

*Full Length Research Paper*

# Application of probiotic on egg production and egg quality of chukar partridge

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**A total of 304 mixed sex chukar partridge (*Alectoris chukar*) chicks were reared in an environmentally controlled cages, which were divided into two groups, control (not supplemented) and probiotic (PrimaLac<sup>®</sup> supplemented) treatments with four replicates per treatment. The birds were fed PrimaLac<sup>®</sup> at the rate of 1.0 and 0.5 g/kg of feed from 1 to 3 and 4 to 40 weeks of age, respectively. At 32 weeks of age, 64 female chukar partridges with uniform body weight were selected to determine the effects of PrimaLac<sup>®</sup> on egg production over 33 to 40 weeks and egg quality characteristics over the last two days of 40 weeks of age. Addition of probiotic PrimaLac<sup>®</sup> increased ( $P < 0.05$ ) egg weight and specific gravity, but concomitant decrease ( $P < 0.05$ ) in egg shape index. The application of PrimaLac<sup>®</sup> also increased ( $P < 0.05$ ) egg shell thickness, egg shell weight and egg albumen weight. However, in terms of egg production and yolk weight, no significant differences were detected in the two groups ( $P > 0.05$ ), although these values were numerically greater in probiotic treated birds.**

**Key words:** Chukar partridge, egg production, egg quality, probiotic.

## INTRODUCTION

Chukar partridge is the common name of a game bird classified as *Alectoris chukar* species of the Phasianidae family (Cetin and Kirikci, 2000). Partridges are exploited for eggs, meat and hunting preserves. The wild partridge population in many countries, especially the industrialized, has decreased considerably due to increase in illegal hunting, environmental pollution, number of predators, general use of herbicides and insecticides and loss of habitat (Rueda et al., 1992) over the last few decades. Thus, there is need to investigate different aspects affecting the partridge egg production and quality to repopulate of partridge subspecies.

The microflora in the gastrointestinal tracts of poultry plays a key role in normal digestive processes and in maintaining the animal's health. Some feed additives can substantially affect this microbial population and their health promoting effects. Recently, concerns about some

unwanted harmful side effects caused by antibiotics (Botsoglou and Fletouris, 2001) has grown in many countries, so that there is an increasing interest in finding alternatives to antibiotics in poultry production. Some possible alternatives are dietary supplementation of prebiotic, probiotic, organic acids and medical plant or their by-products (Langhout, 2000). Probiotics which mean "for life" in Greek contain yeast cells, bacterial cultures or both and their use has gained widespread interest since Turtuero's (1973). Probiotics defined as live microbial feed supplements beneficially improve the intestinal microbial balance in host animal (Isolauri et al., 2001).

The proposed mode of action for the beneficial effect of probiotics are as follows: High affinity for attachment to the mucosal wall, adjusting to immune responses (Patterson and Burkholder, 2003), providing digestive enzymes, stimulating synthesis vitamins of the B-group and enhancement of growth of nonpathogenic facultative anaerobic and Gram positive bacteria by producing inhibitory compounds like volatile fatty acids and hydrogen peroxide that inhibit the growth of harmful bacteria enhancing the host's resistance to enteric pathogens

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**Table 1.** The ingredients and calculated nutrient contents of the Chukar partridge starter, grower, holder and breeder control diets<sup>1</sup>.

Ingredient (%)	Starter	Grower	Holder	Breeder
	(0 to 8 weeks)	(9 to 16 weeks)	(17 to 28 weeks)	(29 to 40 weeks)
Corn	43.80	50.18	55.07	44.50
Soybean meal, 44% CP	47.00	29.85	11.32	25.40
Wheat bran	0.00	6.50	20.00	7.72
Barley	2.06	7.68	10.00	8.00
Vegetable oil	2.50	0.76	0.85	3.60
Limestone	0.60	0.96	0.85	6.50
Dicalcium phosphate	2.70	2.30	0.90	2.30
Salt (NaCl)	0.50	0.50	0.50	0.50
DL-Methionine	0.25	0.30	0.16	0.33
L-Lysine	0.24	0.62	0.00	0.80
Vitamin mix <sup>2</sup>	0.25	0.25	0.25	0.25
Mineral mix <sup>3</sup>	0.10	0.10	0.10	0.10
<b>Calculated nutrient contents</b>				
ME (kcal / kg)	2800	2700	2700	2700
Crude Protein (%)	25.00	20.00	14.00	18.00
Calcium (%)	1.00	1.00	0.60	3.10
Available phosphorus (%)	0.70	0.60	0.30	0.60
Methionine (%)	0.60	0.60	0.40	0.60
Lysine (%)	1.57	1.50	0.60	1.50

<sup>1</sup>Probiotic PrimaLac<sup>®</sup> was added to the diets at the rate of 1 g per kg of feed for the first 3 weeks and thereafter 0.5 g / kg until 40 weeks of age.

<sup>2</sup>Vitamin mix provided the following per kilogram of diet: vitamin A, 1.000 IU; vitamin D<sub>3</sub>, 3.500 IU; vitamin E, 100 mg; vitamin K<sub>3</sub>, 3 mg; vitamin B<sub>1</sub>, 3 mg; vitamin B<sub>2</sub>, 6 mg; vitamin B<sub>6</sub>, 5 mg; vitamin B<sub>12</sub>, 0.03 mg; niacin, 45 mg; calcium pantothenate, 15 mg; folic acid, 1 mg; biotin, 0.15 mg; ethoxyquin (antioxidant), 150 mg. <sup>3</sup>Trace mineral mix provided the following per kilogram of diet: iron, 60 mg; manganese, 100 mg; zinc, 60 mg; copper, 5 mg; iodine, 2 mg; cobalt, 0.2 mg; selenium, 0.15 mg; choline chloride, 400 mg.

(Fuller, 1989; Jin et al., 1996; Rolf, 2000; Sun, 2005). In line with the study of Nahashon et al. (1994) and Turtuero and Fernandez (1995), administration of probiotic into the diet enhances egg production and egg quality. These results are consistent with Haddadin et al. (1996) who reported that egg production, size and qualities were improved by addition of probiotic. In contrary, inclusion of the probiotic Bioplus in laying hens diet did not influence on egg production and egg weight (Mahdavi et al., 2005).

Literature review indicates the effect of probiotics on partridge egg production and quality has not been tested yet and it is necessary for detailed research to reach sufficient amount of knowledge related in this field. Therefore, the aim of this study was to determine the effects of dietary probiotic PrimaLac<sup>®</sup> supplementation on egg production and some egg quality parameters of chukar partridge (*A. chukar*).

## MATERIALS AND METHODS

### Birds, diets and management

Three hundred and four mixed sex chukar partridge chicks were accommodated in electrically heated brooder batteries for the first 5 weeks and grower batteries till the end of 28 weeks of age. Then

they were transferred to breeding cages at 29 weeks to the end of egg laying period. The chicks were fed diets containing 25, 20, 14 and 18% CP and 2800, 2700, 2700, 2700 kcal / kg ME from hatch to 8, 9 to 16, 17 to 28 and 29 weeks of age to the end of egg laying period. Formulation and composition of the starter, grower, holder and breeder diets are presented in Table 1, according to nutrient requirement of chukar partridge recommended by Woodard (1982) and Ernst et al. (2007). The birds were randomly allocated to two dietary treatments of not supplemented (control) and supplemented (probiotic) groups in a completely randomized design considering each treatment was replicated 4 times with 38 birds per replicate. Then sixty four (eight per replicate), not vaccinated but healthy and nimble, 32 weeks of age female chukar partridge (*Alectoris chukar*) with uniform ( $P > 0.05$ ) body weight were selected after sexed at the same age. Every eight females were lodged in one well ventilated cage as a replicate.

The probiotic PrimaLac<sup>®</sup> was added to the diets at the rate of 1 and 0.5 g / kg of feed for 1 to 3 and 4 to 40 weeks of age. This commercial microbial culture (PrimaLac<sup>®</sup>) included a minimum presence of  $1.0 \times 10^8$  CFU of friendly bacteria *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilus* and *Enterococcus faecium* per gram (PrimaLac<sup>®</sup>, Star Labs, St. Joseph, MO). PrimaLac<sup>®</sup> has been shown to resist extremes in temperature and humidity and remains stable up to a year after its container is opened. Its unique formulation ensures that a large portion of what is ingested actually gets into the bowel or gastrointestinal tract and is not destroyed in the crop or stomach. Therefore, its desired effect is achieved.

The cages were enclosed with plates at the sides, and between two cages a space as half long as one cage length was kept empty

**Table 2.** Effect of dietary probiotic Primalac<sup>®</sup> supplementation on egg production and external egg quality parameters of chukar partridge (mean  $\pm$  SE)<sup>1</sup>.

Parameter	Treatment	
	Control	Primalac <sup>®</sup>
Egg production (%)	43.20 $\pm$ 2.12	44.73 $\pm$ 2.83
Egg weight (g)	20.99 $\pm$ 0.32 <sup>b</sup>	22.57 $\pm$ 0.18 <sup>a</sup>
Specific gravity	1.05 $\pm$ 0.03 <sup>b</sup>	1.07 $\pm$ 0.04 <sup>a</sup>
Shape index	77.77 $\pm$ 0.97 <sup>a</sup>	75.38 $\pm$ 1.12 <sup>b</sup>

<sup>a-b</sup>Means  $\pm$  SE in a column with different superscript are significantly different (P < 0.05). <sup>1</sup>Number of eggs = 32.

**Table 3.** Effect of dietary probiotic Primalac<sup>®</sup> supplementation on internal egg quality parameters of chukar partridge (mean  $\pm$  SE)<sup>1</sup>.

Parameter	Treatment	
	Control	Primalac <sup>®</sup>
Shell thickness (mm)	0.206 $\pm$ 0.009 <sup>b</sup>	0.223 $\pm$ 0.007 <sup>a</sup>
Shell weight (g)	2.13 $\pm$ 0.07 <sup>b</sup>	2.30 $\pm$ 0.05 <sup>a</sup>
Yolk weight (g)	7.76 $\pm$ 0.20	8.09 $\pm$ 0.22
Albumin weight (g)	11.10 $\pm$ 0.37 <sup>b</sup>	12.18 $\pm$ 0.34 <sup>a</sup>
Yolk color (Roch)	8.00 $\pm$ 0.12	8.00 $\pm$ 0.15

<sup>a-b</sup>Means  $\pm$  SE in a column with different superscript are significantly different (P < 0.05). <sup>1</sup>Number of eggs = 32.

to avoid undesired spreading of the bacteria. Laying of egg commenced within 32 weeks of age in all the groups. Continuous lighting exposure was provided during the first week of age and afterwards the light (L): dark (D) program was followed as 20L: 4D and 8L: 16D for weeks 2 to 16 and 17 to 28 (holding period), respectively. Lighting was provided 12 h/day at week 29 and increased 1 h/week, and then fixed when 16 h L/day were reached at 33 weeks of age. The temperature was maintained at 34°C initially and reduced by 2.8°C per week until the temperature 20°C was reached. For the remainder of study, this temperature was constant. Free access to water from nipple drinkers and feed was provided *ad libitum*.

#### Data collection

The eggs were collected three times daily during the research period of 33 to 40 weeks to record the egg production. 32 eggs from each group (8 eggs / cage) were obtained to assess the egg quality parameters at the last two days of 40 weeks of age. Eggs were stored at room temperature during sampling. The eggs weighed and then sampled. The specific gravity was determined by using the formula according to the Archimet method (Hempe et al., 1988): Specific gravity = weight in air / (weight in air – weight in distilled water). The weight and specific gravity of eggs were calculated at the same day they were collected.

The shape index was measured by a micrometer with the following formula (Yannakopoulos and Tserveni-Gousi, 1986): Shape index = (width / length)  $\times$  100. The eggs were then broken one by one on a glass plate with a waiting period of 5 min. The shells of the broken eggs were washed under gently flowing tap water to release albumen residues, and were then air-dried and weighed. Shell thickness was a mean value of measurements at 3 locations on the egg (air cell or blunt edge, equator, and sharp or pointed edge) by using a micrometer with the following formula (Yannakopoulos and Tserveni-Gousi, 1986): Shell thickness =

(sharp end thickness + equator thickness + blunt end thickness) / 3. The yolks separated from the albumens and the weights of them were recorded. Yolk color was determined with a Roche fan color. The experimental protocols were reviewed and approved by the Animal Care Committee of the Ferdowsi University of Mashhad.

#### Statistical analyses

All percentage data were subjected to arcsine transformation. The results were statistically analyzed by t-test using SAS and the significance level was set at P  $\leq$  0.05.

## RESULTS AND DISCUSSION

In this study, the effects of dietary probiotic PrimaLac<sup>®</sup> supplementation on egg production and external egg quality (Table 2) and internal egg quality (Table 3) of chukar partridge are presented. Incorporation of probiotic PrimaLac<sup>®</sup> improved (P > 0.05) egg production although not at a significant value, increased (P < 0.05) egg weight and specific gravity, and concomitant decreased (P < 0.05) shape index. The application of this probiotic increased all the internal egg quality parameters except the yolk color. This increase in shell thickness, shell weight and albumen weight were significant (P < 0.05). Statistically significant difference was not detected in yolk weight (P > 0.05), though value was greater in probiotic treated birds.

Egg production and quality are important determinations of the optimum length of a laying partridge cycle and these are presented in Tables 2 and 3. These

determinations are influenced in partridge by housing systems (Ozbey and Esen, 2007), different rates of mating (Cetin, 2002), environmental circumstances such as lighting program (Kirikci et al., 1999) and layer weight (Kirikci et al., 2007).

### Egg production and external egg quality

Partridges normally lay 8 to 16 eggs in natural habitat (Turan, 1990), but breeding domesticated wild partridges under human care is more advantageous as they produce eggs or meat efficiently in small breeding areas within short time and without great investments than their wild ancestors. Chukar partridges located on the ground have an egg production percentage of 47% and egg weight of 19.31 g (Cetin et al., 1997). The findings show a quadratic increase in egg production in chickens supplemented with 0.0, 100.0 and 150.0 mg probiotic (*Lactobacillus*, *Bifidobacterium*, *Aspergillus*, and *Torulopsis* spp. at  $27 \times 10^9$  cfu / 10 g) per kilogram of diet during the peak period (Mohan et al., 1995). This study supports the earlier reports that suggested a negative correlation between the egg weight and the shape index (Poyraz, 1989; Grunder et al., 1991; Ozbey and Esen, 2007). Egg weight is one of the main criteria of external egg quality due to its effects on hatching rate, hatching weight, growth and fattening performance (Castilla et al., 2009). Song et al. (2000) indicated that shape index of the partridge egg is 78.00. Egg shape is critical for the egg crushing strength, but it is a hereditary trait that is associated mainly with hen genotype and age (Solomon, 1991).

### Internal egg quality

There is a positive coefficient of correlation between egg weight and yolk weight (Suk and Park, 2001; Hartman and Wilhelmson, 2001; Krawczyk, 2009). Albumen and eggshell are the main components forming the egg (Kirikci et al., 2007) and weights of these components increased in birds dosed with probiotic PrimaLac<sup>®</sup>, hence the higher egg weight in these birds. By the values of the egg yolk color in this study, there was no variation in the yolk color of the eggs between two the groups. The reason for this lack of variation might be due to feeding the same amount of pigments present in the experimental diets. It may also imply that probiotic supplementation could not increase the normal absorption of the pigments of the diet or its transfer into the egg yolk.

The reason for the variable effects of probiotics on poultry might be attributed to variations in gut flora and environmental conditions (Mahdavi et al., 2005), ability or inability of probiotics to colonize in gastrointestinal tract and competitively exclude the pathogenic bacteria (Jin et al., 1997), amount of stress in flock (Lyon, 1987), strain

and concentration of probiotic, time and method of use (Dizaji and Pirmohammadi, 2009).

### Conclusion

Addition of probiotic PrimaLac<sup>®</sup> increased ( $P < 0.05$ ) egg weight and specific gravity and decreased shape index ( $P < 0.05$ ). The application of probiotic PrimaLac<sup>®</sup> also increased ( $P < 0.05$ ) shell thickness, shell weight and albumen weight. Under the conditions of this study, it was therefore concluded that supplementation of PrimaLac<sup>®</sup> might be a good source of probiotic to increase the internal and external quality of chukar partridge eggs.

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### REFERENCES

- Botsoglou NA, Fletouris DJ (2001). Drug Residues in Foods. Pharmacology, Food Safety and Analysis. New York, Marcel Dekker. pp. 541-548.
- Castilla AM, Martinez de Aragon J, Herrel A, Moller S (2009). Eggshell thickness variation in red-legged partridge (*Alectoris rufa*) from Spain. The Wilson J. Ornith. 121(1): 167-170.
- Cetin O, Kirikci K (2000). Alternative poultry raising. Pheasant-Chukar. Selcuk Univ. Found. Press, Konya, Turkey.
- Cetin O, Kirikci K, Gulsen N (1997). Some productivity characteristics of Chukar partridges (*A. chukar*) in different management conditions. J. Vet. Sci. 13(2): 5-10.
- Cetin O (2002). Egg production and some hatchability characteristics of rock partridges (*Alectoris graeca*) mated at different rates. Turk. J. Vet. Anim. Sci. 26(5): 1009-1011.
- Dizaji SB, Pirmohammadi R (2009). Effect of saccharomyces cerevisiae and Bioplus 2B on performance of laying hens. Int. J. Agric. Biol. 11(4): 495-497.
- Ernst RA, Woodard AE, Vohra P, Cardona C (2007). Raising game birds. Oakland: University of California Division of Agriculture and Natural Resources Publication 8155. <http://anrcatalog.ucdavis.edu>.
- Fuller R (1989). Probiotics in man and animals. J. Appl. Bacteriol. 66(5): 365-378.
- Grunder AA, Fairfull RW, Hamilton RMG, Thompson BK (1991). Correlations between measures of eggshell quality or percentage of intact eggs and various economic traits. Poult. Sci. 70: 1855-1860.
- Haddadin MS, Abdulrahim SM, Hashlamoun EAR, Robinson R (1996). The effect of *Lactobacillus acidophilus* on the production and chemical composition of hen's egg. Poult. Sci. 75: 491-494.
- Hartman C, Wilhelmson M (2001). The hen's egg yolk: A source of biologically active substances. Worlds Poult. Sci. 57(1): 13-27.
- Hempe JM, Laukx RC, Savage JE (1988). Rapid determination of egg weight and specific gravity using a computerized data collection system. Poult. Sci. 67(6): 902-907.
- Isolauri E, Sutas Y, Kankaanpaa P, Arvilommi H, Salminen S (2001). Probiotics: Effects on immunity. Am. J. Clin. Nutr. 73(2): 444S-450S.
- Jin LZ, Ho YW, Abdullah N, Jalaludin S (1996). Influence of dried *Bacillus subtilis* and lactobacilli cultures on intestinal microflora and performance in broilers. Asian-australas. J. Anim. Sci. 9: 397-404.
- Jin LZ, Ho YW, Abdullah N, Jalaludin S (1997). Effect of adherent *Lactobacillus* culture on growth, weight of organs and

- intestinal microflora and volatile fatty acids in broilers. Anim. Feed Sci. Technol. 70: 197-209.
- Kirikci K, Tepeli C, Cetin O, Gunlu A, Yilmaz A (1999). Some production characteristics of Rock partridges (*A. graeca*) under different sheltering and lighting conditions. Vet. Bil. Derg. 15(1): 15-22.
- Kirikci K, Gunlu A, Cetin O, Garip M (2007). Effect of hen weight on egg production and some egg quality characteristics in the partridge (*Alectoris graeca*). Poult. Sci. 86(7): 1380-1383.
- Krawczyk J (2009). Quality of eggs from Polish native Greenland partridge chicken-hens maintained in organic vs. backyard production system. Anim. Sci. Papers Rep. 27(3): 227-235.
- Langhout P (2000). New additives for broiler chickens. World Poultry-Elsevier. 16(3): 22-27.
- Lyon TP (1987). Probiotics: an alternative to antibiotics. Pig. News Info. 8(2): 157-164.
- Mahdavi AH, Rahmani HR, Pourreza J (2005). Effect of probiotic supplements on egg quality and laying hens performance. Int. J. Poult. Sci. 4(4): 488-492.
- Mohan B, Kadirvel R, Bhaskaran M, Natarajan A (1995). Effect of probiotic supplementation on serum/yolk cholesterol and on eggshell thickness in layers. Br. Poult. Sci. 36(5): 799-803.
- Nahashon SN, Nakaue, HS, Mirosh LW (1994). Production variables and nutrient retention in Single Comb White leghorn laying pullets fed diets supplemented with direct-fed microbials. Poult. Sci. 73(11): 1699-1711.
- Ozbey O, Esen F (2007). The effects of different breeding systems on egg productivity and egg quality characteristics of rock partridges. Poult. Sci. 86(4): 782-785.
- Patterson JA, Burkholder K (2003). Application of prebiotics and probiotics in poultry production. Poult. Sci. 82(4): 627-631.
- Poyraz O (1989). Phenotypic correlations between egg characteristics about shell quality. J. Lalahan Livest. Res. Inst. 29(1-4): 66-79.
- Rolf RE (2000). The role of probiotic cultures in the control of gastrointestinal health. J. Nutr. 130(2): 396-402.
- Rueda MJ, Baragano JR, Notario A (1992). Alimentacion natural de la perdiz roja (*Alectoris rufa*). La Perdiz Roja, Gestion del Habitat. Fundacion La Caixa. Aedos, Barcelona, Spain. pp. 27-39.
- SAS Institute (1999). SAS User's Guide. Release 8.2 Ed. SAS Institute Inc, Cary, NC.
- Solomon SE (1991). Egg and eggshell quality. Wolfe Publishing Ltd. p. 149.
- Song KT, Choi SH, Oh HR (2000). A comparison of egg quality of pheasant, chukar, quail and guinea fowl. Asian Australasian. J. Anim. Sci. 13(7): 986-990.
- Suk YO, Park C (2001). Effect of breed and age of hens on the yolk to albumen ratio in two different genetic stocks. Poult. Sci. 80(7): 855-858.
- Sun X (2005). Broiler performance and intestinal alterations when fed drug-free diets. Thesis. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.
- Turan N (1990). Tukiye nin av ve Yaban Hayvanlari: Kuslar, Orman Gen. Mud. Egitim Dairesi Baskanligi Yayinlari, Ankara.
- Turtuero F (1973). Influence of implantation of *Lactobacillus acidophilus* in chicks on the growth, feed conversion, malabsorption of fats syndrom and intestinal flora. Poult. Sci. 52: 197-203.
- Turtuero F, Fernandez E (1995). Effect of inclusion of microbial culture in barley-based diets fed to laying hens. Anim. Feed. Sci. Technol. 53(3-4): 255-265
- Woodard AE (1982). Raising chukar partridges. Oakland: University of California Division of Agriculture and Natural Resources. CA 95616.
- Yannakopoulos AL, Tserveni-Gousi AS (1986). Quality characteristics of quail eggs. Br. Poult. Sci. 27(2): 171-176.