND Y. W. HO. 2009. "Effects of Lactobacillus Cultures on Performance of Laying Hens, and Total Cholesterol, Lipid and Fatty Acid Composition of Egg Yolk." *Journal of the Science of Food and Agriculture* 89: 482–486. doi:10.1002/jsfa.v89.3.
- RITZI, M. M., W. ABDELRAHMAN, M. MOHNL, AND R. A. DALLOUL. 2014. "Effects of Probiotics and Application Methods on Performance and Response of Broiler Chickens to an Eimeria Challenge." *Poultry Science* 93, no. 11 (2014): 2772–2778.
- SAS, I. 2001. *SAS/WATTM User's Guide*. Cary NC: SAS Institute.
- SHAMOTO, K., AND K. YAMAUCHI. 2000. "Recovery Responses of Chick Intestinal Villus Morphology to Different Refeeding Procedures." *Poultry Science* 79: 718–723. doi:10.1093/ps/79.5.718.
- SOBCZAK, A., AND K. KOZŁOWSKI. 2015. "The Effect of a Probiotic Preparation Containing Bacillus Subtilis Atcc Pta-6737 on Egg Production and Physiological Parameters of Laying Hens." *Annals of Animal Science* 15: 711–723. doi:10.1515/aoas-2015-0040.
- SULTAN, K. H., AND S. Y. ABDUL-RAHMAN. 2011. "Effect of Probiotic on Some Physiological Parameters in Broiler Breeders." *International Journal of Poultry Science* 10: 626–628. doi:10.3923/ijps.2011.626.628.
- TANG, S. G. H., C. C. SIEO, R. KALAVATHY, W. Z. SAAD, S. T. YONG, H. K. WONG, AND Y. W. HO. 2015. "Chemical Compositions of Egg Yolks and Egg Quality of Laying Hens Fed Prebiotic, Probiotic, and Synbiotic Diets." *Journal of Food Science* 80: C1686–C1695. doi:10.1111/1750-3841.12947.
- TIMMERMAN, H., C. KONING, L. MULDER, F. ROMBOUTS, AND A. BEYEN. 2004. "Monostrain, Multistrain and Multispecies Probiotics—A Comparison of Functionality and Efficacy." *International Journal of Food Microbiology* 96: 219–233. doi:10.1016/j.ijfoodmicro.2004.05.012.
- TOMA, M. M., AND J. POKROTNIKS. 2006. "Probiotics as Functional Food: Microbiological and Medical Aspects." *Acta Universitatis Latviensis* 710: 117–129.
- TUOHY, K. M., C. J. ZIEMER, A. KLINDER, Y. KNÖBEL, B. L. POOL-ZOBEL, AND G. R. GIBSON. 2002. "A Human Volunteer Study to Determine the Prebiotic Effects of Lactulose Powder on Human Colonic Microbiota." *Microbial Ecology in Health and Disease* 14: 165–173. doi:10.1080/089106002320644357.
- WANG, Y., AND Q. GU. 2010. "Effect of Probiotic on Growth Performance and Digestive Enzyme Activity of Arbor Acres Broilers." *Research in Veterinary Science* 89: 163–167. doi:10.1016/j.rvsc.2010.03.009.
- WEBSTER, A., AND J. HURNTK. 1990. "Behavior, Production, and Well-Being of the Laying Hen. 1. Effects of Movable Roosts, Relationship of Cage Mates, and Genetic Stock." *Poultry Science* 69: 2118–2127. doi:10.3382/ps.0692118.
- WILLIAMS, N. T. 2010. "Probiotics." *American Journal of Health-System Pharmacy* 67(6): 449–458.
- WISHART, G. 1985. "Quantitation of the Fertilising Ability of Fresh Compared with Frozen and Thawed Fowl Spermatozoa." *British Poultry Science* 26: 375–380. doi:10.1080/00071668508416825.
- ZAGHARI, M., M. DERAKHSHANI DIBA, H. MORAVEJ, AND N. ZAHROOJIAN. 2017. "Estimation of Metabolizable Energy Equivalency of Bacillus Subtilis Spore for Male Broiler Chickens." *Journal of Livestock Science and Technologies* 5: 9–18.
- ZAGHARI, M., N. ZAHROOJIAN, M. RIAHI, AND S. PARHIZKAR. 2015. "Effect of Bacillus subtilis spore (GalliPro®) Nutrients Equivalency Value on Broiler Chicken Performance." *Italian Journal of Animal Science* 14: 3555. doi:10.4081/ijas.2015.3555.
- ZHANG, Z., AND I. KIM. 2014. "Effects of Multistrain Probiotics on Growth Performance, Apparent Ileal Nutrient Digestibility, Blood Characteristics, Cecal Microbial Shedding, and Excreta Odor Contents in Broilers." *Poultry Science* 93: 364–370. doi:10.3382/ps.2013-03314.
- ZHOU, X., Y. WANG, Q. GU, AND W. LI. 2010. "Effect of Dietary Probiotic, Bacillus Coagulans, on Growth Performance, Chemical Composition, and Meat Quality of Guangxi Yellow chicken." *Poultry Science* 89: 588–593. doi:10.3382/ps.2009-00319.