EFFECT OF SYNBIOTIC ON INTESTINAL HISTOMORPHOMETRY AND GROWTH RATE IN QUAILS, EXPERIMENTALLY INFECTED WITH FIELD STRAIN OF *SALMONELLA GALLINARUM*

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ABSTRACT: This study was planned to investigate the effect of synbiotic on intestinal histomorphometry and the growth rate of quails experimentally infected with a field strain of Salmonella gallinarum. Day old Japanese quails (120), with an average body weight of 6.09±1.1 g, were randomly assigned into 4 groups A, B, C, and D. Quails of groups A and B were given synbiotic on a daily and weekly basis respectively, along with challenge while group C was negative control and group D was the positive control group. A total of 60 organ samples of the intestine were collected from apparently healthy and freshly dead quails respectively to isolate the field strain of Salmonella gallinarum. Bacterial isolation and molecular identification were performed in accordance with laboratory diagnostic culture techniques and PCR. Results from the current study indicate that nonantibiotic feed additives such as synbiotics boosted the gut histomorphometric parameters including the villus height, villus width, and crypt depth under the challenge of Salmonella gallinarum. There was a significant increase in all these parameters due to synbiotic feeding except for the negative control group which showed the lowest values. On day 21, DD achieved a maximum villus height of 636.88 ± 65.93b µm in the duodenal mucosa, whereas, maximum villus width of 182.01 ± 15.40c µm in duodenal mucosa was achieved by group AA. Maximum villus height of 276.89 ± 21.16b µm and crypt depth of 26.66 ± 1.15a µm in jejunum mucosa were recorded in group AA. Statistical results by using the technique of one-way ANOVAs indicated that there was a significant increase in morphmetric parameters of duodenal and jejunum mucosa in the groups fed synbiotic as compared to the negative control. Hence results illustrate that there is an overall increase in histological parameters of duodenal and jejunum mucosa in the groups fed synbiotic as compared with the negative control group. This study supports the beneficial effects of synbiotic on intestinal health and the growth rate of Japanese quails by improving FCR.

Keywords: Japanese quails; synbiotic; salmonella gallinarum; histomorphometry.

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INTRODUCTION

The poultry industry is playing a pivotal role to fulfill the protein needs of consumers throughout the world however with the increase in growth of this sector, the poultry industry is facing many challenges including various infectious diseases. For the last few decades, salmonellosis is considered as the biggest challenge for the poultry industry, as it is reported to cause huge mortality and production losses. Moreover, consumption of salmonella-contaminated poultry meat/products poses a great risk to public health. Conventionally, antibiotics were being used in the feed industry to control/treat various infectious diseases and to enhance the bird's performance. However, continues use of antibiotics has concerns regarding its residual effects on the carcass of birds and the development of antibiotic resistance in bacteria (Sørum and Sunde 2001). Therefore, it is a dire need to explore alternative non-antibiotic organic products to control the diseases. Thus, non-antibiotic feed additives such as synbiotic are being considered a good option to replace the antibiotics in poultry feed to achieve good results. Subsequently, till today a list of products, comprising probiotics, prebiotics, and synbiotics have been acknowledged and suggested equally for antibiotic substitutions in farms, and smallholding animal nourishment. Such non-antibiotic feedstuff, herbs, and additives are being deliberated to bridge the slit and formerly some farmers utilizing these products instead of antibiotics for the poultry (Haque et al. 2017; Kabir 2009; Trafalska and Grzybowska 2004).

Recently, quail breeding is becoming popular

and is considered an alternative to the meat-producing industries for many reasons. Protein value of quail's meat is higher as compared to the chicken's meat. A Probiotic is defined as culture of microorganisms that, when offered in specific amount to living micro-organisms, provide health benefits by improving gut morphology (Kabir 2009). Prebiotics are basically an indigestible feed constituent which bring significant effect on refining the gut health of host by nourishing and enhancing the growth of established gut micro flora, selectively motivating the growth activities of bacteria residing in the gut of the organism (Gibson and Roberfroid 1995). A way of enhancing the efficiency of non-antibiotic feed additives preparation may be the combination of both as a mixture of prebiotic and probiotic in the form of synbiotic. Therefore, synbiotic is used as an alternative supplement to antibiotic for the health of birds to improve the production performance (Erdoğan et al. 2010; Sahin et al. 2008; Skvortsova 2010). In Japanese quails, synbiotic seemed to be more effective to improve their performance. Birds fed with synbiotic has shown higher FCR and growth rate as compared to other alternative growth promoters (Babazadeh et al. 2011).

The harmful effects of synthetic feed additives and antibiotic have caused the general interest of consumers to shift towards organic meat and market preference. Thus, the current study aimed to evaluate the beneficial effects of synbiotic on quail's growth and performance under Salmonella gallinarum challenged condition.

Objectives: To determine the effect of synbiotic on growth rate and FCR of quail, experimentally infected with Salmonella gallinarum.

The main objective of this study is to determine gut health, growth performance and immune status of Salmonella gallinarum infected birds fed on synbiotic.
To understand how synbiotic affect Salmonella gallinarum intestinal colonization and intestinal integrity in birds.

MATERIALS AND METHODS

Experimental station & housing conditions: A total of 120 quails (Coturnix coturnix japonica) were procured from the avian research and training center and were raised in an experimental shed at the avian research and training center, Lahore. These quails were completely randomized into four groups (30 quails in each) namely groups AA, BB, CC, and DD.

Four pens of size (2 × 1 m2) each were taken for the rearing of quails. All the pens were first thoroughly cleaned and then rice husk was added in it as litter. These pens were placed in an experimental room at Avian Research and Training Center and strict biosecurity measures were maintained. Room temperature was set at 96°F on day one and then was decreased gradually@ 5°Fper week until it reached 75°F for the rest of the experiment. Birds were provided a starter ration having 3100 kcal/kg (Metabolizable Energy) and 23% crude protein in mash form.

Treatments: The probiotics used contained a strain of Bacillus subtitles, 1 x ⟦10⟧^11 CFU/lb. and was mixed in the feed @1 lb. /ton. Prebiotics a unique Beta-(1, 3)-Glucan products derived from algae, were added @ 1g/kg in the feed. To prepare Synbiotic, probiotic and prebiotic were mixed together thoroughly in a prescribed ratio of 2:1.Group AA, BB, and CC were challenged with Salmonella gallinarum (SG) at 1 ×⟦10⟧^7CFU/0.25 ml per bird through an oral gavages at the start of the experiment. **The** Challenge organism (Salmonella gallinarum) used in this experiment was isolated from infected quails. SG was cultured on Salmonella Shigella (SS) agar to get fresh colonies of SG. These colonies were processed in selenite broth to prepare 1 x 107 CFU approx. using Miles and Misra technique (SLACK and Wheldon 1978) and Serial dilution of SG was checked by back titration. Quails were infected on day 1 via the oral route.

Experiment treatment groups: Procured quails were randomly divided into 4 groups AA, BB, CC, and DD respectively and each group had 30 quails. The treatments were as follows: Group AA was given synbiotic from day one along with a challenge + basal diet. Group BB was given synbiotic on weekly basis along with a challenge + basal diet. Group CC was the negative control group (challenge + basal diet) and Group DD was the positive control group (just basal diet with synbiotic). Salmonella was identified through different molecular tests at the start of the experiment and afterward at different stages experimental study whereas intestinal morphometric parameters (Villus length, villus width, villus surface area, and crypt depth) were measured by micrometry. The collected data from both experiments were analyzed using the statistical technique of comparing more than two groups i.e. Analysis of variance (ANOVA) through SPSS 14.0.

Identification and evaluation of salmonella by Polymerase Chain Reaction: DNA extraction was performed by boiling method. A pure single bacterial colony was taken from the culture plate and mixed with 100µl of distilled water. The mixture was boiled for 10 minutes and then immediately placed on ice for cold stock then performed centrifugation for 10 mints at 10000 rpm. The supernatant was collected. This supernatant contained DNA used for PCR.

A total of 25µl mixture was prepared. PCR mixture was prepared by using Master Mix (12.5µl), nucleus-free water (8.5µl), forward and reverse primer (1µl each), and extracted DNA template (2µl)

Gene invA was amplified as follows by 20 cycles of denaturation. Initial denaturation was at (94c, 1 minute), denaturation (94c, 1 minute), annealing (62c, 30 second), elongation (72c, 30 second) and final extension (72c, 7 minutes). The final holding temperature was 4c. A 1.5% TAE agarose gel was used for gel electrophoresis of the invA gene. invA is a specific primer used in this study to detect Salmonella gallinarum (Pal et al. 2017).

Experiment 2: Production performance & intestinal morphometric analysis: This experiment consists of determining the effects of synbiotic on salmonella in the intestine and checking the growth performance, FCR, and Morphometric evaluation of the intestine.

Feed intake of each group was recorded on a daily basis and the weight gain of birds of each group was recorded on weekly basis. The mortality record of each group was maintained. On the basis of feed consumption data and weight gain records, the weekly FCR of each group was calculated (Petek and Dikmen 2004).

For the intestine morphometric analysis, four birds from each group were taken randomly at 72 hours of age, 14th day, and 21 days of age and slaughtered humanely. An approximately one-centimeter section of the distal ending from the lower jejunum as well as from the center of the duodenal loop from each slaughtered bird was aseptically collected after carefully washing the tissue with normal saline for the removal of any intestinal content residues. The steps comprise the fixation of the tissue, dehydration of the sample, clearing, sectioning post embedding, and lastly the careful staining of the sectioned tissue (Athanassopoulou et al. 1999). The stained slides were then examined under 4X and 10X magnification and pictures were taken and analyzed by PixelPro software. Villus length was measured from the top of the villus to the top of the lamina propria. Crypt depth was measured from the base upward to the region of transition between the crypt and villus. For this experiment, results are given as the average value calculated from 5 sections each of duodenum and jejunum of 2 birds per group at designated evaluation days. All values are expressed as Mean ± Standard deviation. Villus width was measured at the widest area of each villus.

RESULTS AND DISCUSSION

Microscopic examination of grams staining revealed the pinkish-colored, gram-negative, short rod-shaped bacteria which were arranged in single and paired form (see figure 1). Grayish black, round, and smooth colonies on the Salmonella shigella agar plate showed the presence of Salmonella which is further confirmed by culturing pure colonies of salmonella taken from the SS agar plate and growing on the XLD agar plate (see figure 3). On Xylose Lysine Deoxycholate agar media salmonella produced colonies of pinkish color from the periphery and dark black from the center (see figure 2). Salmonella gallinarum was identified by using the molecular technique of polymerase chain reaction. invA gene of 284 bp was used in this technique having sequence. F: 5' - GTG AAA TTA TCG CCA CGT TCG GGC AA - 3'. R: 5' – TCA TCG CAC CGT CAA AGG AAC C - 3'. Result of PCR on gel electrophoresis (see figure 4).

The result of morphometric analysis of the intestine was evaluated by calculating the average value of five sections of duodenum and jejunum of two birds/groups on their designated days of evaluation. On 3rd day of the study, there was a significant difference in the mean of all the parameters of morphometric analysis of duodenal mucosa between all six groups. Group DD showed the highest value of villus height of 636.88 ± 65.93b µm in duodenal mucosa. Whereas, maximum villus width of 182.01 ±15.40c µm in duodenal mucosa was achieved by group AA. Maximum villus height of 276.89±21.16b µm and crypt depth of 26.66±1.15a µm in jejunum mucosa was recorded in group AA.

Results show that there is an overall increase in histological parameters of the mucosa of duodenum and ileum in the groups fed non-antibiotic feed additives as compared with control positive and negative. Synbiotic showed the maximum positive effects (see table 1, 2). Hence this study suggests that a combination of non-antibiotic feed additives will be beneficial for the intestinal health of broiler quails but there is a need for more research on combinations of non-antibiotic feed addition.

FCR of each group was calculated on weekly basis from day 1st to the 22nd day. FCR was calculated by dividing feed consumed over weight gain on the 7th, 15th, and 22nd day respectively. There was no significant difference between the groups of avg. weight gain values except for group CC which is not treated with synbiotic showed a significant decrease in weight gain. While in FCR measurements, group DD showed the highest FCR followed by group AA. Group BB showed an avg. FCR value and group CC have the lowest FCR. Weight gain was measured in grams (see tables 3, and 4).

In the present study, treatment with probiotics improved the intestinal morphology (height of duodenal and jejunum villus) and increased the population of Bifido bacterium and Lactobacillus in small intestinal in the presence of Salmonella enterica serovar gallinarum challenged strain which ultimately results into more feed intake and weight gain in the treatment group (Madden and Hunter 2002). (Song et al. 2014). Clostate (probiotic) has been reported to maintain the balance of microflora in the intestinal tract and inhibit pathogens (Teo and Tan 2005). Grimes et al. (2008) examined the beneficial effects of probiotics on the production

performance and development of muscle mass.

Aleta (prebiotic) used in this study is reported to improve the general health of the birds by improving vaccination efficiency, helping animals to resists stressful conditions, reducing morbidity and mortality during pathogenic challenges (diseases), and helping to avoid performance reduction in situations of disease and stressful condition (Volman et al. 2008). Gallaher and Khil (1999) investigated that Probiotic in combination with prebiotics (synbiotic) has shown a significant effect on the growth rate, feed conversation ratio, and gut health by improving intestinal histo-morphology in poultry. The synbiotic has been shown more beneficial effects than when used separately (Awad et al. 2009; Awad et al. 2008; Jung et al. 2008). The combination of other commercial preparation of synbiotic such as FloraMax-B11 and Early bird has also been tested to encourage the gut health morphology as well as the muscle mass of birds in comparison with control by each product separately (Biloni et al. 2013; Sultan et al. 2015)

In the present study morphometric analysis showed the significant effect of synbiotic on duodenal and jejunum mucosa of positive control group DD on days 14 and 21 respectively followed by group AA. Group CC showed the highest rate of mortality as well as the lowest gut morphology including villus height, villus width, and crypt depth. In the present study, the growth rate in the groups fed with synbiotic on a daily and weekly basis was higher than the birds of the positive and negative control groups. There is a significant difference between the groups fed on synbiotic and the negative control group. The beneficial effects of synbiotic on the performance parameters of Japanese quails including FCR and body weight are in the agreement with previous research studies (Kabir et al. 2004; Mountzouris et al. 2007; Samli et al. 2007). Groups fed synbiotic supplemented diet showed better FCR compared with the negative control group. Moreover, birds fed synbiotics on a daily basis and the positive control group showed significant improvement in FCR as compared to birds fed on a weekly basis and the negative control. Thus, the results indicated that consumption of synbiotic was more effective than negative groups in FCR and growth rate of Japanese quails.

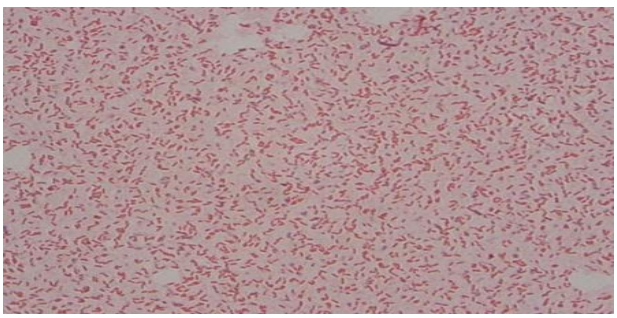

Figure 1 Salmonella at 10x.

Figure 2 Salmonella colonies on XLD agar plate.

Figure 3 Salmonella colonies on XLD agar plate.

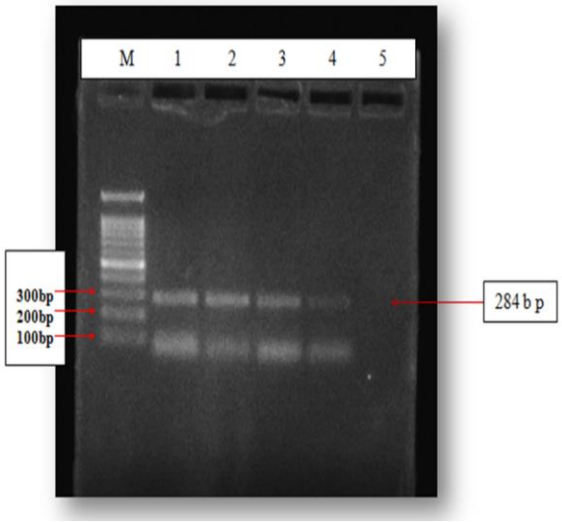

Figure 4 PCR amplification of gene invA of salmonella gallinarum isolate. Lane M: 100 bp Ladder, Lane 1, 2, 3, and 4: Positive sample for invA gene, Lane 5: Negative control.

Table 1 Morphological analysis of duodenal mucosa (Mean ± SD).

3 day			
Treatment	Villus height (µm)	Crypt width (µm)	Villus depth (µm)
1. Group AA	425.52 ± 29.08[a]	77.20 ± 4.8[a]	20.99 ± 1.87[ab]
2. Group BB	432.70 ± 25.96[a]	72.81 ± 7.45[a]	20.75 ± 2.61[ab]
3. Group CC (control negative)	393.39 ± 38.63[a]	70.10 ± 7.44[a]	17.79 ± 3.36[a]
4. Group DD (control positive)	401.11 ± 41.29[a]	80.09 ± 10.01[a]	23.29 ± 2.67[b]
14 day			
1. Group AA	524.88 ± 25.37[b]	112.49 ± 11.40[b]	58.43 ± 6.82[c]
2. Group BB	524.87 ± 25.36[b]	110.60 ± 13.08[b]	54.76 ± 6.22[c]
3. Group CC (control negative)	398.04 ± 55.54[a]	70.37 ± 6.58[a]	40.47 ± 4.44[b]
4. Group DD (control positive)	532.57 ± 48.78[b]	138.29 ± 28.13[c]	24.54 ± 1.25[a]
21 day			
1. Group AA	632.98 ± 61.07[b]	182.01 ± 15.40[c]	70.19 ± 17.47[c]
2. Group BB	593.78 ± 42.94[b]	135.92 ± 28.71[b]	63.78 ± 7.53[bc]
3. Group CC (control negative)	405.40 ± 36.43[a]	75.94 ± 12.57[a]	43.05 ± 7.22[a]
4. Group DD (control positive)	636.88 ± 65.93[b]	167.04 ± 13.56[c]	54.75 ± 5.18[ab]

[a–c] Values within columns with no common superscript differ significantly at $P < 0.05$

Table 2 Morphmetric analysis of jejunum mucosa (Mean ± SD).

3 day			
Treatment	Villus height (µm)	Crypt width (µm)	Villus depth (µm)
1. Group AA	203.61 ± 24.59[a]	52.42 ± 4.07[a]	17.30 ± 3.41[a]
2. Group BB	206.34 ± 23.12[a]	53.06 ± 9.71[a]	19.66 ± 2.23[a]
3. Group CC (control negative)	173.80 ± 31.54[a]	46.53 ± 3.95[a]	17.28 ± 2.84[a]
4. Group DD (control positive)	264.91 ± 44.26[b]	49.53 ± 9.26[a]	20.01 ± 3.73[b]
14 day			
1. Group AA	214.97 ± 15.72[a]	71.15 ± 7.05[a]	23.60 ± 1.76[a]
2. Group BB	218.60 ± 13.98[a]	72.45 ± 14.63[a]	23.05 ± 1.85[a]
3. Group CC (control negative)	213.24 ± 14.65[a]	67.68 ± 10.49[a]	20.79 ± 4.01[a]
4. Group DD (control positive)	258.86 ± 30.02[b]	74.51 ± 15.09[a]	22.45 ± 3.84[a]
21 day			
1. Group AA	276.89 ± 21.16[b]	87.19 ± 6.57[b]	26.66 ± 1.15[a]
2. Group BB	248.88 ± 24.15[ab]	84.07 ± 11.42[ab]	25.27 ± 3.57[a]
3. Group CC (control negative)	237.21 ± 27.28[a]	71.86 ± 11.40[a]	23.35 ± 3.29[a]
4. Group DD (control positive)	270.03 ± 34.13[ab]	84.68 ± 11.92[ab]	26.55 ± 4.97[a]

[a–b] Values within columns with no common superscript differ significantly at $P < 0.05$

Table 3 Feed Conversion Ratio (Mean ± SD).

Groups	**1st Week**	**2nd Week**	**3rd week**
1. Group AA	1.29 ± 0.28	1.31 ± 0.45	1.301 ± 0.37
2. Group BB	1.36 ± 0.66	1.42 ± 0.81	1.403 ± 0.61
3. Group CC (control negative)	1.64 ± 0.93	1.85 ± 0.84	2.008 ± 1.08
4. Group DD (control positive)	1.18 ± 0.29	1.17 ± 0.11	1.122 ± 0.06

Table 4 Weight Gain (Mean).

Groups	Weight gain 1st Week (gm)	Weight gain2nd Week (gm)	Weight gain 3rd week (gm)
1. Group AA	14.25	35.42	31.58
2. Group BB	18.26	28.74	32.78
3. Group CC (control negative)	16.86	24.97	30.28
4. Group DD (control positive)	15.33	31.42	32.95

Conclusions: Results of the present study showed that the use of synbiotic in the basal diet of quails has a significant effect on gut health and production performance of Japanese quails. Therefore, synbiotics should be used in place of antibiotics to minimize the mortality rate and improve the FCR of salmonella-challenged quails, and overcome the issue of a ban on antibiotics and antibiotic resistance.

REFERENCES

Athanassopoulou F, Prapas T, Rodger H (1999) Diseases of Puntazzo puntazzo Cuvier inmarine aquaculture systems in Greece. Journal of Fish Diseases. 22 (3): 215-218.

Awad W, Ghareeb K, Abdel-Raheem S, Böhm J (2009) Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poultry Science. 88 (1): 49-56.

Awad W, Ghareeb K, Böhm J (2008) Intestinal structure and function of broiler chickens on diets supplemented with a synbiotic containing Enterococcus faecium and oligosaccharides. International Journal of Molecular Sciences. 9 (11): 2205-2216.

Ayasan T, Ozcan BD, Baylan M, Canogullari S (2006) The Effects of dietary inclusion of probiotic protexin on egg yield parameters of japanese quails (Coturnix coturnix Japonica). Int. J. Poult. Sci. 5 (8): 776-779.

Babazadeh D, Vahdatpour T, Nikpiran H, Jafargholipour M, Vahdatpour S (2011) Effects of probiotic, prebiotic and synbiotic intake on blood enzymes and performance of Japanese quails (Coturnix japonica). Indian Journal of Animal Sciences. 81 (8): 870.

Bengmark S (2001) Pre-, pro-and synbiotics. Current Opinion in Clinical Nutrition & Metabolic Care. 4 (6): 571-579.

Biloni A, Quintana C, Menconi A, Kallapura G, Latorre J, Pixley C, Layton S, Dalmagro M, Hernandez-Velasco X, Wolfenden A (2013) Evaluation of effects of EarlyBird associated with FloraMax-B11 on Salmonella Enteritidis, intestinal morphology, and performance of broiler chickens. Poultry Science. 92 (9): 2337-2346.

Erdoğan Z, Erdoğan S, Aslantaş Ö, Celik S (2010) Effects of dietary supplementation of synbiotics and phytobiotics on performance, caecal coliform population and some oxidant/antioxidant parameters of broilers. Journal of animal physiology and animal nutrition. 94 (5): e40-e48.

Fooks LJ, Fuller R, Gibson GR (1999) Prebiotics, probiotics and human gut microbiology. International dairy journal. 9 (1): 53-61.

Gallaher DD, Khil J (1999) The effect of synbiotics on colon carcinogenesis in rats. The Journal of nutrition. 129 (7): 1483S-1487S.

Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. The Journal of nutrition. 125 (6): 1401-1412.

Grimes J, Rahimi S, Oviedo E, Sheldon B, Santos F (2008) Effects of a direct-fed microbial (Primalac) on turkey poult performance and susceptibility to oral Salmonella challenge. Poultry Science. 87 (7): 1464-1470.

Haque MI, Ahmad N, Miah MA (2017) Comparative analysis of body weight and serum biochemistry in broilers supplemented with some selected probiotics and antibiotic growth promoters. Journal of Advanced Veterinary and Animal Research. 4 (3): 288-294.

Jung S, Houde R, Baurhoo B, Zhao X, Lee B (2008) Effects of galacto-oligosaccharides and a Bifidobacteria lactis-based probiotic strain on the growth performance and fecal microflora of broiler chickens. Poultry Science. 87 (9): 1694-1699.

Kabir S (2009) The role of probiotics in the poultry industry. International Journal of Molecular Sciences. 10 (8): 3531-3546.

Kabir S, Rahman MM, Rahman M, Rahman M, Ahmed S (2004) The dynamics of probiotics on growth performance and immune response in broilers. Int. J. Poult. Sci. 3 (5): 361-364.

Madden J, Hunter J (2002) A review of the role of the gut microflora in irritable bowel syndrome and the effects of probiotics. British Journal of Nutrition. 88 (S1): s67-s72.

Mountzouris K, Tsirtsikos P, Kalamara E, Nitsch S, Schatzmayr G, Fegeros K (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 (2): 309-317.

Pal S, Dey S, Batabyal K, Banerjee A, Joardar SN, Samanta I, Isore DP (2017) Characterization of Salmonella Gallinarum isolates from backyard poultry by polymerase chain reaction detection of invasion (invA) and Salmonella plasmid virulence (spvC) genes. Veterinary world. 10 (7): 814.

Petek M, Dikmen S (2004) The effects of prestorage incubation of quail breeder eggs on hatchability and subsequent growth performance of progeny. Animal Research. 53 (6): 527-534.

Roberfroid MB (2000) Prebiotics and probiotics: are they functional foods?–. The American journal of clinical nutrition. 71 (6): 1682S-1687S.

Sahin T, Kaya I, Unal Y, Elmali DA (2008) Dietary supplementation of probiotic and prebiotic combination (Combiotics) on performance, carcass quality and blood parameters in growing quails. Journal of Animal and Veterinary Advances. 7 (11): 1370-1373.

Samli HE, Senkoylu N, Koc F, Kanter M, Agma A (2007) Effects of Enterococcus faecium and dried whey on broiler performance, gut histomorphology and intestinal microbiota. Archives of animal nutrition. 61 (1): 42-49.

Sanders ME (2003) Probiotics: considerations for human health. Nutrition reviews. 61 (3): 91-99.

Singh H, Nordskog A (1982) Significance of body weight as a performance parameter. Poultry Science. 61 (10): 1933-1938.

Skvortsova L (2010) Use of prebiotics in rearing broiler chicks. Russian Agricultural Sciences. 36 (3): 194-197.

SLACK MP, Wheldon D (1978) A simple and safe volumetric alternative to the method of Miles, Misra and Irwin for counting viable bacteria. Journal of medical microbiology. 11(4): 541-545.

Song J, Xiao K, Ke Y, Jiao L, Hu C, Diao Q, Shi B, Zou X (2014) Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. Poultry Science. 93 (3): 581-588.

Sørum H, Sunde M (2001) Resistance to antibiotics in the normal flora of animals. Veterinary research. 32 (3-4): 227-241.

Trafalska E, Grzybowska K (2004) Probiotics--an alternative for antibiotics? Wiadomosci lekarskie (Warsaw, Poland: 1960). 57 (9-10): 491-498.

Vali N (2009) Probiotic in quail nutrition: a review. Internaabout of Poultry Science. 8 (12): 1218-1222.

Volman JJ, Ramakers JD, Plat J (2008) Dietary modulation of immune function by β-glucans. Physiology & behavior. 94 (2): 276-284.