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Multi-strain probiotic improved feed conversion ratio and selected health indicators in Japanese quail*

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Use of multi-strain probiotics in poultry nutrition provides advantages, including promoting microbial diversity in the birds' gut. Less investigated, as compared to broilers, is how application of probiotics affects performance and health of Japanese quails. Up to date effects of either probiotics based on a single microorganism or using a single dose of probiotic were investigated. The aim of the study was to investigate the effect of gradual levels of multi-strain probiotic applied in the Japanese quails' diet on their performance, carcass, microbiological and immunological parameters. Total of 240 Japanese quail mixed sex chicks were randomly selected into four treatments: 0.15, 0.10, 0.05 g/l of the probiotic and control group not provided with probiotic. Body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), carcass characteristics, selected gut microbiome species and sheep red blood cell (SRBC) immunoglobulin IgT, IgG and IgM antibodies were determined. Repeated measures ANOVA was performed on parameters related to productive performance of the birds, while one-way ANOVA on all other parameters using SAS software v 9.4. FI was the

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highest in the control group (16.84±2.19) and did not differ significantly between other groups (from 16.53±2.12 to 15.18±1.86). FCR was the highest in the control and the lowest dose group (3.15±0.28 and 3.07±0.27), as compared to two other groups of birds which received multi-strain probiotic (2.8±0.23 and 2.77±0.24, respectively). Birds in the control group and birds which received the lowest dose of the probiotic had lower counts of IgT (5.63±0.26 and 5.75±0.25, respectively) and IgG (4.00±0.38 and 4.13±0.3, respectively), as compared to two other treatment groups which received the multi-strain probiotic. Birds which did not receive the multi-strain probiotic had significantly lower levels of *Lactobacillus* (6.98±0.01), as compared to other birds, while those in the control group and which received lowest amount of probiotic had significantly higher levels of *E.Coli* (6.67±0.01). Multi-strain probiotic had overall positive effect on the selected parameters of Japanese quails' performance and health, while efficacy varied depending on dosage.

KEY WORDS: probiotics / performance / immune response / gut microbiota / Japanese quail

The meat of Japanese quail is lean, tender, and flavorful [Vali 2008]. It has a delicate taste, often compared to that of chicken, but with a gamier flavor [Ioniţă *et al*. 2010]. Moreover, Japanese quails are valued for their fast growth, efficient feed conversion, high-quality meat, good disease resistance and high environment adaptability [Minvielle 2004]. These characteristics make them an attractive choice for producers also in more extensive production systems, which are gaining popularity among consumers [Nasr *et al*. 2017] like others game meat [Horbańczuk *et al.* 1998, 2007, 2008, Cooper *et al*. 2004, 2008].

Nutritional requirements of Japanese quails are high due to their rapid growth rates [Shim and Vohra 1984], which imposes feeding them with good quality and balanced diet. Probiotics and neutraceuticals [Tewari *et al.* 2017, Yeung *et al.* 2018, 2020abc, 2021ab, 2022, Wang *et al*. 2020, Li *et al*. 2021, Mozos *et al*. 2021, Chopra *et al.* 2022] in other than Japanese quails poultry species, are known to be used primarily to increase the bioavailability and digestibility of nutrients contained in the feed, as well as to develop a beneficial intestinal microflora [Alagawany *et al*. 2018]. It however remains much less investigated how application of specific probiotics may affect performance, carcass, microbiological and immunological parameters in Japanese quails.

Among very few existing scientific reports in this area, studies investigated effects of either probiotics based on a single microorganism or using a single dose of probiotic. For instance, the impact of dietary supplementation of 2 types of single-strain probiotics based on either *Bacillus toyonensis* or *Bifidobacterium bifidum* on growth performance, carcass traits, meat quality, and bacteriology of Japanese quail was recently explored [Abd El-Hack *et al*. 2021]. Moreover, effect of phytogenic feed additive in one dose on performance, mortality and carcass traits, meat quality, intestinal morphology, and microbial population of Japanese quail was investigated [Bagherzadeh Kasmani *et al*. 2018, Kalafova *et al*. 2018, Soomro *et al*. 2019, Hazrati *et al*. 2020].

Multi-strain probiotics offer several potential advantages [Fuller 1989]. Different strains of bacteria have unique characteristics and functions, where a wider range of beneficial bacteria is introduced into the gut, promoting microbial diversity [Lokapirnasari *et al*. 2017]. The gut microbiota is a dynamic ecosystem that can be influenced by various factors, such as changes in diet or environmental factors [Pieczyńska *et al.* 2020, 2022, Sztandarski *et al*. 2022]. A diverse gut microbiota is associated with improved overall health and a balanced immune system. The gut is a complex environment, and not all strains of bacteria are equally effective at colonizing it [Pan and Yu 2014]. By including multiple strains in a probiotic supplement, the chances of some strains successfully establishing themselves in the gut are increased. This can improve the overall effectiveness of the probiotic. Moreover, different strains of bacteria can work together synergistically, meaning their combined action produces more significant benefits than each strain individually [Taksande *et al*. 2009]. Multi-strain probiotics often include strains that complement each other's functions, such as one strain that produces certain substances that enhance the growth or activity of another strain. Each strain of bacteria has its own unique set of potential health benefits [Vali 2009]. Some strains may support digestive tract health, while others may contribute to immune function, nutrient absorption, or even mental health [Strompfova *et al*. 2005, Chimote *et al*. 2009]. This can help maintain a healthy microbial balance even during periods of stress or disruption [Alagawany *et al*. 2018].

Certain strains of bacteria have been extensively studied and shown to have specific benefits. A multi-strain probiotic allows for targeted supplementation, as strains known for their efficacy in addressing specific health concerns can be selected. Strains like *Lactobacillus acidophilus, Lactobacillus delbrueckii* and *Bifidobacterium bifidum* are commonly included for digestive health support. In turn, *Bacillus subtilis* and *Lactobacillus rhamnosus* microbial strains inhibit the growth of pathogenic microorganisms, prevent infections and support the immune system [Marchewka *et al*. 2022].

The effectiveness of any probiotic supplement, including multi-strain probiotics, can vary depending on factors such as the specific strains used, the dosage, the individual's health status, and the overall composition of their gut microbiota. Therefore, to have a complete overview of the effect of a multi-strain probiotic it is necessary to include in the experimental design gradual dietary levels. Understanding the dose-response relationship can help determine the optimal dosage for specific benefits.

The aim of the study was to investigate the effect of gradual dietary levels of a multi-strain probiotic applied to the Japanese quails on their performance, carcass, microbiological and immunological parameters. We hypothesized that measured parameters will be improved for the birds which will receive higher doses of the probiotic (0.15 and 0.10 g/l of the probiotic), as compared to birds receiving 0.05 g/l of the probiotic and those in the control group (no probiotic received).

Material and methods

Animals and management

The study was performed using 240 Japanese quails. Day-old chicks were purchased from a commercial hatchery and then placed in 0.5 x 0.5 m (providing a floor area/bird of 0.016 m^2) cages of 15 individuals where they remained until 42 days of age. Cages were centrally positioned in a thermostatically-controlled curtain side-walled poultry barn. The cage floors were covered with wood shaving as litter, and the birds remained in the cages for the duration of the experiment. Temperature in the experimental room where birds were maintained ranged from 39°C at the beginning of the experiment when birds were placed in the cages and decreased progressively to 28°C at 4 weeks of age after which it was maintained at this level until the end of the experiment. Temperature was maintained using thermostatically-controlled gasoline heaters. Humidity in the experimental room where birds were maintained was on the level of 55-65%. Controlled lighting and tunnel ventilation were included in the barn. Lighting was provided by 43 W fluorescent tubes in ceiling fixtures. During the first two days of birds' life the light was on at all times and from the third day of birds' life it was deflated from 1 hour until 16 hours a day. Air circulation within the poultry barn was facilitated by 2 wall-mounted fans at one end of the barn to establish tunnel ventilation. The approval of the Ethics Committee of Rasht Branch Islamic Azad University for all experimental procedures was received (No. 911130).

Diets and treatments

Throughout the experiment (1-42 days) birds received one phase diet (Tab. 1). Sixteen mixed sex groups of 15 one day old chickens were assigned into four treatments and four replicates of each treatment. Four levels of probiotic (Bioguil, Zistyarvarna Co., Iran): 0 g / l (control group); 0.05 g / l; 0.10 g / l and 0.15 g / l were added to the drinking water, depending on the treatment group. Probiotic included 10^{11} CFU/g *Lactobacillus acidophilus, Bifidobacterium bifidum, Lactobacillus delbrueckii, Bacillus subtilis,* and *Lactobacillus rhamnosus*.

At 42 days, two randomly selected birds per replicate were randomly selected and sacrificed for carcass evaluations and *ileum* microflora parameters. Another 2 randomly selected birds per replicate of the same age were sacrificed for measuring immune response.

Performance traits

Body weight and feed intake were measured weekly throughout the experiment. Additionally, the feed conversion ratio was calculated by dividing the feed intake by the weight gain.

Carcass traits

The whole carcass, without giblets and the weight of the cut organs (breasts, thighs, heart, gizzard, liver) were weighed. The following formula was applied to calculate the carcass parameters:

$$[(\textit{weight of component(s)/eviscerated carcass weight}) \times 100]$$

Table 1. Feed ingredients and chemical composition of the experimental diets of the Japanese quail

Feed composition (g/kg*)	1-42 days of birds' age
Corn	519.38
Soybean Meal	400.38
Fish meal	40.0
NaCl	2.44
Soybean oil	17.5
CaCO3	10.78
Dicalcium phosphate	3.72
Vitamin premix	2.5
Mineral premix	2.5
DL-Methionine	0.7
Lysine-HCl	0.1
Metabolizable Energy (ME. kcal/kg)	2900
Crude protein (%)	24
Lysine (%)	1.47
Arginine (%)	1.65
Methionine + Cysteine (%)	0.91
Threonine (%)	0.96
Tryptophan (%)	0.29
Potassium (%)	0.97
Calcium (%)	0.85
Available Phosphorus (%)	0.38
Sodium (%)	0.15

*Fresh weight basis.

Microbiological analysis

From collected section of the *ileum* amount of *Escherichia coli* and *Lactobacillus* bacteria was determined according to Dibaji *et al.* [2014]. The *ileum* samples were placed in sterile tubes with phosphate-buffered saline (PBS) and then shaken for half an hour to separate the gastrointestinal contents from the bacteria. 1 ml of the obtained suspension was transferred to a new tube with 9 ml PBS. About 1 drops of each sample was spread on the appropriate agar surface. The inoculated plates were then incubated for 48 hours in aerobic conditions. A bacterial colony counter was used to indicate the number of detected bacterial colonies. Log colony forming units (CFU/g) were used to convert all quantitative data.

Immunological analysis

Immunological parameters were measured according to the methods described by Seidavi *et al*. [2014]. Selected birds were injected subcutaneously of Sheep Red Cell Antibody (SRBC) at 28 and 35 days of age. Total SRBC titers were determined by the haemagglutination test. Immunoglobulins G (IgG) and M (IgM) were also determined at 42 days of age.

Statistical analysis

A generalized linear mixed model analysis (repeated measures ANOVA) was performed on parameters related to productive performance of the birds (FI, FCR and BWG), in order to determine the effect treatment as a fixed factor and birds' age as a repeated measure, as well as their interaction. No significant effect of the treatment by birds' age interaction was detected, hence it was excluded from the final model. A one-way ANOVA was performed on all other analysed parameters with a treatment as fixed effect. There were no outliers present in the dataset. Normality and homogeneity of residual variance assumptions were checked and met by all variables under investigation. The validity of the models was tested using Akaike's information criterion. PROC GLIMMIX of SAS v 9.4 (SAS Institute Inc., Cary, NC, USA) including the Tukey adjustment option was used to conduct the analysis. The least square means for all significant effects in the models ($p \leq 0.05$) were computed using the LSMEANS option. The trend of a significant effect was considered for $p<0.10$.

Results and discussion

Performance traits

The effect of treatment and birds' age on selected performance traits (FI, FCR and BWG) are presented in Table 2. Significant effect of the treatment was found on the FI and FCR. FI was the highest in the control group and did not differ significantly between the groups which were administered the probiotic regardless of the dose. FCR was higher in the control and lowest dose treatment group (0.05 g/l of the probiotic),

Table 2 Feed intake (FI), feed conversion ratio (FCR) and body weight gain (BWG) of Japanese quail according to the applied amount of probiotic and birds' age

Item		Parameter					
		FI		FCR		BWG	
		mean	SE*	mean	SE	mean	SE
Treatment	control (0 g/l)	16.84^{A}	2.19	3.15^{A}	0.28	4.84	0.33
	0.05 g/l	16.53^{AB}	2.12	3.07^{A}	0.27	4.90	0.34
	0.10 g/l	15.26^{B}	1.86	2.80^{B}	0.23	4.99	0.32
	0.15 g/l	15.18^{B}	1.86	2.77^{B}	0.24	5.02	0.32
Birds' age	1st week of age	2.55^{F}	0.05	1.53^{E}	0.05	1.70^{D}	0.06
	2nd week of age	7.93^{E}	0.11	1.75^{E}	0.03	4.54^{C}	0.07
	3rd week of age	12.55^{D}	0.23	2.29^{D}	0.07	5.51^{B}	0.11
	4th week of age	18.08^{C}	0.30	3.24^{C}	0.07	5.58^{B}	0.07
	5th week of age	23.68^{B}	0.49	3.88^{B}	0.09	6.12^{A}	0.11
	6th week of age	30.95^{A}	1.02	4.99^{A}	0.13	6.18^{A}	0.08
Effects		p-value					
treatment		0.0027		<.0001		0.3053	
birds' age		<.0001		<.0001		<.0001	

$^{AB...}$Means within each column for treatment and birds' age bearing different superscripts differ significantly $p<0.05$.
**SE – standard error.

as compared to two other groups of birds which received the probiotic (0.10 and 0.15 g/l). No significant effect of the treatment was found on the BWG. In all treatment groups FI, FCR and BWG significantly increased each week over the course of experiment, except for FCR in the period between week 1 and 2 and BWG in the period between week 3 and 4 when no significant changes were observed.

Carcass traits

No significant effect of the treatment applied on any of the measured carcass characteristics was observed (Tab. 3).

Microbiological analysis

The effect of treatment on immunological response of the birds to SRBC challenge is presented in Table 4. Significant differences were observed between treatments in IgT (total) anti-SRBC and IgG anti-SRBC. Birds in the control group and birds which received the lowest dose of the probiotic (0.05 g/l) were characterised with lower levels of those parameters, as compared to two other treatment groups of birds which received the probiotic (0.10 and 0.15 g/l). No significant effect of the treatment on IgM anti-SRBC was detected.

Immunological analysis

The effect of treatment on ileum microflora is presented in Table 5. Birds which did not receive the probiotic (control group) were characterised with the significantly lower levels of *Lactobacillus* presence, as compared to other birds. On the other hand birds in the control group and those, which received the lowest amount of probiotic (0.05 g/l) were found with significantly higher levels of *E.Coli* presence in their *ileum*.

Table 3. Carcass characteristics at 42nd days of age in Japanese quail according to the applied amount of probiotic

Item		Parameter											
		empty abdomen carcass*		breast**		thigh**		heart**		gizzard**		liver**	
		mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Treatment	control (0 g/l)	59.19	2.76	39.52	1.68	26.97	1.40	0.86	0.04	1.91	0.12	2.55	0.20
	0.05 g/l	59.85	1.95	40.76	1.43	27.15	1.95	0.87	0.03	1.92	0.11	2.56	0.10
	0.10 g/l	60.69	1.05	40.80	0.84	28.38	1.53	0.93	0.04	1.93	0.11	2.60	0.18
	0.15 g/l	61.71	3.32	40.91	1.53	28.48	1.55	0.96	0.08	1.94	0.14	2.60	0.17
Effect		p-value											
treatment		0.8943		0.8833		0.8668		0.355		0.988		0.995	

*Relative weight to live body weight.
**Relative weights to empty abdomen carcass weight.
***SE – standard error.

In the current study stated hypothesis, that measured parameters will be improved for the birds which received higher doses of the probiotic (0.10 and 0.15 g/l of the probiotic), as compared to birds in the control group and birds receiving 0.05 g/l of

Table 4. Immune responses at 42nd days of age in Japanese quail according to the applied amount of probiotic

Item		IgT (total) anti- SRBC*		IgG anti- SRBC		IgM anti- SRBC	
		mean	SE**	mean	SE	mean	SE
Treatment	control (0 g/l)	5.63^B	0.26	4.00B	0.38	1.63	0.32
	0.05 g/l	5.75^B	0.25	4.13B	0.30	1.63	0.26
	0.10 g/l	7.13^A	0.35	5.63A	0.26	1.50	0.19
	0.15 g/l	7.38^A	0.26	5.75A	0.37	1.63	0.26
Effects		p-value					
treatment		0.0001		<.0005		0.9822	

$^{AB...}$Means within each column for treatment bearing different superscripts differ significantly p<0.05.
*SRBC – Sheep red blood cell.
**SE – standard error.

Table 5. *Ileum* microflora parameters at 42nd days of age in Japanese quail according to the applied amount of probiotic

Item		*Lactobacillus*		*Escherichia coli*	
		mean	SE*	mean	SE
Treatment	control (0 g/l)	6.98^B	0.01	6.67^A	0.01
	0.05 g/l	7.12^A	0.01	6.65^A	0.02
	0.10 g/l	7.13^A	0.04	6.49^B	0.03
	0.15 g/l	7.16^A	0.01	6.48^B	0.04
Effect		p-value			
treatment		0.0001		<.0005	

$^{AB...}$Means within each column for treatment bearing different superscripts differ significantly p<0.05.
*SE – standard error.

the probiotic, has been partially confirmed. It was confirmed that feed intake of birds provided with the multi-strain probiotic at any dose applied was reduced. On the other hand, feed conversion ratio was improved in groups of birds receiving higher doses of probiotic. Lower feed conversion ratio is an indicator of more efficient utilization of feed. Importantly, all the treatment groups did not differ in their body weight gain levels. Probiotics can enhance earlier establishment of beneficial intestinal microflora, optimize it and hence trigger the symbiotic effect of the host animal's enzymes helping to improve the nutrient digestibility [Lokapirnasari *et al*. 2017, Seifi *et al*. 2018]. Ingestion of probiotic applied in the current study could optimize intestinal flora and thus improve feed conversion ratio. Previously, the average feeding efficiency depended on the type of use probiotic and was higher only in some of the groups supplemented with probiotics [Taksande *et al*. 2009].

The results obtained in the present study show that dietary supplementation of probiotics significantly increased the immune response to immunological challenge. Serum IgT and IgG concentration was significantly higher in response to the SRBC injection in the two treatment groups provided with higher doses of the probiotic, as compared to the lowest dose group and control group. Commensal bacteria or their products which interact closely with cells within the birds' gut-associated lymphoid tissue play a role in the development of immune response [Haghighi *et al*. 2005, Cox and Dalloul 2015]. In a previous study no effect of probiotic provision was identified on the IgT levels, which could be related to non-targeted selection of the microorganisms in the provided probiotic [Seifi *et al*. 2018]. Overall, IgG plays a crucial role in the immune response of quail. It contributes to pathogen recognition, neutralization, opsonization, immune memory, and the passive transfer of immunity to offspring. These functions collectively help to defend against infections, maintain health, and develop immunity over time. Our results showed that the application of the probiotic enhanced the immune function as indicated by IgG, underlying its beneficial effects on the birds in the current experiment. The effects of probiotics on immunoglobulins can vary depending on the specific strains used, the dosage, and the individual's immune system. Not all strains of probiotics have the same immunomodulatory effects, which may explain no observed effect of the applied probiotic on the levels of IgM in the current study.

In the present study, probiotic application increased the *Lactobacillus* spp. count in the ileum microflora, while *Escherichia coli* counts were lower in two treatment groups provided with higher probiotic doses. The addition of probiotics to the diet caused a reduction in the number of harmful microorganisms which is beneficial, as it improves metabolism, having a positive effect on growth and retention of nutrients [Mahrose *et al*. 2019]. *Lactobacillus* spp. strains in quail can contribute to a healthy gut environment. They help create an acidic pH in the gut, which inhibits the growth of pathogenic bacteria. *Lactobacillus* spp. species also produce organic acids, such as lactic acid, that can further promote a favorable gut environment by supporting the growth of beneficial bacteria and discouraging the proliferation of harmful microorganisms [Brisbin *et al*. 2011]. Certain *Lactobacillus* species have been shown to modulate the immune response in quail. They can stimulate the production of immunoglobulins, including IgG, promoting a balanced immune function. By interacting with the gut-associated lymphoid tissue, *Lactobacillus* strains can enhance the immune system's ability to defend against pathogens and maintain gut health. Certain pathogenic strains of *E. coli*, such as avian pathogenic *E. coli* (APEC) or enteropathogenic *E. coli* (EPEC), can cause infections in Japanese quail. These strains may have virulence factors that enable them to adhere to and invade the Japanese quail's intestinal epithelial cells, leading to symptoms of gastrointestinal illness.

Efficacy of a probiotic in broilers varied depending on factors such as dosage, administration methods, and the health status of the birds, as affected by the environmental conditions, housing and management or in the general production system [Marchewka *et al*. 2023]. Therefore, the application of the probiotic used in the current study should be investigated with regard to above listed aspects also in Japanese quail.

Conclusion

In conclusion, we found a significant effect of the multi-strain probiotic in all doses on: increased *Lactobacillus* counts and reduced feed intake, while maintaining equal body weight gains in all treatments and of the two highest doses on: improved feed conversion ratio, reduction of *E. coli* count and increased Japanese quails' immunity, as indicated by IgT and IgG. Therefore, overall current study has showed that the probiotic including 1011 CFU/g *Lactobacillus acidophilus*, *Bifidobactrium bifidum*, *Lactobacillus delbrueckii*, *Bacillus subtilis*, and *Lactobacillus rhamnosus* had positive effect on the selected parameters of Japanese quails' performance and health.

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