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The Effect of Dietary Energy and Protein Levels on Growth Performance and Antibody Responses of Offspring of Laying Japanese Quails

Research Article

M. Aami Azghadi[1*], H. Kermanshahi[1] and A. Golian[1]

[1] Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

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*Correspondence E-mail: aamiazghadi@stu.um.ac.ir

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ABSTRACT

An experiment was conducted to study the effect of metabolizable energy (ME) and protein of Japanese quails diets on offspring performance and antibody production against sheep red blood cell (SRBC). A total of 432 Japanese quails (13 week-old) were divided into nine treatments. Each treatment comprised four replicates of twelve birds (9 female and 3 male). Nine diets including three levels of metabolizable energy (2750, 2900 and 3050 kcal ME/kg diet) each at three levels of crude protein (18, 20 and 22% CP/kg) in a 3 × 3 factorial design were formulated. Offspring live weight was significantly lower in week 1, 2, 3 and through the whole of growth period when quails fed diets contained 2750 kcal/kg energy and 18% protein. Quail feeding with different levels of energy and protein did not affect feed intake of offspring. The feed conversion ratio of offspring was significantly higher when quails fed diet with 2750 kcal/kg ME and 18% protein during the experimental periods. Quail fed diet containing 2750 kcal/kg ME with 18 and 20% protein decreased antibody production of offspring against SRBC. It was concluded that using 2750 kcal/kg energy with 18 and 20% protein in diets of quails may have adverse effects on offspring growth rates and immune responses.

KEY WORDS antibody response, Japanese quail, metabolizable energy, protein.

INTRODUCTION

The meat production performance of Japanese quails has been improved during recent years due to genetic selection. Therefore, there is a need of updating optimal nutritional requirements of Japanese quails with the improvement in genetic makeup to exploit production potentiality. Precise nutrient supply reduces feed cost, wastage of nutrients, environmental pollution and thus improves animal welfare. Energy and protein are primary nutritional requirements of growing quails. These requirements must be met before requirements for other nutrients are addressed. Protein and energy limitation is thought to account for much of the variation in immune function among individuals because the vertebrate immune response is reliant upon significant supplies of proteins and amino acids (Klasing, 1998). During an immune response, animals often experience negative nitrogen balance through an energy-dependent proteolytic response in skeletal muscle (Lochmillerand and Deerenberg, 2000). This accelerated breakdown of proteins further increases the energetic demands of infected individuals (Hasselgrenand Fischer, 1998). As a result, even mild immune challenges may result in negative nitrogen balance that persists for at least several days (Klasing, 1998). Therefore levels of energy and protein in diet are important for having a good immune response. Symonds *et al.* (2007)

reported that maternal malnutrition (e. g. low protein and energy) during pregnancy effects on fetal development and postnatal growth, and may predispose offspring to diseases later in life. In this paper we studied the specific effects of maternal protein and energy intake on performance and antibody production of offspring.

MATERIALS AND METHODS

Birds and housing

A total of 432 Japanese quails (*Coturnix coturnix japonica*) aged 13 weeks and with uniform body weight (296±12) gain were housed in cages and randomly allocated to nine treatments. Each treatment comprised four replicates of twelve birds (9 females and 3 males). Quails house was provided with programmable lighting and ventilation (22 ˚C, 50% relative humidity and 16/8 h of light/dark circle). The experimental period was six weeks in May and June which environmental temperature was between 18 and 26 ˚C. Feed and water were provided *ad libitum*. The ingredient and nutrient composition of the experimental diets is shown in Table 1 and 2. All of the diets were consisted of National Research Council recommended levels of nutrients (NRC, 1994). The experimental laying quails were fed with three levels of energy (2750, 2900 and 3050 kcal/kg) and three levels of protein (18, 20 and 22%) in a 3×3 factorial design. A diet containing 24% crude protein and 2900 kcal/kg was used for growth period of offspring (Table 2).

Dam performance

Quails were weighed at the beginning and at the end of the experiment (420±12). Feed intake (FI) was measured weekly. Feed conversion ratio (FCR) was calculated as g of feed intake for g of egg production. Eggs were collected daily and egg production was calculated on a bird-day basis.

Hatchability

Random samples of 720 eggs (80 per treatment) were collected biweekly and stored in a storage room at 16 ˚C and 60% relative humidity. Eggs had been stored for up to 6 d when set. Before setting the eggs into incubator cracked eggs, thin shells, dirty and abnormal eggs in size or shape, were eliminated and incubated at 37.9 ˚C and 65% RH in a commercial incubator with automatic egg turning. These eggs were identified individually and placed in plastic trays, which were set randomly in the incubator. On day 14, egg turning was stopped, and eggs were transferred to the hatchery, which maintained the lower temperature (37.4 ˚C) and higher humidity (75% RH) until hatching.

Offspring performance

The newly hatched chicks transferred to rearing house. The temperature of the house was 37 ˚C and it was reduced weekly by 3 ˚C until 24 ˚C. Weighing of birds was performed weekly from hatch to 28 days with a 0.01 g sensitive electronic scale. Feed, but not water, was removed 6 h prior to weighing. A diet containing 24% crude protein and 2900 kcal/kg was used *ad libitum*. Illumination was 24 h/d during rearing period. The ingredient and nutrient composition of the used experimental diets for growth period is shown in Table 2.

Antibody response measurement

All quail dams in each replicate unit were intramuscularly injected with 0.5 mL/bird sheep red blood cell (SRBC) 10% suspension in PBS at d 28 (primary injection) and 35 (secondary injection) of experiment. Blood samples were collected from brachial vein 7 days after each injection. Also, quail offspring were injected with 0.2 mL/bird SRBC 5% at 21 and 28 and then serum samples were achieved 7 days after each injection. The serum from each sample was collected; heat inactivated at 56 ˚C for 30 min and then analyzed for total anti-SRBC antibodies as described by Cheema *et al.* (2003).

Statistical analysis

The experiment was conducted as a completely randomized design with 3 × 3 factorial arrangement with 3 levels of metabolizable energy (2750, 2900 and 3050 kcal ME/kg diet) each at three levels of crude protein (18, 20 and 22%). Each pen was as an experimental unit. The obtained data were subjected to statistical analysis using the general linear model (GLM) procedures of the SAS software (SAS, 2001). Before statistical analysis, univariate procedure of SAS was also used to test the normality of data. Output data are given as means with standard error. Significant differences among the means of treatments were determined by using Tukey test ($P<0.05$).

RESULTS AND DISCUSSION

The performance parameters of quails fed with different energy and protein levels are depicted in Table 3. Results showed that the body weight gain, feed intake, feed conversion ratio, egg mass and egg production were influenced due to interaction of ME and CP levels ($P<0.05$). Body weight gain, egg production, feed intake egg mass were maximum in quails fed by high ME and CP. The FCR improved with increase of dietary energy level and the best FCR was emerged from the diet with

Table 1 Composition of experimental diets of Japanese quail[a]

Ingredients (percentage)	18% CP			20% CP			22% CP		
	2750	2900	3050	2750	2900	3050	2750	2900	3050
Corn (CP=7.89%)	62.74	59.95	56.86	57.56	54.46	51.35	52.84	49.74	46.62
Soybean meal (CP=43.68%)	28.61	29.11	29.67	32.22	32.78	33.35	34.49	35.05	35.61
Soybean oil	-	2.28	4.82	0.41	2.95	5.49	0.86	3.4	5.95
Fish meal (CP=55.32)	1	1	1	2.5	2.5	2.5	5	5	5
Dicalcium phosphate	1.06	1.07	1.07	0.84	0.84	0.85	0.5	0.5	0.51
Limestone	5.61	5.61	5.6	5.57	5.57	5.56	5.51	5.5	5.5
Salt	0.32	0.33	0.33	0.3	0.3	0.3	0.26	0.26	0.26
Vitamin premix[b]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[c]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.02	0.01	-	-	-	-	-	-	-
DL-methionine	0.14	0.14	0.15	0.1	0.1	0.1	0.04	0.05	0.05
Calculated chemical component									
Metabolisable energy, kcal/kg	2750	2900	3050	2750	2900	3050	2750	2900	3050
Crude protein	18	18	18	20	20	20	22	22	22
Calcium	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Available phosphorus	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Sodium	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Lysine	1	1	1	1.14	1.15	1.15	1.3	1.32	1.33
Methionine	0.75	0.75	0.75	0.78	0.78	0.77	0.80	0.80	0.80
Methionine + cysteine	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45

[a] Experimental diets were calculated to contain 18, 20 and 22% CP and 2750, 2900 and 3050 kcal of ME/kg.
[b] Vitamin premix provided the following (per kg of diet): vitamin A (trans-retinyl acetate): 7700 IU; vitamin D_3 (cholecalciferol): 3300 IU; vitamin E (all rac-tocopherolacetate): 10 IU; vitamin K (bisulfate menadione complex): 0.55 mg; Thiamine (thiamine mononitrate): 1 mg; Riboflavin: 4.4 mg; Pyridoxine (pyridoxine HCl): 1 mg; pantothenic acid (D-calcium pantothenate): 5.5 mg; Nicotinicacid: 22 mg; Choline (choline chloride): 275 mg and vitamin B_{12} (cyanocobalamin): 20 ug.
[c] Mineral premix provided the following (per kg of diet): Mn: (MnSO4, H2O) 66 mg; Zn (ZnO): 66 mg; Fe (FeSO4, H2O): 33 mg; Cu (CuSO4, 5H2O): 8.8 mg; I (KI): 0.9 mg; Co: 0.2 mg and Se (Na2SeO3): 0.3 mg.

Table 2 Experimental diets of quail offspring in growth period (1-4 week)[a]

Ingredients	%	Composition	%
Corn (CP:7.89)	50.50	AME: kcal/kg	2900 kcal/kg
Soybean meal (CP:43.68)	42.12	Crude protein	24
Fish meal (CP:55.32)	3.00	Calcium	0.8
Soybean oil	2.07	Available phosphor	0.3
Dicalcium phosphate	0.32	Sodium	0.15
Limestone	1.16	Lysine	1.39
NaCl	0.30	Methionin	0.5
Vitamin premix[b]	0.25	Methionin + cystine	0.88
Mineral premix[c]	0.25	-	-
Clinacox	0.02	-	-
DL-methionin	0.01	-	-

[a] Calculated composition was according to NRC (1994).
[b] Vitamin premix provided the following (per kg of diet): vitamin A (trans retinyl acetate): 7700 IU; vitamin D3 (cholecalciferol): 3300 IU; vitamin E (all-rac-tocopherolacetate): 10 IU; vitamin K (bisulfate menadione complex): 0.55 mg; Thiamine (thiamine mononitrate): 1 mg; Riboflavin: 4.4 mg; Pyridoxine (pyridoxine HCl): 1 mg; pantothenic acid (D-calcium pantothenate): 5.5 mg; nicotinicacid: 22 mg; Choline (choline chloride): 275 mg and vitamin B_{12} (cyanocobalamin): 20 ug.
[c] Mineral premix provided the following (per kg of diet): Mn: (MnSO4, H2O): 66 mg; Zn (ZnO): 66 mg; Fe (FeSO4, H2O): 33 mg; Cu (CuSO4, 5H2O): 8.8 mg; I (KI): 0.9 mg; Co: 0.2 mg and Se (Na2SeO3): 0.3 mg.

3050 kcal ME/kg diet (P<0.05). Increasing of diet energy from 2750-2900 and 3050 in quails were fed with diet include 18% protein improved body weight gain from -13.1 g to -4.08 and 5.48 g thatindicated negative effect of protein shortage can be less in diet with increasing level of energy.

Although egg production significantly increased, other parameters such as feed intake and FCR did not have any alter. To increase protein levels (20%) the effect of energy was decreased.

Also, in treatments with 18% protein and 2750 or 2900 kcal ME/kg the growth weight was similar, but with raising the energy level to 3050 kcal this scale significantly improved.

Egg production variation was the same as body weight gain. Regardless of the diet energy level, egg weight in birds fed with 18% protein in comparison to other birds significantly was less.

Table 3 Effects of different levels of energy and protein on performance of laying Japanese quails

Treatment		Body weight	Feed intake	Feed conversion	Egg mass	Egg production
Energy (kcal/kg)	Protein (g/kg)	gain (g/bird)	(g/bird per day)	(g of feed/g of egg)	(g of egg/bird per day)	(%)
2750	18	-13.01^{d}	29.07^{b}	3.26^{a}	8.94^{b}	72.39^{c}
2750	20	2.78^{b}	29.97^{b}	3.35^{a}	8.97^{b}	72.09^{c}
2750	22	4.09^{b}	32.07^{b}	3.27^{a}	10.01^{ab}	78.74^{c}
2900	18	-4.08^{c}	32.25^{b}	3.29^{a}	10.23^{a}	79.99^{bc}
2900	20	3.02^{b}	34.01^{a}	2.66^{b}	11.08^{a}	83.01^{b}
2900	22	3.69^{b}	33.91^{a}	2.76^{b}	11.18^{a}	84.12^{b}
3050	18	5.48^{b}	31.57^{b}	3.18^{a}	11.02^{a}	82.19^{b}
3050	20	6.50^{a}	34.25^{a}	2.69^{b}	11.12^{a}	84.03^{b}
3050	22	9.61^{a}	33.00^{a}	2.71^{b}	11.49^{a}	86.32^{a}
Main effects						
2750	-	-1.77^{b}	28.06^{b}	3.21^{a}	9.72^{b}	73.14^{c}
2900	-	1.86^{b}	34.71^{a}	3.10^{a}	11.57^{a}	83.96^{b}
3050	-	6.19^{a}	33.11^{a}	2.63^{b}	11.69^{a}	85.65^{a}
	18	-3.87^{b}	31.29	3.06	10.49	80.38^{b}
	20	3.10^{a}	34.53	2.91	11.86	88.21^{a}
	22	6.07^{a}	34.10	2.94	11.95	89.66^{a}
SEM		1.13	0.35	0.16	0.13	0.88
Probability						
Energy	-	0.001	0.015	0.041	0.023	0.019
Protein	-	0.031	0.076	0.127	0. 270	0.037
Energy× protein	-	0.004	0.001	0.039	0.042	0.019

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Offspring live weight at week 1 was lower when quails were fed diets containing 2750 or 2900 kcal/kg energy and 18% protein (Table 3). At week 2, live weight was lower just when quails were fed diet containing 2750 kcal/kg energy and 18% protein. While, at tertiary week of the experiment, Offspring live weight was higher when quails were fed diets containing 3050 kcal/kg energy and 20 and 22% protein. But in week 4 there were no significant differences between live weights of offspring. During growth period of offspring (1-4 week of age) higher live weights have been observed when quails fed diets containing 3050 kcal/kg energy and 20 and 22% protein. Whereas the lowest live weight of offspring was for quails that fed diets containing 2750 kcal/kg energy and 18% protein. No differences in feed intake were observed among treatments in offspring at growth period. Furthermore feed conversion ratio of offspring was higher when quails fed diets containing 2750 kcal/kg energy and 18% protein. Main effect of energy and protein levels of quail's diets was not significant for feed intake. However the live weight gain and FCR differed significantly due to energy and CP levels. Some researchers studied about effect of maternal malnutrition on offspring performance. Langley and Jackson (1994) demonstrated that Offspring of the 6% protein fed rats were smaller than pups from 18, 12 and 9% protein fed rats, over a 21 week period. Spratt and Leeson (1987) showed that when broiler breeders fed low, medium and high energy diets body weights of 20 day old male offspring were 575, 586 and 601 g respectively. However, the energy intake of broiler breeders had no effect on growth weight of female offspring (P>0.01). Furthermore, Lopez and Leeson (1994) studied about effect of feeding low-protein diets to older broiler breeders on egg weight and offspring performance. They demonstrated that egg weight was lower (P<0.01) for birds fed 9 or 11% CP. This effect was reflected in lower chick weight at hatching. However after 49 days of growth, the difference was no longer apparent. Kingori *et al.* (2010) concluded that maternal dietary protein level (differing in protein levels: 100, 120, 140 and 170 g CP/kg DM) has no effect on hatchability and post-hatch offspring feed intake, feed efficiency and growth rate. Brake *et al.* (2003) discussed about relation between energy and protein content of young breeder diets and chick performance. They explained that cumulative ME and CP nutrition during rearing may affect the body reserves available to the young breeder for incorporation into the egg, possibly altering egg lipid composition and subsequent chick performance. Also Rao *et al.* (2009) demonstrated that maternal low protein diet programs effect on post-hatch growth of the offspring. They discussed that the associated alterations in yolk leptin deposition as well as in yolk sac membrane, fetal hypothalamus and muscle gene expression may be involved in mediating such programming effect in the chicken. Guibert *et al.* (2011) revealed that mild stressors applied to laying Japanese quail can increase the emotional reactivity of their chicks and suggested that maternal stress effects on offspring are mediated by changes in egg composition and yolk testosterone levels. On the other hand, the importance of nutritional factors for immune responses has received much attention in the past several years. Olusi *et al.* (1980) studied about deficient protein during gestation. They observed that when rats were fed a protein deficient diet for 4 days prior to mating and then throughout gestation there was a delay in the appearance of IgG in malnourished offspring. Yamaguchi *et al.* (1983) investigated on effect of maternal immune challenge with SRBC on offspring immune status. They concluded that ability to produce antigen- specific antibody of female mice affected in their offspring responses. It is thought that during the neonatal period, maternal antibodies induce T-cell dependent idiotypic responses that prime the offspring immunesystem (Lemke *et al.* 2009). Enting *et al.* (2007) showed the significant differences in IgG and IgM titers in broiler chickens of 35 d of age which indicate that low-density breeder diets can affect adaptive immune responses of offspring. Grindstaff *et al.* (2005) fed adult Japanese quail isocaloric diets containing either the recommended protein content for reproducing adults (20%) or a low protein diet (12%). They suggested that protein limitation may influence offspring immunity indirectly through a reduction in transmission of other egg components and egg size. Although maternal protein restriction did not signiflcantly affect antibody transmission to eggs. These stressors cause hormone changes, declines in feed intake, altered nutrient metabolism and suppressed immune function. The adverse effects of the stressors are additive and every attempt should be made in all poultry operations to lessen the number and intensity of these stressors. Nutrients are known to influence the responses of poultry to a disease challenge. Normally, during such a challenge nutrients are shunted away from growth. For example, body proteins are broken down and amino acids are shunted away from growth and are used by specific cells to synthesize critical proteins which allow the bird to mount a successful immune response to a particular disease challenge. Lymphoid cells increase in their numbers, and acute phase proteins and antibodies also increase through the use of amino acids. Defense mechanisms take priority in a disease challenge and these nutrients help the bird's system overcome the challenge. If specific nutrients are at or below the bird's requirement, then a limited amount of nutrients will be available to meet all of the body's needs during a time of challenge. Our result similarly showed that deficiency in protein and energy of quail diets results in low antibody titer against SRBC in offspring. It may be related to alteration in egg component spatially immunoglobulin. The primary response of antibody titer against SRBC in quails showed no clear differences among any of the treatments (Table 4).

Table 4 Effect of dietary levels of energy and protein of Japanese quail on performance of offspring

Treatment in dam		Feed intake	FCR	Body weight gain				
Energy (kcal/kg)	Protein (%)	(g/bird 0-4 week)	(0-4 week)	(Week 1)	(Week 2)	(Week 3)	(Week 4)	(Week1-4)
2750	18	391.65±10.15	2.96±0.03^{a}	12.14±0.52^{b}	26.04±0.81^{b}	44.64±1.01^{b}	30.17±1.11	112.99±3.27^{c}
2750	20	415.83±9.16	2.39±0.02^{b}	20.23±0.58^{a}	30.29±0.82^{ab}	48.29±1.12^{ab}	31.48±1.32	130.29±3.08^{bc}
2750	22	420.27±11.92	2.37±0.03^{b}	20.13±0.53^{a}	33.93±0.83^{ab}	51.03±1.23^{b}	30.93±1.21	136.02±3.62^{b}
2900	18	419.61±10.29	2.39±0.04^{b}	13.01±0.52^{b}	33.01±0.72^{ab}	46.21±1.27^{b}	29.71±1.07	121.94±3.12^{bc}
2900	20	434.81±9.36	2.19±0.03^{b}	21.98±0.44^{a}	35.97±0.74^{a}	53.17±1.17^{ab}	32.01±0.91	143.13±4.21^{ab}
2900	22	443.27±9.38	2.26±0.05^{b}	22.32±0.49^{a}	36.30±0.79^{a}	52.39±1.02^{ab}	32.30±1.02	143.31±3.89^{ab}
3050	18	411.96±10.19	2.29±0.02^{b}	18.19±0.45^{ab}	32.81±0.85^{ab}	48.73±1.19^{b}	30.03±1.11	129.17±3.12^{bc}
3050	20	424.81±9.26	2.09±0.03^{b}	23.93±0.54^{a}	36.03±0.74^{a}	56.43±1.21^{a}	31.13±1.01	147.52±4.25^{a}
3050	22	422.27±10.48	2.16±0.02^{b}	23.27±0.59^{a}	38.87±0.81^{a}	56.80±1.25^{a}	32.01±1.15	150.91±4.15^{a}
Main effects								
2750	-	400.55±8.23	2.66±0.03^{a}	15.74±0.48	29.92±0.80	44.32±1.10	30.30±0.92	120.29±3.28^{b}
2900	-	430.04±7.13	2.45±0.02^{ab}	17.44±0.58	35.49±0.75	50.41±1.03	31.42±1.12	134.76±4.19^{b}
3050	-	408.17±10.27	2.12±0.02^{b}	20.01±0.53	34.08±0.73	51.28±1.24	30.29±1.09	135.66±3.21^{a}
	18	402.65±9.89	2.46±0.02^{a}	14.39±0.52^{b}	28.91±0.82^{b}	45.96±1.22^{b}	28.06±1.02	117.32±3.80^{b}
	20	418.81±10.06	2.19±0.03^{b}	20.83±0.48^{a}	33.55±0.74^{ab}	53.37±1.28^{ab}	32.32±1.14	140.37±4.09^{a}
	22	427.27±9.28	2.20±0.02^{b}	21.36±0.49^{a}	37.36±0.79^{a}	56.22±1.19^{a}	31.92±1.10	146.80±4.11^{a}
Probability								
Energy	-	0.141	0.037	0.412	0.097	0.241	0.036	0.028
Protein	-	0.169	0.049	0.042	0.036	0.047	0.078	0.019
Energy × protein	-	0.109	0.041	0.039	0.042	0.029	0.173	0.001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

FCR: feed conversion ratio.

However, quails that fed diets containing 2750 kcal/kg energy and 18 and 20% protein, their offspring had lower antibody titer against SRBC for secondary response. On the whole, nutrients intake by quail's dam directly influence offspring immune response.

CONCLUSION

There is no question that a well nourished bird is more immunologically competent and better able to cope with disease challenges than a poorly nourished bird. Based on the results of this study, it can be concluded that use of 2750 kcal/kg energy with 18 and 20% protein may have a negative effect on offspring growth rate and antibody responses. Furthermore increase in offspring growth rate at initial rearing period seems to be mainly related to an increase in nutrient intake of parents.

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