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Supplementation of a Blend of Beneficial Bacteria and Antibodies on Growth Performance, Intestinal Mucosa Morphology and Right Heart Failure of Japanese Quail (*Coturnix coturnix japonica*)

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Abstract

Background: Early nutrition of chicks with beneficial bacteria might help in occupying the inner surface of the intestinal tract. Interference of pathogens in intestinal microbiota is well known as barrier effect, bacterial interference, and competitive exclusion.

Objectives: It was hypothesized that competitive exclusion in Japanese quails with a blend of beneficial bacteria (Aquablend Avian® probiotic) would enhance quails' growth performance and intestinal mucosal morphology. Furthermore, the study was performed at 2100 m above sea level at Shahrekord University, so another hypothesis was the capability of the probiotic for inhibiting right heart failure.

Materials and Methods: One hundred fifty-six Japanese quails were divided into 4 groups: 2 groups (Aquablend and control) at standard environmental temperature and 2 (Aqua-stress and Cont-stress) at cold-hypoxic environmental situation. Aquablend groups received the probiotic in the first 3 days of life in drinking water (0.5 g/100 birds/day).

Results: Feed conversion ratio (FCR) was significantly reduced at the end of the experiment (day 35) in both Aquablend and aqua-stress groups compared to control and cont-stress groups, respectively ($P<0.05$). Carcass characteristics' improvements in treated quails were not significant. However, slight improvements in carcass and spleen relative weights were observed ($P>0.05$). Cont-stress group had higher RV: TV ratio (0.28) and heterophil: lymphocyte (H: L) ratio (1.22) than aqua-stress group: (0.25) and (1.20), respectively ($P>0.05$). Data regarding to intestinal mucosa morphology was controversial but the probiotic was able to elevate duodenum villi surface ($P<0.05$) and also jejunum and ileum lamina propria thickness.

Conclusion: Obtained data suggests that addition of Aquablend Avian® probiotic in the first 3 days of life may improve growth performance and some intestinal mucosa characteristics of Japanese quails. Moreover, the probiotic might reduce right heart failure and stress induced by cold-hypoxic situation.

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Background

Japanese quail (*Coturnix coturnix japonica*) has been considered as an important laboratory animal and also an economically important egg and meat-type poultry worldwide.[1] However, stressful conditions cause great economic losses when large-scale rearing facilities have been utilized for the quails.[2]

Stressful situation often depends on the region of rearing; for instance, cold-hypoxic situation is one of such stressful situations which occur in some mountainous areas like Chaharmahal va Bakhtiari, which is the highest province of Iran. It could be stated that environmental air changes, like cold-stress is one of the most significant adverse factors that might significantly influence metabolism and subsequently the production of broiler chickens.[3]

In biotic conditions to control the infectious and in particular metabolic diseases and to improve the performance, antibiotics have been used widely by producers[4] which have their adverse effects in meat quality and safety. On the other hand, cold-hypoxic environment could hasten the prevalence of right heart failure and ascites syndromes particularly in boilers and to lesser degree in quails, resulting in reduced growth performance and high mortality in birds.[5]

Early nutrition of chicks with beneficial bacteria might help the intestinal tract in occupying the inner surface of gut. Interference of pathogens in the intestinal microbiota is well known as barrier effect, bacterial interference, and competitive exclusion.[6]

Mechanisms that are involved in the inhibition of pathogens consist of competing for nutrients, production of toxic compounds and conditions (bacteriocins, low pH, and volatile fatty acids), competing for attaching to the sites of gut epithelium, and stimulating the immune system.[7-9] Inoculating 1-d old broilers with beneficial bacteria and prebiotics is effective for improving resistance to pathogenic bacteria and intestinal function.[10,11]

Aquablend Avian® probiotic is a blend of beneficial bacteria and antibodies including *Lactobacillus*, *Streptococcus* and *Bifidobacterium* strains which provides broilers with essential microflora and specific antibodies against predominant poultry pathogens.[12]

Objectives

To the best of our knowledge, the effects of blends of beneficial bacteria on right heart failure in cold stress situation were not evaluated in Japanese quail; so the study aimed to figure out the effects of Aquablend Avian® probiotic (a blend of beneficial bacteria and antibodies) on the growth performance, intestinal mucosa morphology, heterophil: lymphocyte (H: L) ratio and right heart failure in Japanese quails reared in normal and cold hypoxic environment.

Materials and Methods

Animals, Management and Treatments

The experiment was performed in experimental poultry farm of Shahrekord University, Iran, which is located at 2100 m above sea level. Quails were kept, raised, treated and transported based on the approved rules for the human treatment of animals accepted by the Institutional Animal Care and Use Committee of Shahrekord University.

One hundred fifty-six day-old Japanese quails (*Coturnix coturnix japonica*) from both sexes were divided into 12 floor pens. Each pen measured 1.4 m^2 (13 quails/pen) and bell drinker and a feed trough was provided in each pen. For making deep litter system wood shavings were used as the litter material. Twenty-three hours of light was available for the birds in the first 7 days and then decreased to 20 hours at the end of the experiment for all groups. At day 1, environmental temperature was 36°C and was gradually reduced to 24°C at the end of the experiment for normal temperature groups. At the end of the second week, 2 groups were transferred to another area with the environmental temperature of 10-13°C till the end of the experiment in order to induce cold-hypoxic situation.

Quails were divided into 4 groups (3 replicates/ group), with 39 birds in each group. Treatments were designed as follows:

Group 1 (Aquablend): quails were fed basal corn–soya bean meal based diet (Table 1), and received 0.5 g/100 birds/day (0.065 g/replicate/day) of Aquablend Avian® (Agranco Corp., Gables International Plaza 2655 S Le Jeune Rd., Suite 805 Coral Gables, Fl 33134 USA), during the first 3 days of life, in the drinking water.

Group 2 (control): birds were fed basal corn–soya bean meal based diet, but without any probiotic addition in the drinking water.

Group 3 (aqua-stress): the group received the same amount of Aquablend Avian® as group 1, but was transferred to cold environment at the end of the second week. Dietary formula was the same as others.

Group 4 (cont-stress): quails were fed the same basal corn–soya bean meal based diet without inclusion of probiotic, but were subjected to stressful situation of cold environment from day 15.

Measurements

Body weight (BW) gain and food intake were measured weekly in each pen, as shown in the entire trial. Feed conversion ratio (FCR) data were also calculated and corrected to exclude mortality BWs in the entire trial. On day 35th, 12 birds/treatment were selected randomly for blood collection. Blood samples (3 mL) were collected from the brachial vein and one drop was smeared on a slide. Approximately, after 2-4 hours of fixation with methyl alcohol, the smears were stained by May-Grünwald and Giemsa stains.[13] A 100 granular and non-granular leucocytes, were measured on the slide using light microscopy, and H:L ratios were determined.

Moreover, after bleeding, the selected quails were euthanized for carcass processing. Assessed data were hot eviscerated carcass, liver, spleen and lung weight. To calculate right to total ventricular weight ratio (RV: TV ratio), hearts were also harvested and the ventricles were dissected and weighed separately.

The intestinal morphometric parameters including

Table 1. Ingredients of the Experimental Diet (g/kg)

Ingredients	Corn-Based Diet
Corn seed	509.6
Soybean meal	438.4
Soybean oil	20.6
Di-calcium phosphate	8.3
Calcium carbonate	12.6
Salt	1.6
Mineral-vitamin premix[a]	5
DL-methionine	1.4
Vitamin D_3[b]	1
Vitamin E[c]	1.5

[a] Each kilogram of the mineral-vitamin premix contains vitamin A 200000 IU; vitamin D_3 80000 IU; vitamin E 1600 mg; vitamin K_3 35 mg; vitamin C 1200 mg; vitamin B_1 30 mg; vitamin B_2 130 mg; vitamin B_{12} 700 mcg; nicotinic acid 1300 mg; pantothenic acid 225 mg, choline chloride 8200 mg, biotin 3300 µg; and Mn 1200 mg; Zn 1000 mg; Fe 1800 mg; Cu 400 mg; Se 8 mg; iodine 38 mg; and Ca 180 g.

[b] Each kg of vitamin D3 (cholecalciferol) contains 100000 IU.

[c] Each kg of vitamin E (alpha-tocopherol) contains 100000 IU.

villus sizes (length, width, surface area and lamina propria thickness) were assessed in the duodenum, jejunum and ileum of each euthanized quail at the end of the experiment. Briefly, after fixation of the mentioned segments of intestine in Clark solution, sections were stained by periodic acid–Schiff (PAS) reagent, and then rows of villi were separated in thickness of the sections, transferred over glass slides and covered with a cover-slip. Eyepiece graticules (10×) and magnification of 100× were used in microscope to measure villus sizes in these samples. The villus length and width were used to calculate villus surface area according to formula 2π×(villus width/2)×(villus length), as described by Sakamoto et al.[14] The space between base of the villus and top of the muscularis mucosa was measured to find out the lamina propria thickness.

Statistical Analysis

Results were analyzed using SigmaPlot 12.0 package. One-way analysis of variance (ANOVA) test was used to understand the significant differences among groups and *P* values < 0.05 were considered as significant differences.

Results

There was significant decrement in FCR in Aquablend group compared to other groups at fourth and fifth weeks ($P<0.05$) which means lower costs per each kilograms of meat production. Additionally, aqua-stress group gained better FCR than cont-stress groups in fourth ($P<0.05$) and 5th ($P>0.05$) weeks. Moreover, in the last 2 weeks, FCR of control and aqua-stress groups were significantly ($P<0.05$) lower than cont-stress group (Table 2).

Carcass and organs relative weights were not significantly different at the end of the experiment among 4 treatments (Table 3). However, quails in Aquablend group had slightly higher percentage of carcass and spleen weights relative to BW ($P>0.05$).

Figures 1, 2 and 3 manifest villus surface area and lamina propria thickness of duodenum, jejunum and ileum in the 4 groups. As it is noticeable in Figure 1, Aquablend group had the highest surface area compared to control ($P<0.05$), but the lamina propria thickness was not significantly different among groups ($P>0.05$) except cont-stress group which was the least ($P < 0.05$). On the other hand, jejunum villi morphology results were different among groups and there were not any statistically significant differences among groups, although Aquablend group showed slightly thicker lamina propria compared to control ($P>0.05$) and both of them were significantly thicker than the 2 cold-hypoxic groups ($P<0.05$) (Figure 2). Meanwhile, ileum surface area of control group was increased more than other groups. However, the increment was not significant in comparison to Aquablend and aqua-stress groups ($P>0.05$), but similar to jejunum lamina propria, ileum lamina propria for Aquablend group was slightly higher than others.

RV: TV ratio was highest in cont-stress group and lowest in both standard temperature groups. Aqua-stress group gained lesser amount of RV: TV ratio (0.25) than cont-stress group (0.28) ($P>0.05$). In addition, H: L ratio was higher in cont-stress group (1.22) than that in aqua-stress group (1.2) ($P>0.05$) and the differences between Aquablend and cont-stress groups were significant

Table 2. Weekly Feed Conversion Ratio in 4 Groups at Day 35

	Aquablend	Control	Aqua-stress	Cont-stress
Week 1	1.57 ± 0.003	1.63 ± 0.167	-	-
Week 2	2.03 ± 0.295	2.30 ± 0.303	-	-
Week 3	2.08 ± 0.132	2.46 ± 0.269	2.53 ± 0.035	2.73 ± 0.24
Week 4	3.53 ± 0.117 [a]	4.53 ± 0.202 [b]	4.54 ± 0.152 [b]	5.60 ± 0.381 [C]
Week 5	3.70 ± 0.085 [a]	4.63 ± 0.174 [b]	4.85 ± 0.361 [b]	5.28 ± 0.374 [b]

[a,b,c] Different letters within a row demonstrate statistically significant differences ($P<0.05$).

Table 3. Percent of Relative Weight of Carcass, Liver, Spleen, and Lung to Live Weight at Day 35 in 4 Groups

	Carcass (%)	Liver (%)	Spleen (%)	Lung (%)
Aquablend	66.28 ± 0.65	2.45 ± 0.188	0.078 ± 0.008	0.72 ± 0.051
Control	65.43 ± 0.68	2.48 ± 0.107	0.072 ± 0.005	0.74 ± 0.030
Aqua-stress	64.60 ± 0.90	2.42 ± 0.171	0.071 ± 0.006	0.76 ± 0.028
Cont-stress	63.75 ± 1.26	2.57 ± 0.146	0.066 ± 0.006	0.75 ± 0.030

[a,b,c] Different letters within a row demonstrate statistically significant differences ($P<0.05$).

Table 4. RV: TV and H: L Ratios in 4 Groups at Day 35

	Aquablend	Control	Aqua-stress	Cont-stress
RV: TV	0.24 ± 0.015	0.24 ± 0.014	0.25 ± 0.010	0.28 ± 0.013
H: L	1.12 ± 0.018 [a]	1.13 ± 0.024 [ac]	1.20 ± 0.017 [bc]	1.22 ± 0.016 [b]

[a,b,c] Means within a row with the same letter are not significantly different ($P<0.05$).

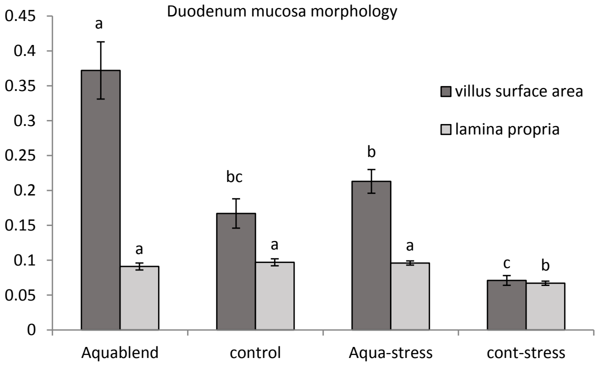

Figure 1. Duodenum Villus Surface Area and Lamina Propria Thickness in 4 Groups at Day 35.
[a,b,c] Different letters within a row demonstrate statistically significant differences ($P<0.05$).

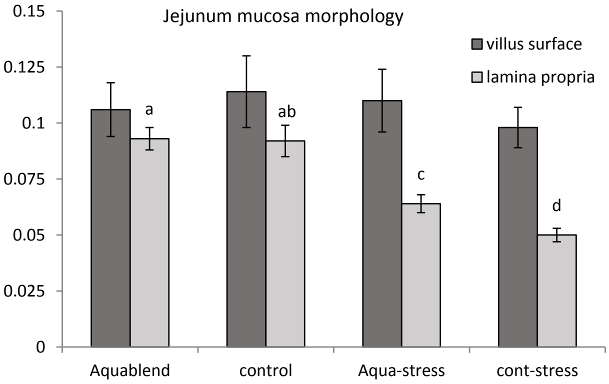

Figure 2. Jejunum Villus Surface Area and Lamina Propria Thickness in 4 Groups at Day 35
[a,b,c] Different letters within a row demonstrate statistically significant differences ($P<0.05$).

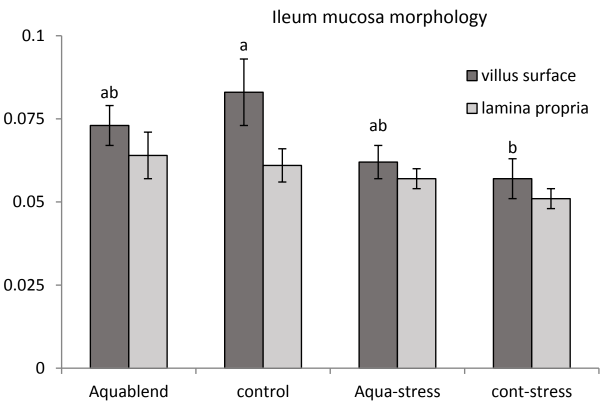

Figure 3. Ileum Villus Surface Area and Lamina Propria Thickness in 4 Groups at Day 35.
[a,b,c] Different letters within a row demonstrate statistically significant differences ($P<0.05$).

($P<0.05$) (Table 4).

Discussion

The primary role of probiotics in the first days of birds' lives is to inhibit the pathogens from occupying inner intestinal surface as competitive exclusion which can facilitate growth and hold considerable promise for the health care industry.

Growth performance results in this study revealed that addition of Aquablend Avian® probiotic to drinking water during the first 3 days of life could apparently decrease feed: gain ratio in comparison with control group at the end of the experiment ($P<0.05$). On the other hand, Aquablend decreased FCR in quails which were raised under cold-hypoxic stress situation and the decrement was enough to be statistically significant in fourth week compared to cont-stress group and also to help this group not to have any significant difference with control group. Effects of beneficial bacteria on BW gain and FCR has been described to be controversial. There are some results in agreement with our data which showed advantageous effects of probiotics and synbiotics on BW and FCR.[15-18] On the other hand reports suggesting no impact of probiotics on BW are also available.[19,20] This conflict might have connection with dosage, concentration, strain and form of bacteria used and also the methods of using probiotics.[21]

Based on our data although not significant, Aquablend group had slightly higher relative weights of carcass and spleen. Furthermore, aqua-stress group was able to increase the percent of carcass, spleen and lung in comparison with cont-stress group, although not significant. Several studies have shown that synbiotics, prebiotics and probiotics had no significant positive effects on carcass yield of quails and broilers.[19,22-25]

There have been such controversial results in this study regarding intestinal villi morphology. The villi surfaces were bigger in duodenum segments of Aquablend fed quails. Solis de los Santos et al[26] reported that the largest increases of villi was in duodenum villi which was significantly elevated in prebiotic-treated birds. The jejunum and ileum surface area of cont-stress quails were reduced significantly compared to aqua-stress birds ($P<0.05$), which was also in agreement with the study worked by Solis de los Santos et al.[26] In addition, lamina propria thickness of ileum and jejunum was elevated in Aquablend and aqua-stress groups compared to control and cont-stress groups, respectively. Lamina propria thickness demonstrates intestinal health because it consists of dendritic cells that survey the contents of the lumen and immunize the bird against infection by stimulating the adaptive immune response, modifying mucin production and defensin secretion, increasing gut motility, and IgA production.[27] Thin scattered lamina propria is more quickly penetrated by pathogens; so it is an essential component of the immune system.[28]

Results regarding right heart failure index shows that RV/TV ratio was higher in cont-stress group versus aqua-stress group which might show the inhibition of right ventricular failure and ascites syndrome in aqua-stress group. However, signs of ascites syndrome were not observable in quails as significant hydropericardium and mortality. Although occurrence of ascites syndrome is more common in broilers than in other poultry [5].

Saffar and Khajali[29] discussed that the lower number of mortality in probiotic-receiving groups due to ascites syndrome might correlate with decreased ammonia production in the intestine. Our data is also in agreement with those of Solis de los Santos et al[26] that declared the first connection of gut development to ascites syndrome in broiler chicks.

Data about H: L ratio was also represented to be the highest in cont-stress and the lowest in Aquablend group that shows lesser stress in Aquablend group under normal temperature. It is suggested that probiotic could alleviate the stress of feed deprivation and enhance the immune-competence of chickens[30] which is in agreement with the data presented here. Both normal environment groups gained fewer H: L ratio than two cold hypoxic environment groups. Moreover, cont-stress group showed slightly higher amount of H: L ratio which might reveal higher stress induced by cold-hypoxic situation than aqua-stress group. Totally both Aquablend groups showed better response to physiological responses.

Conclusion

This study showed that addition of Aquablend Avian probiotic® in the first 3 days of life can significantly reduce FCR, either in normal, or in cold-stressful situations. This report is the first one to induce right heart failure in Japanese quail by cold hypoxic situation to evaluate the effects of inclusion of probiotic on reducing RV: TV ratio. Treated birds were also more resistant to stressful conditions while comparing H: L ratio. Data regarding to intestinal mucosa morphology was controversial but Aquablend Avian® probiotic was able to elevate duodenum villi surface and also jejunum and ileum lamina propria thickness.

Authors' Contributions

Study concept and design: AKZM; Acquisition of data: MHMH, SST, HP and AH; Analysis and interpretation of data: AKZM and MHMH; Drafting of the manuscript: AKZM and MHMH.

Ethical Approval

The Institutional Animal Care and Use Committee of Shahrekord University approved the study.

Conflict of Interest Disclosures

The authors declare that they have no confict of interests.

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