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Increased Egg Production of Japanese Quail (*Cortunix japonica*) by Improving Liver Function Through Turmeric Powder Supplementation

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Abstract: An experiment was conducted to study the effects of period of turmeric powder supplementation and ration quality on egg production in quails. One hundred and fifty female quails were assigned into a completely randomized design with a 2 x 5 factorial arrangement. The first factor was ration quality consisted of two levels i.e., ration with high carbohydrate (standard protein) and ration with high protein (standard carbohydrate) contents. The second factor was a period of turmeric supplementation (at a dosage of 54 mg/quail/day) consisted of 5 levels i.e. (1) Without turmeric supplementation (control) (2) 30 days at the age of 14 to 44 days (3) 30 days at the age of 45-75 days (4) 30 days at the age of 7-8 months and (5) 8.5 months at the age of 14 days to 9 months. Each experimental unit consisted of 15 quails. Parameters measured were feed and water consumptions, total number of egg production, follicle hierarchy, liver functions, oviduct weight and length and blood metabolites. The results showed that quails supplemented with turmeric for 30 days prior to sexual maturity and for 8.5 months started before sexual maturity gave the highest egg production (20 and 17%, respectively), liver function, vitellogenin secretion, follicle development and blood metabolites. Ration with high carbohydrate with standard protein content gave the best egg production, liver function and blood metabolites. It was concluded that improvement of liver functions could increase total egg production by increasing yolk precursors synthesis and their depositions in the developing follicles.

Key words: Turmeric powder, egg production, liver functions, follicle hierarchy, blood metabolite, Japanese quail

INTRODUCTION

Total number of egg production in fowl is determined by the rate of ovulation of mature and developing follicles in the ovary during sexual maturity and laying period. Ovulation of developing follicles, in turn, is determined by the maturation of the ovum which is determined by the availability and deposition of yolk components in the developing follicles. The initial decline in egg production is due to a reduction in the rate of recruitment of follicles into the yellow-yolky follicular hierarchy but towards the end of the laying year a steep drop in the rate of layoff individual birds may be caused by an increase in the incidence of follicular atresia (Williams and Sharp, 1978a, b). The availability and deposition of yolk components or ovogenesis during follicular development, in turn, is affected by the synthesis and production of vitellogenin by the liver cells (Deeley *et al.*, 1975; Burley *et al.*, 1993) under the stimulation of estrogen (Bergink *et al.*, 1974; Burns *et al.*, 1978; Geert *et al.*, 1976; Gruber *et al.*, 1976; Jost *et al.*, 1978; Williams and Sharp, 1978) produced by the developing follicles. The other components of egg were synthesized

by the cells of the other parts of oviduct (magnum, isthmus and shell gland) and were added into the ovulated ovum during the transport in the oviduct before oviposition. Albumen is a protein which is synthesized, excreted and accumulated in the epithelial cells and tubular gland cells in the magnum of the oviduct and the egg shell is synthesized in the uterus or shell gland. Therefore, egg production in fowl could be improved by improving the number (quantity) and synthetic activity (quality) of liver cells involved in vitellogenin synthesis. Production of vitellogenin by the liver is also affected by the availability of substrate and nutrients provided by the rations consumed by the fowls. The quality of ration fed to the fowl could affect the availability of substrate for egg synthesis that ultimately affect the growth and characteristics of eggs (Watson, 2002).

Curcumin is the active compound in turmeric powder which is shown to have a lot of molecular targets in the cells (Zhou *et al.*, 2011) that could affect cell functions in the body. Previous reports showed that the hepatoprotector activities of curcumin could prevent the

damage of liver cells (Aggarwal *et al.*, 2007; Gowda *et al.*, 2008; Inokuma *et al.*, 2012; Rivera-Espinoza and Muriel, 2009; Somanawat *et al.*, 2013; Somchit *et al.*, 2005; Yarru *et al.*, 2009). The ability of curcumin to inhibit several factors like nuclear factor-kappaB which modulates several pro-inflammatory and profibrotic cytokines as well as its anti-oxidant properties (Reyes-Gordillo *et al.*, 2007; Reyes-Gordillo *et al.*, 2008; Yarru *et al.*, 2009; Inokuma *et al.*, 2012; Somanawat *et al.*, 2013) provide a rational molecular basis to use it in hepatic disorders (Riviera-Espinoza and Muriel, 2009). Rangsz and Ahangaran (2011) reported that turmeric extract (*Curcuma longa*) could provide protection against the negative effects of aflatoxin on performance of broiler chickens. Early report showed that turmeric supplementation had no adverse effect on egg production and egg quality in laying hens (Keshavarz, 1976). However, Kermanshahi and Riasi (2006) reported the increase in blood lipid and egg lipid contents in laying hens supplemented with turmeric powder.

Functions of liver in laying fowls decrease with the increase age and with the advance of egg production. Our previous study in laying quails showed that at the initial decline in egg production and with the advance of laying period, functions of liver cells decreased as indicated by the increased serum SGPT and SGOT concentrations (Saraswati *et al.*, 2013a). Our previous study showed that the optimum concentration of turmeric powder supplementation to improve liver function was 54 mg/quail/day (Saraswati *et al.*, 2013c). Improvement of liver function by turmeric powder supplementation increased vitellogenin synthesis and follicular development as indicated by improvement of follicular hierarchy and egg production (Saraswati *et al.*, 2013c). This experiment was designed to study the effect of period of turmeric powder supplementation and ration quality on total number of egg production and quality in quails to obtain the best time of turmeric powder supplementation in improving egg production.

MATERIALS AND METHODS

Experimental design: One hundred and fifty female quails, age 14 days, were assigned into a completely randomized design with a 2 x 5 factorial arrangement. The first factor was the quality of ration consisted of two levels i.e. (1) ration with high carbohydrate and standard protein (22.67%) content (high carbohydrate ration or ration A) and ration with standard carbohydrate and high protein (25.16%) content (high protein ration or ration B). The second factor was the period of turmeric powder supplementation (54 mg/quail/day) consisted of 5 levels i.e. (1) Without turmeric supplementation during the experiment as a control (2) Turmeric supplementation for a month before sexual maturity (age 14-44 days) (3) Turmeric supplementation for a month during sexual

maturity (age 45-75 days) (4) Turmeric supplementation for a month at the age of 7-8 months (age of decreased egg production) and (5) Turmeric supplementation during the whole period of experiment (for 8.5 months at the age of 14 days to 9 months). Each experimental unit consisted of 15 quails. The experimental quails were supplemented with turmeric powder at a dosage of 54 mg/quail/day according to the duration of the treatment. The experimental quails were maintained until the age of 9 months. During the experiment, feed and drinking water were provided ad libitum. Feed intake and drinking water consumption were measured daily during the whole experiment (8.5 months). Egg production was measured during the whole experiment (age of 9 months). Egg weight, yolk index, egg shell index, haugh unit and chemical composition of the egg (fat, cholesterol and protein) were measured in the eggs laid at the ages of 9 months. At the age of 9 months, the experimental quails were sacrificed for measurement of liver parameters (weight, the liver histology, liver cell DNA and RNA content), ovaries and oviduct weights and blood samples were taken for measurement of vitellogenin, SGPT and SGOT, cholesterol and triglyceride concentrations.

Parameters measured: The eggs laid at the ages of 9 months were collected for determination of egg quality (yolk index, eggshell index and Haugh unit) and chemical composition (cholesterol, triglyceride and protein concentrations). Feed intake and daily drinking water consumption were measured daily. Body weights were measured at the beginning of the experiment (age 14 days) and at the end of experiment at the age of 9 months to obtain body weight gains during the whole experiment. At the age of 9 months, liver weight and percentage of liver weight to body weight, abdominal and pectoral fat weights (as indicator body fat deposition) were determined. At the age of 9 months, the weights of ovaries and oviduct and the lengths of magnum, isthmus and uterus were measured. The concentration of vitellogenin in the serum was determined by using stacking gel (Laemmli, 1970). The concentrations of SGPT and SGOT in the serum were measured by Reitman and Frankel procedure (Bigoniya *et al.*, 2009). Serum cholesterol concentrations were measured by using CHO-PAP method (Elwakkad *et al.*, 2012). Serum triglycerides concentrations were determined by GPO-PAP method (Bekal *et al.*, 2011). The diameter of hepatocyte was measured by paraffin method with haematoxylin and eosin staining (Anonymous, 2008). Liver DNA concentration (as an indicator of the number of cells per g liver tissue) and RNA concentration (as an indicator of synthetic activity per g liver tissue) were measured by method used by Manalu and Sumaryadi (1998). The hierarchy of follicles, egg weight, yolk index, eggshell index and haugh unit were determined by

method explained by Silversides (1994). Cholesterol concentration in the egg was determined by Liebermann Burchard procedure, protein concentration in the egg was determined by Kjeldahl and fat concentration of the egg was measured by Soxhlet method (Puwastien *et al.*, 2011).

Data analysis: The data obtained were analyzed using Analysis of Variance (ANOVA) for the effect of main factor (period of turmeric supplementation and feed quality) and their interaction followed Duncan test.

RESULTS

Egg production: Quails fed high carbohydrate ration had higher total number of egg production until 9 months of age as compared to those fed high protein ration ($p<0.05$) (Table 1). Supplementation of turmeric powder, regardless of period of administration, increased the total number of egg production until 9 months of age ($p<0.05$). There was an interaction between the period of turmeric powder administration and ration quality ($p<0.05$). Supplementation of turmeric powder in quails fed high protein ration did not improve total number of egg production ($p>0.05$). Supplementation of turmeric powder for 30 days prior to sexual maturity (at age of 14-44 days) and for 8.5 months started prior to sexual maturity (at the age of 14 days to 9 months) in the quails fed high carbohydrate ration gave the highest total number of egg production as compared to the other periods of turmeric powder supplementation. Quails fed high carbohydrate ration (ration A) and supplemented with turmeric powder for 30 days prior to sexual maturity had 20% higher egg production as compared to control (188 vs 157 in control). However, a longer period of turmeric powder supplementation did not significantly increase total number of egg production. Quails fed high carbohydrate ration and supplemented with turmeric powder for 8.5 months started before sexual maturity to the age of 9 months had 17% higher total number of egg production as compared to control (183 vs 157 in control).

The increased in total number of egg production did not decrease egg quality as indicated by egg weight, yolk index, egg shell index and haugh unit (Table 1). Turmeric powder administration and ration quality did not affect egg weight, yolk index, egg shell index and haugh unit. The effects of ration quality and turmeric powder supplementation on chemical composition of the egg were varied (Table 2). Carbohydrate and protein contents of the ration did not affect egg triglyceride concentration but quails fed high carbohydrate ration had higher egg cholesterol and protein concentrations ($p<0.05$). Turmeric powder supplementation for 30 days prior to sexual maturity (at the ages of 14-44 days) and during the decreased egg production period (age 7-8 months) decreased egg triglyceride concentration.

However, turmeric powder supplementation for 30 days during sexual maturity (age 44-75 days) and for 8.5 months (from age 14 days to 9 months) did not affect egg triglyceride concentration. Ration with high carbohydrate content increased cholesterol concentrations of the egg while turmeric powder supplementation, regardless of period of supplementation, decreased cholesterol concentrations of the egg ($p<0.05$). Ration quality and turmeric powder supplementation affected protein content of the egg ($p<0.05$). There was no interaction effect of ration quality and period of turmeric powder supplementation on egg protein concentrations. Quails fed high protein ration had lower egg protein content as compared to those fed with high carbohydrate and low protein ration. Turmeric powder supplementation for a month prior to sexual maturity, at sexual maturity and at the age of 7-8 months did not affect egg protein content. However, turmeric powder supplementation for 8.5 months from age 14 days to 9 months decreased protein content of the egg ($p<0.05$).

Reproductive organs: The increased total number of egg production in the experimental quails fed different qualities of ration and supplemented with turmeric powder was also supported by the processes and parameters of folliculogenesis and oogenesis. Quails with the highest total number of egg production (quails supplemented with turmeric powder before sexual maturity) had also the highest ovarian weight ($p<0.05$) (Table 3). However, quails supplemented with turmeric powder with a longer period (8.5 months) from 14 days to 9 months had lower ovarian weight as compared to control. Quails supplemented with turmeric powder for 30 days at the ages of 45-75 days and 7-8 months numerically had increased ovarian weight ($p>0.05$). Surprisingly, in this experiment, the protein and carbohydrate contents of the ration did not affect the ovarian weight.

Turmeric powder supplementation for 30 days prior to sexual maturity (14-44 days) and at the period of decreased egg production at the age of 7-8 months and for 8.5 months from the age of before sexual maturity (at the age of 14 days to 9 months) increased the number developing follicles in the ovary ($p<0.05$). Parallel to the highest total number of egg production, turmeric powder supplementation prior to sexual maturity gave the highest number of developing follicles in the ovary (Fig. 1). In contrast, administration of turmeric powder during sexual maturity (at the age 45-75 days) did not significantly increased ($p>0.05$) the number of developing follicles in the ovary. Surprisingly, the protein and carbohydrate contents of the ration fed to the experimental quails did not affect the number of developing follicles and the diameters of F1 follicles in the ovary. Turmeric powder supplementation, regardless

Table 1: Total number of eggs, egg weight, yolk index, egg shell index, Haugh unit in 9- month quails supplemented with turmeric powder at different period of times and fed high carbohydrate or high protein ration

	High carbohydrate ration					High protein ration				
	Control	C	D	E	F	Control	C	D	E	F
Total number of eggs	157±2.3 ⁱ	188±1.67 ^a	151±5.72 ^a	167±5.3 ^b	183±9.7 ^a	166±9.61 ^b	164±1.79 ^b	169±1.23 ^b	165±5.5 ^b	160.844 ^{cd}
Egg weight (g)	9.9±0.60	10.60±0.60	10.54±0.62	10.36±0.63	10.28±0.66	10.48±0.63	10.89±0.54	10.54±0.85	10.53±0.70	10.81±0.48
Yolk index	0.43±0.02	0.43±0.03	0.41±0.04	0.44±0.04	0.44±0.06	0.42±0.04	0.42±0.03	0.44±0.02	0.43±0.03	0.44±0.02
Eggshell index	6.84±0.47	6.96±0.8	7.15±0.56	6.98±0.65	7.12±0.53	7.18±0.33	7.52±0.16	7.22±0.59	7.19±0.46	7.28±0.56
Haugh unit	92.22±2.56	91.32±7.62	93.42±3.44	93.65±3.77	94.99±4.37	93.18±2.69	94.39±3.51	96.02±3.27	93.90±4.44	94.35±3.35

Different superscripts in the same row indicate significant differences (p<0.05).

C: Age of 14-44 days, D: Age of 44-75 days, E: Age 7-8 month, F: Age of 14 days-9 month

Table 2: Triglyceride, cholesterol, and protein concentrations (mg/dL) of eggs layed at the age of 9 months in quails supplemented with turmeric powder at different period of times and fed high carbohydrate or high protein ration

	High carbohydrate ration					High protein ration				
	Control	C	D	E	F	Control	C	D	E	F
Triglyceride (mg/dL)	12.44±0.45 ^a	11.26±0.34 ^{cd}	12.16±0.25 ^{ab}	11.08±0.26 ^{cd}	11.87±0.26 ^b	12.82±0.51 ^a	12.68±0.3 ^a	11.52±0.39 ^a	11.03±0.51 ^d	13.04±0.19 ^a
Cholesterol (mg/dL)	460.24±5.74 ^a	432.27±2.83 ^b	401.43±15.08 ^c	374.23±5.74 ^d	359.68±5.05 ^d	426.34±6.02 ^b	395.04±7.64 ^c	355.75±17.93 ^d	340.78±5.26 ^d	338.61±6.21 ^d
Protein (mg/dL)	12.51±0.42 ^{ab}	12.9±0.09 ^a	12.47±0.22 ^{ab}	12.8±0.34 ^a	12.53±0.89 ^{ab}	12.65±0.4 ^a	11.22±0.21 ^c	11.97±0.52 ^b	11.36±0.06 ^b	10.90±0.2 ^a

Different superscripts in the same row indicates significant differences (p<0.05)

C: Age of 14-44 days, D: Age of 44-75 days, E: Age 7-8 month, F: Age of 14 days-9 month

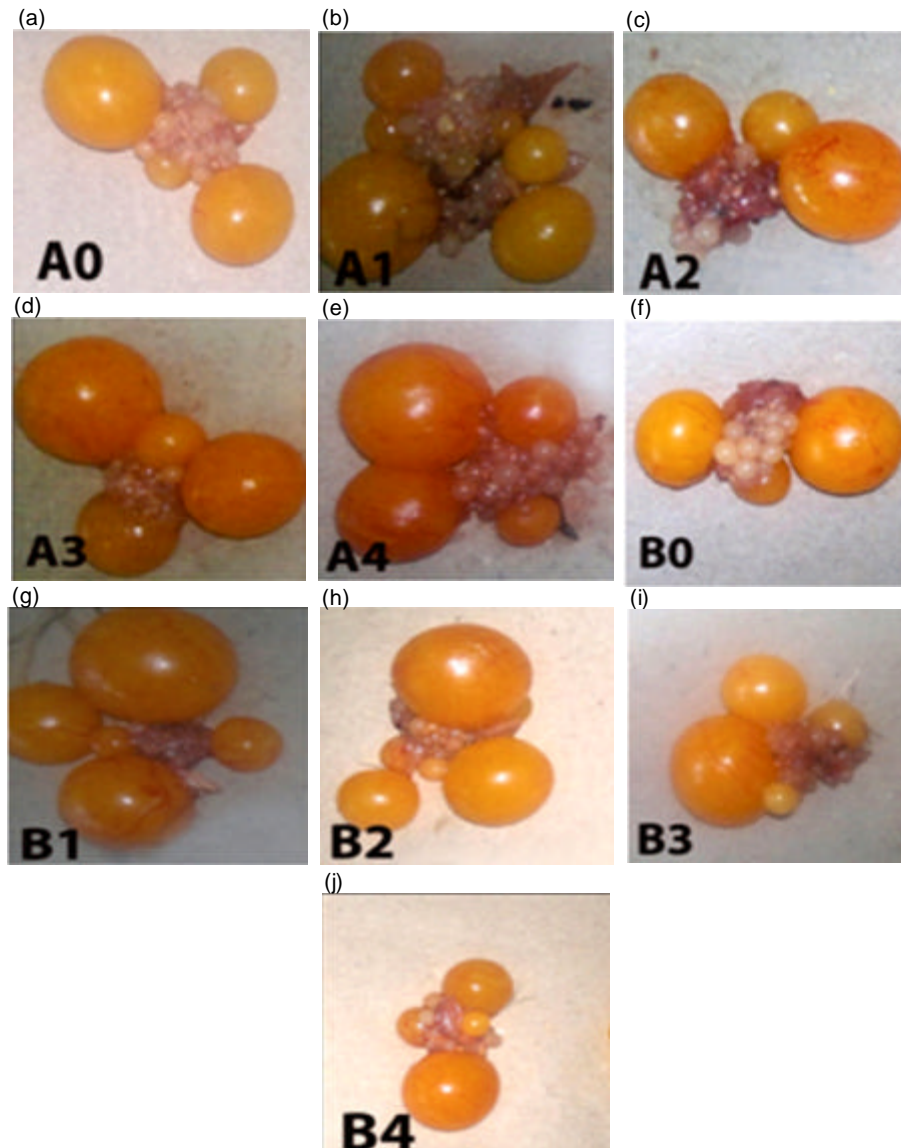


Fig. 1(a-j): Follicular hierarchies in the ovaries of quails at the age of 9 months fed high carbohydrate (ration A) or high protein (ration B) and supplemented with turmeric powder. (a) A0: the ovary of quail fed high carbohydrate ration (ration A) without turmeric powder supplementation during 9 months. (b) A1: the ovary of quail fed high carbohydrate ration (ration A) with turmeric powder supplementation for 30 days prior to sexual maturity (age 14-44 days). (c) A2: the ovary of quail fed high carbohydrate ration (ration A) with turmeric powder supplementation for 30 days during sexual maturity (age 45-75 days). (d) A3: the ovary of quail fed high carbohydrate ration (ration A) with turmeric powder supplementation for 30 days during the decreased egg production (age 7-8 months). (e) A4: the ovary of quail fed high carbohydrate ration (ration A) with turmeric powder supplementation for 8.5 months (age 14 days to 9 months). (f) B0: the ovary of quail fed high protein ration (ration B) without turmeric powder supplementation during 9 months. (g) B1: the ovary of quail fed high protein ration (ration B) with turmeric powder supplementation for 30 days prior to sexual maturity (age 14-44 days). (h) B2: the ovary of quail fed high protein ration (ration B) with turmeric powder supplementation for 30 days during sexual maturity (age 45-75 days). (i) B3: the ovary of quail fed high protein ration (ration B) with turmeric powder supplementation for 30 days during the decreased egg production (age 7-8 months). (j) B4: the ovary of quail fed high protein ration (ration B) with turmeric powder supplementation for 8.5 months (age 14 days to 9 months)

of period of administration, increased ($p < 0.05$) the diameter of F1 while the ration quality did not affect ($p > 0.05$) the diameter of F1. Turmeric powder supplementation prior to sexual maturity gave the highest diameter of F1 followed by the supplementation for 8.5 months from 14 days to 9 months. The increased diameter of F1 follicle was a result of the rate of yolk deposition during ovogenesis. The increased ovarian weight and the diameter of F1 follicle were not parallel with the growth of the oviduct. The highest total number of egg production in quails fed high carbohydrate ration and supplemented with turmeric powder prior to sexual maturity was primarily associated with the increased egg yolk synthesis and deposition during ovogenesis before ovulation. Ration quality and turmeric powder supplementation did not affect the growth of the oviduct of the experimental quails as were indicated by the non significant difference in oviduct weight and length ($p > 0.05$).

Growth: The increased total number of egg production in quails supplemented with turmeric powder prior to sexual maturity did not related to the effect on feed consumption. In general, regardless of the period of supplementation, turmeric powder supplementation did not affect daily feed consumption, body weight gain and body fat deposition ($p > 0.05$) (Table 4). However, quails supplemented with turmeric powder prior to sexual maturity had higher water consumption ($p < 0.05$) during the whole experiment. In addition, the quality of ration, high carbohydrate or high protein ration, did not affect daily feed consumption, body weight gain and drinking water consumption ($p > 0.05$). However, quails fed with ration with high carbohydrate and standard protein content had higher abdominal and pectoral fat ($p < 0.05$). There was no interaction effect between the time of turmeric powder supplementation and ration quality on daily feed consumption, drinking water consumption, body weight gain and body fat deposition.

Liver function: The increased total number of egg production in quails supplemented with turmeric powder was correlated with the improved liver function, especially in the total number of liver cell per weight of tissue and therefore the total capacity of the hepatocytes to synthesize the substrates for egg yolk synthesis. In general, ration quality and turmeric powder supplementation, regardless of period of supplementation, did not affect liver weight measured at the age of 9 months. However, quails supplemented with turmeric powder prior to sexual maturity had the highest ratio of liver weight to body weight ($p < 0.05$) (Table 5). The number of cell per gram liver tissue, as shown by the DNA content, increased in the quails supplemented with turmeric powder and fed with high carbohydrate and standard protein ration (ration A).

Quails fed ration with high protein and standard carbohydrate content had higher liver cell number when supplemented with turmeric powder prior to sexual maturity and at the age of 7-8 month (during the declining productivity after peak production). Quails fed ration with high carbohydrate ration had higher liver cell number when supplemented with turmeric powder, regardless of period of supplementation. However, turmeric powder supplementation and ration quality did not affect the synthetic activity of the liver cells as was shown by the concentration of RNA per gram liver tissue ($p > 0.05$). The highest percentage of liver weight/body weight ratio and liver DNA content was found in quails supplemented turmeric powder before sexual maturity. These data showed that the increase in total number egg production in quails supplemented with turmeric powder prior to sexual maturity was related to the increased total synthetic capacity of the hepatocytes to produce substrates required for yolk deposition.

The improved liver function in quails supplemented with turmeric powder was confirmed by the decrease in serum SGPT and SGOT concentrations (Table 6). Turmeric powder supplementation decreased ($p < 0.05$) serum concentrations of SGPT and SGOT and supplementation at the age of 7-8 months gave the best result in serum SGPT concentrations. However, supplementation of turmeric powder for 8.5 months from age of 14 days to 9 months gave the best result in the decreased serum SGOT concentrations. Quails fed ration with high protein content had a better liver function, regardless of turmeric powder supplementation, as indicated by the lower serum concentrations of SGPT and SGOT. There was an interaction effect between ration quality and turmeric powder supplementation on serum SGPT and SGOT concentrations.

Quails with the highest total number of egg production (quails supplemented with turmeric powder prior to sexual maturity) were also had the highest vitellogenin synthesis and secretion into the blood (Table 6). The increased liver cell number and functions in quails supplemented with turmeric powder prior to sexual maturity (14-44 days) increased vitellogenin synthesis by the liver cells as indicated by the increased vitellogenin concentrations in the serum ($p < 0.05$). However, turmeric powder supplementation at the age of sexual maturity (45-75 days) reduced serum vitellogenin concentration ($p < 0.05$). Turmeric powder supplementation at the age of 7-8 months and at the ages of 14 days to 9 months did not affect serum vitellogenin concentration. Quails fed high carbohydrate ration had higher serum vitellogenin concentrations as compared to those fed high protein ration.

Liver histology (Fig. 2) also showed that quails fed high carbohydrate ration (ration A) had higher hepatocyte cytoplasmic vacuoles as compared to those fed ration B (ration with high protein content). Quails fed high

Table 3: Ovarian weights, weight and length of the oviduct, the number of follicles, and the diameter of F1 at the age of 9 months in quails supplemented with turmeric powder at different period of times and fed high carbohydrate or high protein ration at the age of 9 months

	High carbohydrate ration					High protein ration				
	Control	C	D	E	F	Control	C	D	E	F
Ovarian weight (g)	4.35±1.16 ^a	6.29±1.31 ^a	4.67±1.65 ^{ab}	5.44±1.35 ^{ab}	5.22±1.09 ^{ab}	5.26±1.90 ^{ab}	5.61±1.26 ^a	5.74±1.86 ^a	4.99±1.22 ^{ab}	3.47±1.78 ^b
Oviduct weight (g)	8.6±1.65	9.19±1.75	9.36±6.30	6.14±1.76	6.89±1.58	8.49±1.92	7.59±1.49	8.20±2.45	7.59±2.17	8.31±3.9
Oviduct length (cm)	30.68±5.18	27.30±4.05	28.11±8.10	26.77±4.95	28.31±3.52	31.08±4.89	36.12±9.85	26.90±3.72	26.79±4.13	36.85±8.88
A*	4.25±0.71 ^c	5.75±0.71 ^{ab}	3.25±1.39 ^d	5.90±1.29 ^{ab}	5.85±1.52 ^{ab}	3.2±1.3 ^a	6.07±1.12 ^a	5.07±0.9 ^{bc}	5.14±0.9 ^{bc}	4.33±1.22 ^{cd}
Diameter of (F1) (cm)	1.45±0.14 ^d	1.71±0.07 ^{bc}	1.65±0.03 ^{cd}	1.76±0.18 ^{abc}	1.93±0.21 ^a	1.53±0.19 ^{cd}	1.86±0.15 ^{ab}	1.78±0.26 ^{abc}	1.66±0.25 ^{bcd}	1.47±0.43 ^d

Different superscripts in the same row indicate significant differences (p<0.05).

A*: The number of developing follicles, C: Age of 14-44 days, D: Age of 44-75 days, E: Age 7-8 month, F: Age of 14 days-9 month

Table 4: Daily feed consumption, body weight gain, pectoral and abdominal fat weight and water consumption of 9-months quails supplemented with turmeric powder at different period of times and fed high carbohydrate or high protein ration

	High carbohydrate ration					High protein ration				
	Control	C	D	E	F	Control	C	D	E	F
DFI (g)	28.42±7.09	29.18±5.49	32.13±7.64	32.77±12.06	31.44±7.24	36.78±16.08	35.11±7.28	32.34±6.98	42.3±13.98	26.78±10.4
BWG (g)	142.73±14.89	155.0±17.16	146.67±17.32	147.27±31.65	155.0±15.67	146.67±14.14	153.64±12.86	144.62±16.64	148.57±3.78	138.89±22.61
PAFW (g)	4.26±2.57 ^{bc}	4.14±2.38 ^{bc}	5.1±3.22 ^{abc}	5.42±2.61 ^{ab}	5.59±2.47 ^a	3.53±2.25 ^c	5.41±2.66 ^{ab}	3.20±2.39 ^c	3.19±1.23 ^c	2.26±0.89 ^c
DWC (ml)	45.53±6.39 ^c	65.01±10.49 ^a	49.37±12.09 ^c	59.38±8.05 ^{ab}	57.38±10.51 ^{ab}	45.85±3.79 ^c	55.08±3.76 ^{abc}	54.21±5.39 ^{abc}	56.74±0.77 ^{abc}	53.30±8.06 ^{abc}

Different superscripts in the same row indicate significant differences (p<0.05).

C: Age of 14-44 days, D: Age of 44-75 days, E: Age 7-8 month, F: Age of 14 days-9 month. DFI: Daily feed intake, BWG: Body weight gain, PAFW: Pectoral and abdominal fat weight, DWC: Daily water consumption

Table 5: Percentage of liver weight/body weight, liver weight, DNA and RNA contents of the liver at the age of 9 months in quails supplemented with turmeric powder at different period of times and fed high carbohydrate or high protein ration

	High carbohydrate ration						High protein ration					
	Control	C	D	E	F	Control	C	D	E	F		
LWBW	2.84±0.41 ^{abc}	2.99±0.5 ^{ab}	2.84±0.54 ^{abc}	2.6±0.72 ^{bc}	2.37±0.49 ^c	2.9±0.92 ^{ab}	3.32±0.82 ^a	2.57±0.67 ^{bc}	2.58±0.68 ^{bc}	2.69±0.62 ^{bc}		
LW (g)	4.88±0.82	5.22±0.86	5.62±1.48	4.66±1.77	4.59±1.44	4.53±1.07	5.35±1.70	5.59±1.04	4.53±1.21	4.67±1.27		
A*	2.61±0.35 ^{ab}	2.66±0.13 ^a	2.66±0.09 ^a	2.59±0.14 ^{ab}	2.67±0.09 ^a	2.14±0.17 ^c	2.65±0.29 ^a	2.28±0.17 ^c	2.64±0.16 ^{ab}	2.40±0.13 ^b		
B*	50.04±2.82	50.45±2.81	50.8±3.07	52.78±3.46	55.16±2.88	54.75±1.25	48.2±1.25	46.59±1.8	47.34±1.02	46.28±1.22		

Different superscripts in the same row indicate significant differences ($p < 0.05$).

A*: Liver DNA concentration (mg/g tissue), B*: Liver RNA concentration (mg/g tissue), C: Age of 14-44 days, D: Age of 44-75 days, E: Age 7-8 month, F: Age of 14 days-9 month.

LWBW: Liver weight/body weight, LW: Liver weight

Table 6: Levels of vitellogenin, SGPT, SGOT, glucose, triglycerides, cholesterol and protein in the blood of 9-month quails supplemented with turmeric powder at different period of times and fed high carbohydrate or high protein ration

	High carbohydrate ration						High protein ration					
	Control	C	D	E	F	Control	C	D	E	F		
Vitellogenin (mg/ml)	11.79±3.54 ^c	37.40±4.68 ^a	13.44±7.69 ^a	17.15±3.74 ^d	28.70±2.97 ^b	27.89±5.75 ^b	24.54±5.01 ^{bc}	14.74±3.13 ^b	18.71±2.91 ^c	7.55±3.89 ^c		
SGPT (U/L)	36.99±0.34 ^a	34.98±0.32 ^c	36.15±0.15 ^c	34.24±0.45 ^d	35.91±0.24 ^b	35.71±0.47 ^b	33.47±0.49 ^c	34.52±0.45 ^d	33.03±0.26 ^d	32.67±0.28 ^d		
SGOT (U/L)	32.39±0.31 ^a	31.94±0.39 ^{ab}	33.56±0.2 ^a	31.35±0.17 ^b	32.82±0.39 ^a	31.92±0.25 ^{ab}	31.61±0.64 ^{bc}	30.36±0.21 ^c	32.07±0.31 ^{ab}	30.28±0.2 ^c		
Glucose (mg/dl)	134.5±28.29 ^a	262.0±32.72 ^a	189.5±4.04 ^d	112.5±32.15 ^c	241.0±0.13 ^b	209.0±22.33 ^c	200.5±20.25 ^d	230.00±30.23 ^b	75.5±7.51 ^c	254.5±0.29 ^{ab}		
Triglyceride (mg/dl)	119.68±1.48 ^a	117.88±0.26 ^c	115.19±1.03 ^d	109.94±0.36 ^c	106.78±0.61 ^b	122.52±0.88 ^a	116.98±0.33 ^c	115.16±1.01 ^d	111.80±0.35 ^c	110.58±0.36 ^c		
Cholesterol (mg/dl)	137.04±0.42 ^a	127.13±1.43 ^c	119.10±0.72 ^a	106.21±1.11 ^a	102.16±1.46 ^b	129.39±0.83 ^b	124.25±1.57 ^a	114.50±0.81 ^c	105.69±0.59 ^b	97.54±1.63 ^c		
Protein (mg/dl)	10.14±0.28 ^c	10.91±0.14 ^d	9.92±0.31 ^c	10.94±0.31 ^d	11.50±0.24 ^c	10.40±0.19 ^c	11.58±0.37 ^b	11.97±0.32 ^b	12.13±0.26 ^b	12.90±0.14 ^a		

Different superscripts in the same row indicate significant differences ($p < 0.05$).

C: Age of 14-44 days, D: Age of 44-75 days, E: Age 7-8 month, F: Age of 14 days-9 month

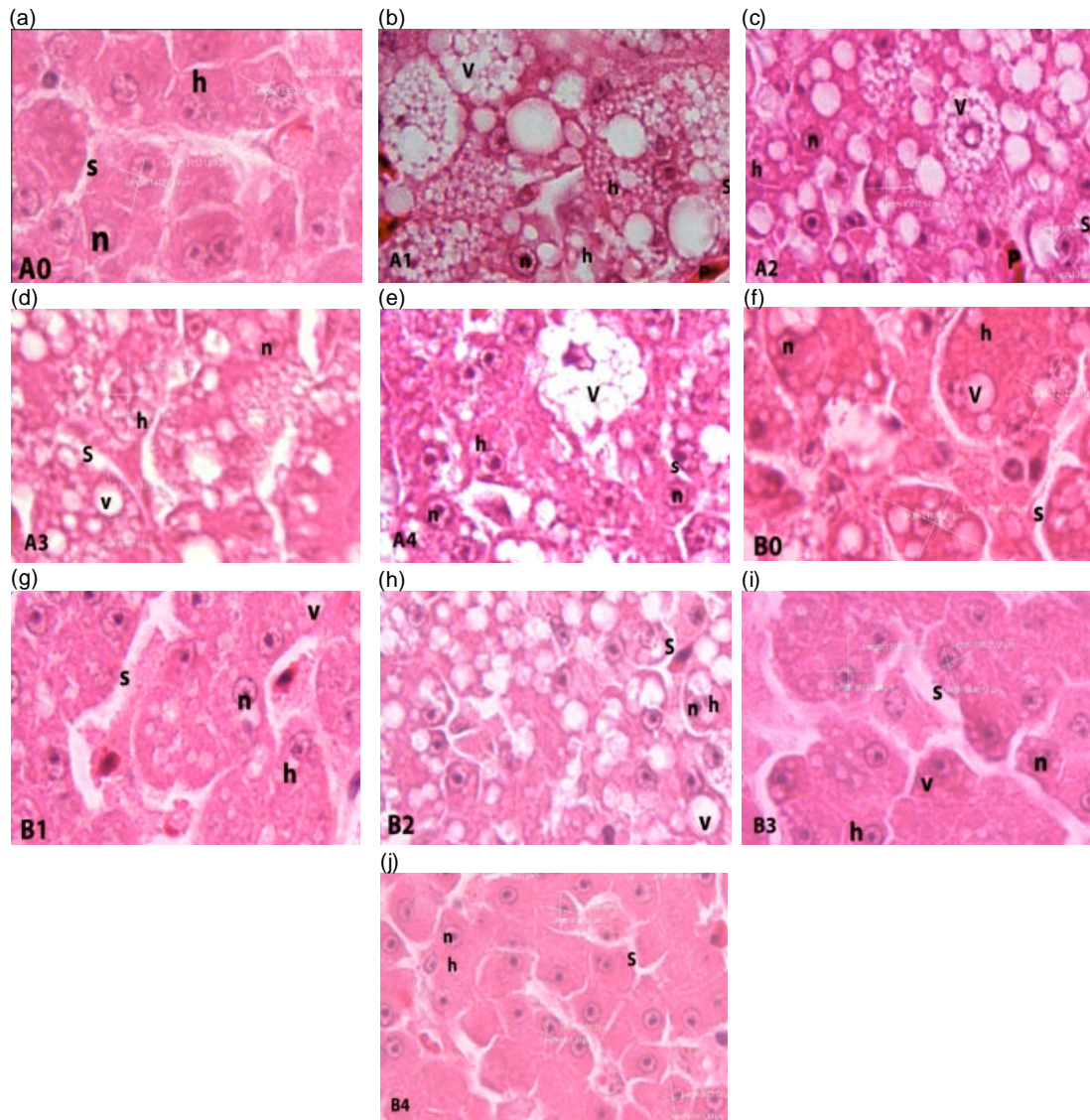


Fig. 2(a-j): Hepatocytes of quails at the age of 9 months fed high carbohydrate (ration A) or high protein (ration B) and supplemented with turmeric powder. (a) A0: the hepatocytes of quail fed high carbohydrate ration (ration A) without turmeric powder supplementation during 9 months. (b) A1: the hepatocytes of quail fed high carbohydrate ration (ration A) with turmeric powder supplementation for 30 days prior to sexual maturity (age 14-44 days). (c) A2: the hepatocytes of quail fed high carbohydrate ration (ration A) with turmeric powder supplementation for 30 days during sexual maturity (age 45-75 days). (d) A3: the hepatocytes of quail fed high carbohydrate ration (ration A) with turmeric powder supplementation for 30 days during the decreased egg production (age 7-8 months). (e) A4: the hepatocytes of quail fed high carbohydrate ration (ration A) with turmeric powder supplementation for 8.5 months (age 14 days to 9 months). (f) B0: the hepatocytes of quail fed high protein ration (ration B) without turmeric powder supplementation during 9 months. (g) B1: the hepatocytes of quail fed high protein ration (ration B) with turmeric powder supplementation for 30 days prior to sexual maturity (age 14-44 days). (h) B2: the hepatocytes of quail fed high protein ration (ration B) with turmeric powder supplementation for 30 days during sexual maturity (age 45-75 days). (i) B3: the hepatocytes of quail fed high protein ration (ration B) with turmeric powder supplementation for 30 days during the decreased egg production (age 7-8 months). (j) B4: the hepatocytes of quail fed high protein ration (ration B) with turmeric powder supplementation for 8.5 months (age 14 days to 9 months). h: hepatocyte, n: nucleus, s: sinusoid, v: vacuola

carbohydrate ration without turmeric powder supplementation (A0) showed no vacuoles in the cytoplasm of the hepatocytes. This result indicated a lower synthetic activity of the hepatocyte. Parallel to the other observations, quails supplemented with turmeric powder before sexual maturity had the highest vacuoles in the cytoplasm as an indicator of high activity in synthesis of yolk precursor. Turmeric powder supplementation at the age of decreased egg production (age 7-8 months) and fed with ration with high carbohydrate (A3) showed high number of vacuoles in the cytoplasm as an indicator of improved liver function. Supplementation of turmeric powder for 8.5 months from the age of 14 days to 9 months, showed the large number of vacuoles in the hepatocytes.

Blood chemistry: The serum glucose concentration was higher ($p<0.05$) in quails fed high protein ration as compared to those fed high carbohydrate ration (Table 6). However, there was an interaction between ration quality and turmeric powder supplementation on serum glucose concentrations. Turmeric powder supplementation for 30 days prior to sexual maturity (age of 14-44 days) and for 8.5 months at the ages of 14 days to 9 months increased serum glucose concentrations ($p<0.05$). Turmeric powder supplementation at the age of 45-75 days did not significantly increase serum glucose concentration ($p>0.05$). In contrast, turmeric powder supplementation at the age of 7-8 months significantly decreased serum glucose concentration ($p<0.05$).

Turmeric powder supplementation, regardless of period of supplementation, decreased ($p<0.05$) serum triglyceride concentration in the experimental quails. Quails fed high protein ration had higher serum triglyceride concentrations as compared to those fed high carbohydrate ration. There is an interaction effect between ration quality and the period of turmeric powder administration on serum triglyceride concentrations. The highest decline in serum triglyceride concentrations was found in quails supplemented with turmeric powder for the longest duration i.e., for 8.5 months at the age 14 days to 9 months.

Supplementation of turmeric powder in quails decreased ($p<0.05$) serum cholesterol concentrations. Quails fed high carbohydrate ration had higher serum cholesterol concentrations ($p<0.05$) as compared to those fed high protein ration. There is an interaction between ration quality and the period of turmeric powder supplementation on the serum cholesterol concentrations. Supplementation of turmeric powder for around 8.5 months at the age of 14 days to 9 months gave the highest decrease in serum cholesterol concentrations.

Turmeric powder supplementation, regardless of duration and period of supplementation, decreased

serum protein concentration ($p<0.05$). Quails fed high protein ration had higher serum protein concentrations ($p<0.05$) as compared to those fed high carbohydrate ration. There was an interaction between ration quality and period of turmeric powder supplementation on serum protein concentrations.

DISCUSSION

The results of this experiment confirmed that turmeric powder supplementation before sexual maturity could improve hepatocytes functions in laying quails for 9 months. This implies that the effects of curcumin in turmeric powder not only improves liver disorders or damage (Aggarwal *et al.*, 2007; Rivera-Espinoza and Muriel, 2009) but also protect and prevent liver damage with the advance of age and egg production in fowls (Saraswati *et al.*, 2013a) as was also reported in rats (Reves-Gordillo *et al.*, 2007, 2008). The improvement of hepatocyte functions and activities by turmeric powder supplementation prior to sexual maturity could improve vitellogenin synthesis during sexual maturity and egg laying period. With normal estradiol and progesterone secretions during laying period (Saraswati *et al.*, 2013b), improvement of total number of functional hepatocytes in the quails supplemented with turmeric powder consequently increases the total number of egg production through the improvement of vitellogenin synthesis and deposition in the developing follicles. The highest increase in total number of egg production in quails supplemented with turmeric powder prior to sexual maturity could be explained by the effect of turmeric powder supplementation on liver cell functions. Quails supplemented with turmeric powder had higher concentrations of cell number per weight of liver tissue with higher number of cytoplasmic vacuoles of hepatocytes and lower serum SGPT and SGOT concentrations as indicator of a better liver cell structure and functions (Saraswati *et al.*, 2013c). The improved liver cell structure and function was also shown in the increased vitellogenin concentration in the serum as a precursor for egg yolk deposition in the developing follicle before ovulation. The increased egg yolk deposition increased the number of developing follicle and the diameter of F1 and the weight of the ovary.

However, the increased total number of egg production in quails supplemented with turmeric powder prior to sexual maturity was not related to the increased secretion of estrogen and progesterone or the estrogenic effect of curcumin on the reproductive tracts and liver functions during sexual maturity and egg production. Turmeric powder supplementation in laying hens also increases follicular growth and development and shortens the time from two consecutive oviposition without any change in progesterone secretions but slightly depresses estrogen secretion (Saraswati *et al.*, 2013b). Even though the progesterone and estrogen

profiles in the experimental quails were not measured, the effects of turmeric powder supplementation on the increased total number of egg production was not through the increase in progesterone and estrogen secretions or phytoestrogen in the turmeric powder since the weight and length of oviduct (infundibulum, magnum, isthmus and shell gland) were not changed in quails supplemented with turmeric powder. The growth and development of the infundibulum, magnum, isthmus and uterus are controlled by estrogen and progesterone. Quails supplemented with 54 mg turmeric powder per day for 30 days prior to sexual maturity produced the highest total number of egg for 9 months without any change in length and weight of the reproductive tract. Even though there was no effect of turmeric powder supplementation on the weight and length of the reproductive tracts, the functions of magnum and uterus in producing albumen and egg shell, respectively, did not change. Egg weight, yolk index, haugh unit, egg shell index and protein concentrations of the egg did not decrease with the increased total number of egg production by the turmeric supplementation. Egg protein primarily contributed by the albumen so the function of the epithelial cells of the magnum was not affected by the turmeric powder administration.

In general, high carbohydrate ration with standard protein content had a better effect on egg production as compared to standard carbohydrate with high protein ration. This effect could be related to the high cholesterol concentration in the quails fed with high carbohydrate and standard protein ration. Increased intake of glucose in high carbohydrate ration could stimulate lipogenic enzymes gene expression (Girard *et al.*, 1994; Fofelle *et al.*, 1996; Girard *et al.*, 1997; Kahn, 1997) that finally increased lipid and cholesterol synthesis in the liver. Cholesterol is also a marker of synthetic activity of the liver cells under the stimulation of estrogen during egg production. Even though high protein ration increased serum glucose, triglyceride and protein concentrations, these increases did not correlate well with the increased ovogenesis as indicated by the increase in follicle hierarchy and total number of egg production. In this experiment, the effect of ration quality on serum glucose, triglyceride and cholesterol concentrations was primarily due to the absorption of the nutrient in the intestine and their availabilities in the circulation while turmeric powder supplementation was assumed to increase nutrient uptake by the liver from circulation for vitellogenin synthesis (Deeley *et al.*, 1975). In laying hens, there is an indication that turmeric powder decreases nutrient absorption in the epithelial cells of the intestine and this effect implies that turmeric powder supplementation could be used as a feed supplement in laying hens for manipulating egg composition (Kermanshahi *et al.*, 2006).

Administration of turmeric powder before sexual maturity (age 14 days) was shown to have the best effect on liver functions and egg production. It was assumed that active compounds in the turmeric powder could stimulate hepatocyte growth and decreased hepatocyte destruction. Curcuminoids in turmeric powder has antihepatotoxic effect, due to the nature of the compound that inhibited lipid peroxidation in the cell membrane and protects hepatocytes by inhibiting NF-kappaB, proinflammatory cytokines production and oxidative stress (Reyes-Gordillo *et al.*, 2007) and prevents and reverses cirrhosis (Reyes-Gordillo *et al.*, 2008) in the rats. Curcumin acts as a free radical scavengers, inhibits the generation of reactive oxygen species such as superoxide anion, H₂O₂, nitrite radicals by activating macrophages that play an important role in the inflammatory process (Chattopadhyay *et al.*, 2004). Curcumin has antioxidant activity by inhibiting the activity of inflammatory enzymes or by increasing the synthesis of glutathione (Sreejayan and Rao, 1996). The role of turmeric powder is also supported by liver histology that showed that no damage to hepatocytes (Fig. 2). Curcumin found in turmeric powder can function as hepatocyte growth factor (Aggarwal *et al.*, 2007) to stimulate hepatocyte growth and development and as a hepatoprotector agent to protect the integrity of the hepatocytes and modulate the growth and cellular responses (Somchit *et al.*, 2005) and to recure the acute liver cell damage by CCl₄ (Park *et al.*, 2000; Reyes-Gordillo *et al.*, 2007, 2008). Therefore, quails supplemented with turmeric powder prior to sexual maturity or during 8.5 months before sexual maturity had the best liver cell number and function which support vitellogenin synthesis for yolk deposition which in turn reflected in the best follicular growth and development (Fig. 1) and the best total number of egg production. In general, the improved total number of egg production in quails supplemented with turmeric powder was not through the increased feed intake and nutrient availability for egg synthesis since turmeric powder supplementation decreased triglyceride, cholesterol and protein concentrations in the serum. Graham (2009) who reported that curcumin could increase the activity of lipoprotein lipase which further increases fat absorption by the cells. This is probably a reason of the effects of curcumin on decreasing levels of triglycerides in the blood. Turmeric powder also showed hypolipidemic effects by inhibiting the secretion of hepatic triglyceride (Chattopadhyay *et al.*, 2004). The decreased serum concentrations of triglyceride, cholesterol and protein could be a result of high uptake by the liver for vitellogenin synthesis or the decrease in absorption in the intestine. Cholesterol is obtained from food or synthesized through a pathway present in virtually all cells of the body. Cholesterol is a precursor for the synthesis of acetyl CoA which can be formed from

glucose, fatty acids or amino acids (Marks *et al.*, 1996). However, quails with the higher serum glucose concentrations during turmeric powder supplementation had also the highest total number of egg production. The increase in serum glucose concentration and a high total number of egg production was not related to the increased feed intake since feed intake was not affected by the turmeric powder supplementation. The high serum glucose concentration in quails with the highest total number of egg production was probably due to the increased gluconeogenesis in the liver. Curcumin has a similar effect of insulin which increases blood glucose homeostasis (Seo *et al.*, 2008). Turmeric powder can reduce 50% of chronic diabetes in mice (Ralet *et al.*, 2010). Quails fed ration with high carbohydrate and supplemented with turmeric powder prior to sexual maturity had the highest serum glucose concentration and total number of egg production. The higher body fat weight in the quails fed high carbohydrate ration was probably due to the higher carbohydrate content. Excess of carbohydrates in the ration can be converted into fat stored in liver and adipose tissue (Girard *et al.*, 1994; Foulle *et al.*, 1996; Girard *et al.*, 1997; Kahn, 1997). Glucose can also be converted into cholesterol (Marks *et al.*, 1996). Triglycerides, cholesterol and protein are used as constituents of vitellogenin in the form of VLDL particles, via endocytosis is used to prepare the yolk (Deeley *et al.*, 1975; Watson, 2002). The increased in drinking water consumption in quails supplemented with turmeric powder before sexual maturity was not clear, however could be related to the increase in egg production since at the time of eggshell formation, there is an increase in water demand (Sauveur and Mongin, 1978).

Conclusion: Based on the results of this study it was concluded that turmeric powder supplementation prior to sexual maturity could be used to increase total number of egg production in quails by improvement in liver function and synthetic activity with the increased vitellogenin synthesis and follicular growth and development. Ration with high carbohydrate and standard protein had better effect on egg production. Turmeric powder supplementation in laying fowls could be used to produce low cholesterol eggs.

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