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Effects of Food Protein Levels on Haematological Parameters of Growing Japanese Quails (*Coturnix Coturnix* Japonica Temminck & Schlegel, 1849)

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ABSTRACT

The study aims to determine the impact of dietary crude protein levels on the hematological parameters of Japanese quail from growth to ovipositor. To this end, five feeds with different crude protein levels (18, 20, 22, 24 and 26%) were supplied to 700 quails of three weeks of age. These were subdivided into six batches, including three batches of females and three batches of males for each feed group. After subjecting the quails to diets containing the different protein levels, four samples were taken at the fourth, fifth, sixth and seventh week of age. The samples taken were analyzed using an SYSMEX XN 350 automated hematological analyzer. The results of this investigation indicated that non-significant differences (P > 0.05) were observed in hematological parameters in both female and male quails. This study showed that dietary crude protein levels had no impact on the health status of Japanese quails.

Keywords: Japanese quails, Crude protein, Hematological parameters.

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INTRODUCTION

The Japanese quail Coturnix japonica is a hardy, small bird characterized by rapid growth, early sexual maturity, short generation interval, and a high ovipositor (Mills et al., 1997). It is therefore considered to be a laboratory model species (Huss et al., 2008). Also, the Japanese quail has economic importance as its flesh and eggs that are sold are valued for their unique flavor and high nutritional value (Huss et al., 2008). Indeed, a variation in protein intake can modify production, and consequently influence energy requirements because the birds' appetite is closely linked to their energy needs. Therefore, diets should be formulated to contain the right amount of crude protein and an adequate amount of energy for optimal feed utilization by the birds. In addition, variations in protein levels in the feed may affect the health of Japanese quail (Cortunix coturnix japonica). Hematological blood analysis is a valuable tool for assessing animal health and contributes to both diagnosis and clinical surveillance of the disease (Karesh et al., 1997). Hematological parameters also allow the detection of blood abnormalities due to the malfunction of the body. In addition, nutritional deficiencies, which condition livestock productivity and their ability to make use of natural resources, can be validly assessed by blood parameters. Several studies have been conducted on the effects of dietary proteins on the immune system of quails (Sharifi et al., 2013).

However, in Ivory Coast very few studies have been conducted on the effects of different protein levels in food on the hematological parameters of Japanese quail. It is therefore important to initiate this study to determine the effects of different protein levels in feed on the hematological parameters of Japanese quail from growth to ovipositor. Specifically, this work aims to make a comparative study of hematological parameters obtained with five types of diet on male and female Japanese quails at different ages.

Table 1. Composition of dietary treatments.

Ingrédients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Maize grain (yellow)	54.5	54.8	49	43	39
Wheatoffal	15.5	10.5	10.5	10.5	9.5
K B C 30	26.5	33.0	37	42	46
Bone meal	0.5	0.5	0.5	0.5	0.5
Palm oil	3	1.2	3	4	5
TOTAL	100	100	100	100	100
Calculated analysis					
Metabolizable energy (Kcal/Kg)	2799.4 (2800)	2799.3 (2800)	2800.8 (2800)	2804.15 (2800)	2801.5 (2800)
Crude protein %	18.05 (18)	19.99 (20)	22.03 (22)	24.02 (24)	25.94 (26)
Crude fiber %	4.25	4.06	4.22	4.45	4.15
Fat matter %	4.21	4.25	4.27	4.32	4.32
Calcium %	1.10	1.19	1.32	1.45	1.55
Sodium %	0.41	0.43	0.43	0.43	0.43
Phosphore %	3.61	3.14	3.04	3.04	2.77
Lysine %	3.00	3.00	3.01	3.04	3.03
Méthionine %	1.53	1.54	1.51	1.48	1.45

MATERIALS and METHODS

Experimental site

The study took place in a farm located in the commune of Daloa (6°53'north, 6°27'west) precisely in the Lobia district. The area has an average annual rainfall of 1317 mm with an average temperature of 25.6°. The farm has an area of 500 m^2 with a livestock building of 10m/ 4m dimension.

Birds and husbandry

700 quails of two weeks old from a commercial farm in Abidjan (Abata) were transported to the experimental farm in Daloa. The animals were transported in an air-conditioned Renault van. The animals were raised on the ground and fed with the same chick starter diet from FACI (Ivorian company specializing in animal nutrition) for one week. Five groups of 120 quail of three weeks of age were formed after sorting and weighing. The quails used at the beginning of the experiment had an average weight of 73.22 ± 0.61 g for males and 79.64 ± 1.28 g for females. Japanese quails were randomly divided into five groups of 40 birds with three replicates. Each group was subdivided into two lots with 20 females and 20 males. The birds were raised in cages of identical size (100cm x 100cm x 80 cm). During the trial, each group was fed a diet according to the dietary protein level (Table 1). Group 1,2,3,4 and 5 were fed on 18, 20, 22, 24 and 26 % protein, respectively. The drinking water and the feed are measured and served daily at 8:00 am. During the study, the animals did not undergo any drug treatment.

Hematological parameters

In each of the birds, blood samples were taken from the jugular vein of each group in the fourth, fifth, sixth and seventh week, early in the morning between 6:30 and 8:30 a.m. Blood from each of the birds, 2 ml was collected in tubes containing EDTA anticoagulants (purple tube). These samples were used to perform a blood count (CBC) using a Sysmex XN 350 automated system. A blood smear stained with MGG (May Grünwald Giemsa) was used to determine the leukocyte count.

Statistical analysis

The results were expressed as the means associated with their standard error. For the evaluation of the various study parameters, the hematological data obtained were statistically processed using the STATISTICA 7.1 computer program by analysis of variance (ANOVA). The significance of the analyses was defined as a probability threshold of less than 5%. The ANOVA test will be complemented by the Tukey post-ANOVA test to identify the variable(s) with highly significant differences in the different values.

RESULTS AND DISCUSSION

The results for the effects of protein levels in feed on hematological parameters of growing Japanese quail are presented in Tables 2-5 for the overall (male + female) and Tables 2a, 3a, 4a and 5a for each sex. The mean values of hematological parameters are shown that the variation in dietary crude protein did not have a significant effect (P0.05) on the indices of the different parameters in all animals. Otherwise, the results also report that no significant difference was observed in both female and male quails during the experimental period.

Effect of dietary crude protein variation on leukocytes

The different diets did not affect leukocytes ($P > 0.05$). Indeed eosinophilic, basophilic and heterophilic polynuclear cells, lymphocytes, monocytes and H/L ratio remained almost stable between the different sexes (female ($P > 0.05$) and male ($P > 0.05$)).

The protein levels used during our study would seem to cover the protein requirements of quails and not alter their health status. Indeed the mean values were within

Table 2. Variation in hematological parameters of each group of quails at 28 days of age.

Blood parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	*P values*
RBC (10^6/mm^3)	2.27 ± 0.02	2.29 ± 0.02	2.30 ± 0.02	2.21 ± 0.02	2.35 ± 0.02	> 0.05
Hemoglobin (g/dl)	9.30 ± 0.16	9.59 ± 0.13	9.38 ± 0.10	9.15 ± 0.11	9.67 ± 0.12	> 0.05
Hematocrit (%)	38.94 ± 0.50	39.35 ± 0.48	38.84 ± 0.41	37.74 ± 0.50	40.21 ± 0.36	> 0.05
MCV (fl)	171.91 ± 1.85	171.71 ± 1.48	169.14 ± 1.74	165.28 ± 2.10	177.29 ± 1.72	> 0.05
MCH (ρg)	41.20 ± 0.92	41.94 ± 0.71	40.86 ± 0.50	40.08 ± 0.52	42.71 ± 0.67	> 0.05
MCHC (g/dl)	24.01 ± 0.54	24.47 ± 0.46	24.21 ± 0.33	23.54 ± 0.42	24.93 ± 0.40	> 0.05
Platelets (10^3/mm^3)	9.53 ± 0.16	9.63 ± 0.18	9.44 ± 0.18	9.44 ± 0.17	9.88 ± 0.18	> 0.05
WBC (10^3/mm^3)	19.26 ± 0.23	18.80 ± 0.24	19.03 ± 0.29	18.39 ± 0.22	19.58 ± 0.22	> 0.05
Eosinophil (%)	0.01 ± 0.001	0.01 ± 0.002	0.01 ± 0.001	0.008 ± 0.001	0.010 ± 0.001	> 0.05
Basophil (%)	0.48 ± 0.05	0.50 ± 0.06	0.47 ± 0.06	0.52 ± 0.05	0.53 ± 0.05	> 0.05
Monocyte (%)	0.82 ± 0.02	0.81 ± 0.02	0.83 ± 0.02	0.79 ± 0.02	0.81 ± 0.02	> 0.05
Heterophil (%)	40.69 ± 0.33	40.14 ± 0.34	40.34 ± 0.33	38.80 ± 0.34	41.16 ± 0.35	> 0.05
Lymphocyte (%)	58.00 ± 0.33	58.54 ± 0.34	58.28 ± 0.33	56.54 ± 0.34	60.83 ± 0.35	> 0.05
H/L ratio	0.70 ± 0.01	0.69 ± 0.01	0.69 ± 0.01	0.66 ± 0.01	0.70 ± 0.01	> 0.05

Table 3. Variation in hematological parameters of each group of quails at 35 days of age.

Blood parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	*P values*
RBC (10^6/mm^3)	2.35 ± 0.03	2.34 ± 0.02	2.36 ± 0.02	2.35 ± 0.03	2.36 ± 0.02	> 0.05
Hemoglobin (g/dl)	9.32 ± 0.12	9.28 ± 0.14	9.135 ± 0.11	9.46 ± 0.11	9.26 ± 0.11	> 0.05
Hematocrit (%)	41.22 ± 0.51	41.39 ± 0.42	41.10 ± 0.46	41.75 ± 0.50	41.39 ± 0.46	> 0.05
MCV (fl)	175.71 ± 2.55	176.90 ± 1.24	176.89 ± 2.11	177.94 ± 2.20	175.78 ± 2.10	> 0.05
MCH (ρg)	39.27 ± 0.61	39.77 ± 0.77	38.75 ± 0.62	40.41 ± 0.72	39.39 ± 0.62	> 0.05
MCHC (g/dl)	29.73 ± 0.45	22.51 ± 0.46	22.31 ± 0.36	22.75 ± 0.38	22.45 ± 0.33	> 0.05
Platelets (10^3/mm^3)	9.67 ± 0.1 6	9.46 ± 0.18	9.33 ± 0.15	9.75 ± 0.15	9.43 ± 0.17	> 0.05
WBC (10^3/mm^3)	19.96 ± 0.19	20.00 ± 0.19	19.96 ± 0.20	19.99 ± 0.18	19.96 ± 0.19	> 0.05
Eosinophil (%)	0.009 ± 0.002	0.011 ± 0.001	0.011 ± 0.002	0 .011 ± 0.003	0.009 ± 0.001	> 0.05
Basophil (%)	0.58 ± 0.05	0.60 ± 0.06	0.64 ± 0.66	0.59 ± 0.05	0.64 ± 0.06	> 0.05
Monocyte (%)	0.96 ± 0.01	0.96 ± 0.02	0.94 ± 0.01	0.93 ± 0.01	0.93 ± 0.02	> 0.05
Heterophil (%)	40.63 ± 0.30	40.61 ± 0.32	40.59 ± 0.33	40.47 ± 0.33	40.71 ± 0.33	> 0.05
Lymphocyte (%)	57.81 ± 0.31	57.93 ± 0.32	57.93 ± 0.32	58.06 ± 0.33	57.80 ± 0.32	> 0.05
H/L ratio	0.70 ± 0.01	0.70 ± 0.01	0.70 ± 0.01	0.70 ± 0.01	0.71 ± 0.01	> 0.05

Table 4. Variation in haematological parameters of each group of quails at 42 days of age.

Blood parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	*P values*
RBC (10^6/mm^3)	2.44 ± 0.05	2.42 ± 0.05	2.41 ± 0.05	2.47 ± 0.05	2.40 ± 0.05	> 0.05
Hemoglobin (g/dl)	9.22 ± 0.17	9.25 ± 0.17	9.36 ± 0.16	9.32 ±0.19	9.25 ± 0.16	> 0.05
Hematocrit (%)	42.15 ± 1.14	42.53 ± 1.03	42.56± 1.05	42.32 ± 1.11	42.35 ± 1.12	> 0.05
MCV (fl)	173.26 ± 3.98	175.80 ± 2.77	176.85 ± 3.52	171.59 ± 2.77	177.54 ± 3.64	> 0.05
MCH (ρg)	38.02 ± 0.65	38.42 ± 0.74	38.93 ± 0.45	37.95 ± 0.62±	38.89 ± 0.81	> 0.05
MCHC (g/dl)	22.14 ± 0.45	21.95 ± 0.40	22.21 ± 0.389	37.95 ± 0.62±	22.09 ± 0.42	> 0.05
Platelets (10^3/mm^3)	9.69 ± 0.17	9.96 ± 0.18	9.62 ± 0.17	9.60 ± 0.15	9.65 ± 0.17	> 0.05
WBC (10^3/mm^3)	19.82 ± 0.23	19.83 ± 0.23	19.85 ± 0.22	19.82 ± 0.23	19.81 ± 0.23	> 0.05
Eosinophil (%)	0.012 ± 0.003	0.011 ± 0.002	0.012 ± 0.002	0.009 ± 0.001	0.01 ± 0.002	> 0.05
Basophil (%)	1.68 ± 0.22	1.69 ± 0.22	1.55 ± 0.20	1.66 ± 0.22	1.85 ± 0.22	> 0.05
Monocyte (%)	1.85 ± 0.06	1.69 ± 0.06	1.79 ± 0.05	1.83 ± 0.06	1.75 ± 0.06	> 0.05
Heterophil (%)	49.44 ± 0.95	49.56 ± 0.95	49.32 ± 0.94	49.25 ± 0.91	49.40 ± 0.95	> 0.05
Lymphocyte (%)	47.02 ± 1.45	47.05 ± 1.13	47.26 ± 1.08	47.25 ± 1.11	46.99 ± 1.14	> 0.05
H/L ratio	1.09 ± 0.05	1.09 ± 0.05	1.07 ± 0.04	1.07 ± 0.04	1.08 ± 0.05	> 0.05

the range reported by Sturkie and Grimminger (1976). These findings confirm the results previously obtained by Padian et al. (2012).

Basophilic values of females at the sixth and seventh weeks of age were high compared to the reference values of Sturkie and Grimminger (1976), which was 0-2%. Similar studies conducted by Khan et al. (2018) on catfish showed that variation in dietary protein levels did not influence basophilic polynuclei.

These high basophilic values could be associated with physiological stress probably due to the egg-laying cycle of adult female quails. Basophils produce and store histamine, which causes vasodilation and increased capillary permeability. They have immediate hypersensitivity reactions (Afolabi et al., 2011).

Basophiles obtained in males at 4 to 7 weeks of age and females at 4 and 5 weeks of age were within the norms defined by Sturkie and Grimminger (1976). The

Table 5. Variation in hematological parameters of each group of quails at 49 days of age.

Blood parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	*P values*
RBC (10^6/mm^3)	2.48 ± 0.05	2.50 ± 0.05	2.49 ± 0.05	2.42 ± 0.05	2.44 ± 0.05	> 0.05
Hemoglobin (g/dl)	8.86 ± 0.15	9.05 ± 0.17	9.08 ± 0.18	8.95 ± 0.19	8.85 ± 0.16	> 0.05
Hematocrit (%)	43.38 ± 1.41	43.62 ± 1.40	43.40 ± 1.50	41.83 ± 1.38	41.87 ± 1.42	> 0.05
MCV (fl)	173.97 ± 3.02	173.21 ± 2.63	173.25 ± 2.96	171.75 ± 2.86	170.67 ± 2.83	> 0.05
MCH (ρg)	35.87 ± 0.42	36.26 ± 0.40	36.58 ± 0.41	37.01 ± 0.44	36.40 ± 0.41	> 0.05
MCHC (g/dl)	20.83 ± 0.48	21. 09 ± 0.41	21.27 ± 0.40	21.72 ± 0.42	21.50 ± 0.42	> 0.05
Platelets (10^3/mm^3)	9.81 ± 0.16	9.71 ± 0.17	9.65 ± 0.18	9.58 ± 0.18	9.37 ± 0.15	> 0.05
WBC (10^3/mm^3)	22.44 ± 0.21	22.47 ± 0.23	22.70 ± 0.29	22.74 ± 0.25	22.51 ± 0.24	> 0.05
Eosinophil (%)	0.011 ± 0.003	0.01 ± 0.002	0.01 ± 0.002	0.012 ± 0.001	0.011 ± 0.002	> 0.05
Basophil (%)	2.08 ± 0.29	2.08 ± 0.28	2.05 ± 0.28	2.31 ± 0.25	2.24 ± 0.28	> 0.05
Monocyte (%)	1.81 ± 0.07	1.77 ± 0.07	1.83 ± 0.08	1.70 ± 0.07	1.74 ± 0.07	> 0.05
Heterophil (%)	51.83 ± 0.98	51.17 ± 1.05	51.43 ± 1.03	52.75 ± 1.01	52.75 ± 0.99	> 0.05
Lymphocyte (%)	44.26 ± 1.21	44.97 ± 1.27	44.67 ± 1.24	43.23 ± 1.21	43.26 ± 1.22	> 0.05
H/L Ratio	1.21 ± 0.05	1.18 ± 0.06	1.20 ± 0.06	1.26 ± 0.05	1.27 ± 0.06	> 0.05

Table 2a. According to the sex.

Blood parameters	Sex	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	*P values*
	Male	2.35 ± 0.01	2.34 ± 0.01	2.34 ± 0.01	2.34 ± 0.01	2.33 ± 0.01	> 0.05
RBC (10^6/mm^3)	Female	2.21 ± 0.01	2.23 ± 0.01	2.22 ± 0.01	2.22 ± 0.01	2.23 ± 0.01	> 0.05
	Male	9.34 ± 0.05	9.34 ± 0.04	9.35 ± 0.04	9.34 ± 0.04	9.34 ± 0.04	> 0.05
Hemoglobin (g/dl)	Female	9.33 ± 0.04	9.34 ± 0.04	9.33 ± 0.03	9.35 ± 0.03	9.35 ± 0.04	> 0.05
	Male	41.25 ± 0.06	41.25 ± 0.06	41.05 ± 0.05	41.26 ± 0.06	41.06 ± 0.05	> 0.05
Hematocrit (%)	Female	37.15 ± 0.06	37.32 ± 0.04	37.29 ± 0.05	37.24 ± 0.04	37.23 ± 0.04	> 0.05
	Male	176.08 ± 0.14	176.21 ± 0.12	176.03 ± 0.15	176.32 ± 0.16	176.36 ± 0.11	> 0.05
MCV (fl)	Female	167.74 ± 0.11	167.63 ± 0.1	167.92 ± 0.12	167.78 ± 0.11	167.32 ± 0.13	> 0.05
	Male	40.01 ± 0.16	39.97 ± 0.1	40.06 ± 0.07	39.97 ± 0.11	40.18 ± 0.1	> 0.05
MCH (ρg)	Female	42.17 ± 0.09	42.00 ± 0.12	42.04 ± 0.08	42.1 ± 0.07	42.04 ± 0.1	> 0.05
	Male	22.72 ± 0.11	22.71 ± 0.07	22.78 ± 0.06	22.74 ± 0.09	22.81 ± 0.08	> 0.05
MCHC (g/dl)	Female	25.16 ± 0.08	25.08 ± 0.09	25.07 ± 0.07	25.13 ± 0.07	24.14 ± 0.06	> 0.05
	Male	9.77 ± 0.06	9.60 ± 0.06	9.54 ± 0.06	9.61 ± 0.05	9.59 ± 0.06	> 0.05
Platelets (10^3/mm^3)	Female	9.67 ± 0.05	9.67 ± 0.06	9.69 ± 0.05	9.70 ± 0.06	9.73 ± 0.05	> 0.05
	Male	17.91 ± 0.02	17.81 ± 0.02	17.97 ± 0.02	17.89 ± 0.02	17.87 ± 0.02	> 0.05
WBC (10^3/mm^3)	Female	20.20 ± 0.01	20.23 ± 0.02	20.21 ± 0.02	20.20 ± 0.01	20.11 ± 0.01	> 0.05
	Male	0.009±0.014	0.01 ± 0.016	0.009±0.014	0.009±0.014	0.009±0.014	> 0.05
Eosinophil (%)	Female	0.009±0.014	0.009±0.014	0.009±0.014	0.009±0.014	0.009±0.014	> 0.05
	Male	0.53 ± 0.08	0.53 ± 0.09	0.52 ± 0.07	0.54 ± 0.08	0.55 ± 0.08	> 0.05
Basophil (%)	Female	0.52 ± 0.07	0.51 ± 0.09	0.52 ± 0.08	0.52 ± 0.06	0.53 ± 0.07	> 0.05
	Male	0.81 ± 0.03	0.80 ± 0.03	0.83 ± 0.03	0.82 ± 0.02	0.82 ± 0.02	> 0.05
Monocyte (%)	Female	0.79 ± 0.02	0.79 ± 0.02	0.78 ± 0.03	0.80 ± 0.03	0.79 ± 0.03	> 0.05
	Male	38.71 ± 0.02	38.72 ± 0.02	38.72 ± 0.02	38.72 ± 0.03	38.34 ± 0.02	> 0.05
Heterophil (%)	Female	42.09 ± 0.02	41.82 ± 0.02	41.92 ± 0.02	41.79 ± 0.02	41.65 ± 0.02	> 0.05
	Male	59.94 ± 0.02	59.96 ± 0.02	59.85 ± 0.02	60.04 ± 0.02	60.27 ± 0.02	> 0.05
Lymphocyte (%)	Female	56.59 ± 0.02	56.86 ± 0.02	56.77 ± 0.02	56.87 ± 0.02	56.98 ± 0.02	> 0.05
	Male	0.65 ± 0.005	0.65 ± 0.005	0.65 ± 0.005	0.64 ± 0.005	0.64 ± 0.005	> 0.05
H/L ratio	Female	0.74 ± 0.004	0.74 ± 0.005	0.74 ± 0.005	0.73 ± 0.004	0.73 ± 0.004	> 0.05

values obtained in this study suggested that there were no conditions of prolonged stress. Furthermore, the non-effect of dietary crude protein on monocytes was also observed by Mohamed (2012). However, our results disagree with those reported by Osama et al. (2015) who showed that monocytes increase when dietary crude protein was increased. These differences could be due to rearing conditions.

In the present study, heterophiles remained almost stable during the intake of dietary crude protein by quail. The results of our investigations conform with the findings of Egbunike et al. (2009). These authors reported no effect of crude protein level and source on hematological parameters. Indeed, heterophiles are the "first responders" at the site of inflammation after infectious contact. They cause an inflammatory reaction attracting lymphocytes (Bennoume et al., 2009). They increase with age and are reared in females. These results were similar as reported by Muhammad (2013). Moreover, the lymphocytes obtained during this study were the same as reported by Ritchie et al. (1994). These values did not vary significantly in the quail groups during the weeks of feed intake. Similar results were recorded in the fourteen-week old mamourah cockerel by Rabie et al. (2017) showed that plasma blood parameters were not influenced by the rations of different dietary protein levels. It appears from this study that the H/L ratio of adult female quails (weeks 6 and 7)

Table 3a. According to the sex.

Blood parameters	Sex	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	*P values*
	Male	2.42 ± 0.01	2.40 ± 0.01	2.42 ± 0.01	2.44 ± 0.01	2.43 ± 0.01	> 0.05
RBC ($10^6/mm^3$)	Female	2.28 ± 0.01	2.29 ± 0.01	2.29 ± 0.01	2.28 ± 0.01	2.29 ± 0.01	> 0.05
	Male	9.33 ± 0.03	9.33 ± 0.05	9.33 ± 0.04	9.33 ± 0.03	9.32 ± 0.04	> 0.05
Hemoglobin (g/dl)	Female	9.34 ± 0.04	9.35 ± 0.05	9.33 ± 0.03	9.34 ± 0.04	9.33 ± 0.04	> 0.05
	Male	43.20 ± 0.05	43.12 ± 0.04	43.24 ± 0.04	43.56 ± 0.04	43.57 ± 0.04	> 0.05
Hematocrit (%)	Female	39.27 ± 0.03	39.42 ± 0.03	39.44 ± 0.02	39.33 ± 0.02	39.41 ± 0.02	> 0.05
	Male	179.11 ± 0.19	179.41 ± 0.14	179.17 ± 0.18	179.09 ± 0.18	179.27 ± 0.18	> 0.05
MCV (fl)	Female	172.31 ± 0.11	172.38 ± 0.09	172.18 ± 0.10	172.48 ± 0.11	172.23 ± 0.11	> 0.05
	Male	38.69 ± 0.11	38.87 ± 0.12	38.69 ± 0.10	38.36 ± 0.10	38.38 ± 0.11	> 0.05
MCH (ρg)	Female	40.94 ± 0.09	40.81 ± 0.07	40.71 ± 0.08	40.94 ± 0.09	40.79 ± 0.08	> 0.05
	Male	21.66 ± 0.07	21.67 ± 0.07	21.65 ± 0.08	21.43 ± 0.05	21.43 ± 0.07	> 0.05
MCHC (g/dl)	Female	23.79 ± 0.07	23.69 ± 0.09	23.66 ± 0.06	23.75 ± 0.06	23.70 ± 0.06	> 0.05
	Male	9.67 ± 0.05	9.46 ± 0.05	9.85 ± 0.05	9.72 ± 0.05	9.49 ± 0.05	> 0.05
Platelets ($10^3/mm^3$)	Female	9.71 ± 0.05	9.58 ± 0.05	9.68 ± 0.06	9.70 ± 0.05	9.62 ± 0.05	> 0.05
	Male	18.97 ± 0.006	18.95 ± 0.007	18.96 ± 0.006	19.01 ± 0.005	18.94 ± 0.006	> 0.05
WBC ($10^3/mm^3$)	Female	20.95 ± 0.004	20.97 ± 0.003	20.95 ± 0.003	20.97 ± 0.003	20.99 ± 0.004	> 0.05
	Male	0.009 ± 0.014	0.01 ± 0.01	0.009 ± 0.014	0.009 ± 0.015	0.009.±.0.014	> 0.05
Eosinophil (%)	Female	0.01 ± 0.016	0.01 ± 0.01	0.01 ± 0.014	0.01 ± 0.014	0.01.±.0.014	> 0.05
	Male	0.52 ± 0.07	0.50 ± 0.09	0.52 ± 0.07	0.53 ± 0.08	0.52 ± 0.09	> 0.05
Basophil (%)	Female	0.66 ± 0.06	0.67 ± 0.06	0.67 ± 0.08	0.70 ± 0.06	0.71 ± 0.06	> 0.05
	Male	0.98 ± 0.02	0.97 ± 0.02	0.97 ± 0.02	0.96 ± 0.01	0.96 ± 0.02	> 0.05
Monocyte (%)	Female	0.94 ± 0.01	0.93 ± 0.01	0.94 ± 0.03	0.91 ± 0.02	0.91 ± 0.03	> 0.05
	Male	39.02 ± 0.02	39.05 ± 0.02	39.06 ± 0.02	39.05 ± 0.03	39.04 ± 0.03	> 0.05
Heterophil (%)	Female	42.10 ± 0.02	42.01 ± 0.02	42.11 ± 0.02	41.98 ± 0.02	42.30 ± 0.01	> 0.05
	Male	59.47 ± 0.02	59.45 ± 0.02	59.37 ± 0.02	59.44 ± 0.02	59.47 ± 0.02	> 0.05
Lymphocyte (%)	Female	56.50 ± 0.02	56.49 ± 0.02	56.32 ± 0.01	56.50 ± 0.01	56.20 ± 0.02	> 0.05
H/L ratio	Male	0.66 ± 0.005	0.65 ± 0.005	0.66 ± 0.005	0.66 ± 0.005	0.65 ± 0.005	> 0.05

Table 4a. According to the sex.

Blood parameters	Sex	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	*P values*
	Male	2.64 ± 0.03	2.64 ± 0.03	2.65 ± 0.03	2.65 ± 0.02	2.64 ± 0.03	> 0.05
RBC ($10^6/mm^3$)	Female	2.23 ± 0.01	2.22 ± 0.01	2.24 ± 0.01	2.23 ± 0.01	2.22 ± 0.01	> 0.05
	Male	9.93 ± 0.04	9.91 ± 0.04	9.96 ± 0.03	9.96 ± 0.05	9.92 ± 0.04	> 0.05
Hemoglobin (g/dl)	Female	8.54 ± 0.02	8.56 ± 0.02	8.62 ± 0.01	8.57 ± 0.02	8.54 ± 0.02	> 0.05
	Male	47.69 ± 0.06	47.71 ± 0.07	47.87 ± 0.07	47.83 ± 0.06	47.74 ± 0.06	> 0.05
Hematocrit (%)	Female	36.51 ± 0.07	36.47 ± 0.07	36.83 ± 0.08	36.70 ± 0.08	36.42 ± 0.07	> 0.05
	Male	182.34 ± 0.30	182.32 ± 0.28	182.18 ± 0.27	182.82 ± 0.3	182.78 ± 0.27	> 0.05
MCV (fl)	Female	164.00 ± 0.19	164.07 ± 0.15	164.42 ± 0.20	164.25 ± 0.15	164.24 ± 0.16	> 0.05
	Male	37.94 ± 0.14	38.01 ± 0.16	37.89 ± 0.11	37.91 ± 0.13	37.96 ± 0.1	> 0.05
MCH (ρg)	Female	38.35 ± 0.06	38.54 ± 0.07	38.50 ± 0.05	38.36 ± 0.06	38.54 ± 0.06	> 0.05
	Male	20.89 ± 0.08	20.84 ± 0.07	20.92 ± 0.08	20.84 ± 0.08	20.85 ± 0.07	> 0.05
MCHC (g/dl)	Female	23.51 ± 0.07	23.59 ± 0.08	23.58 ± 0.08	23.45 ± 0.07	23.53 ± 0.06	> 0.05
	Male	9.78 ± 0.06	9.86 ± 0.05	9.65 ± 0.06	9.61 ± 0.06	9.87 ± 0.05	> 0.05
Platelets ($10^3/mm^3$)	Female	9.49 ± 0.06	9.75 ± 0.05	9.49 ± 0.05	9.54 ± 0.04	9.68 ± 0.05	> 0.05
	Male	18.58 ± 0.009	18.61 ± 0.009	18.65 ± 0.008	18.60 ± 0.009	18.60 ± 0.007	> 0.05
WBC ($10^3/mm^3$)	Famele	21.03 ± 0.004	21.05 ± 0.004	21.03 ± 0.005	21.03 ± 0.004	21.03 ± 0.004	> 0.05
	Male	0.009 ± 0.01	0.01 ± 0.016	0.009 ± 0.01	0.009 ± 0.01	0.009±0.01	> 0.05
Eosinophil (%)	Female	0.01 ± 0.01	0.01 ± 0.010	0.01 ± 0.01	0.01 ± 0.01	0.01±0.01	> 0.05
	Male	0.63 ± 0.07	0.62 ± 0.08	0.60 ± 0.07	0.57 ± 0.07	0.61 ± 0.09	> 0.05
Basophil (%)	Female	2.74 ± 0.07	2.82 ± 0.05	2.58 ± 0.04	2.86 ± 0.04	2.96 ± 0.04	> 0.05
	Male	1.87 ± 0.05	1.86 ± 0.05	1.87 ± 0.05	1.91 ± 0.04	1.95 ± 0.02	> 0.05
Monocyte (%)	Female	1.69 ± 0.05	1.68 ± 0.04	1.69 ± 0.04	1.70 ± 0.04	1.68 ± 0.03	> 0.05
	Male	44.35 ± 0.01	44.42 ± 0.01	44.40 ± 0.01	44.40 ± 0.02	44.33 ± 0.01	> 0.05
Heterophil (%)	Female	54.50 ± 0.01	54.57 ± 0.01	54.37 ± 0.02	54.18 ± 0.02	54.56 ± 0.01	> 0.05
	Male	53.13 ± 0.01	53.08 ± 0.01	53.03 ± 0.02	53.09 ± 0.01	53.09 ± 0.01	> 0.05
Lymphocyte (%)	Female	41.04 ± 0.03	40.90 ± 0.02	41.34 ± 0.02	41.23 ± 0.03	40.78 ± 0.01	> 0.05
	Male	0.83 ± 0.003	0.83 ± 0.002	0.83 ± 0.003	0.83 ± 0.004	0.83 ± 0.003	> 0.05
H/L Ratio	Female	1.32± 0.007	1.33 ± 0.007	1.31 ± 0.006	1.32 ± 0.007	1.34 ± 0.005	> 0.05

was significantly higher than that of adult and younger quails; this could be due to the physiological stress due to oviposition. The same observations were made by Fouad et al. (2008). They showed that the H/L ratio increases with age. The H/L ratio has proven to be a reliable indicator of stress associated with injuries,

Table 5a. According to the sex.

Blood parameters	Sex	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	*P values*
	Male	2.74 ± 0.014	2.74 ± 0.012	2.75 ± 0.01	2.74 ± 0.011	2.74 ± 0.013	> 0.05
RBC (10^6/mm^3)	Female	2.21 ± 0.008	2.22 ± 0.007	2.20 ± 0.009	2.22 ± 0.009	2.22 ± 0.006	> 0.05
	Male	9.87 ± 0.04	9.90 ± 0.04	9.86 ± 0.04	9.88 ± 0.04	9.90 ± 0.03	> 0.05
Hemoglobin (g/dl)	Female	8.22 ± 0.012	8.23 ± 0.012	8.20 ± 0.010	8.23 ± 0.01	8.21 ± 0.01	> 0.05
	Male	50.17 ± 0.1	50.14 ± 0.07	50.44 ± 0.08	50.34 ± 0.09	50.30 ± 0.09	> 0.05
Hematocrit (%)	Female	36.28 ± 0.06	36.25 ± 0.06	36.07 ± 0.05	36.24 ± 0.07	36.26 ± 0.05	> 0.05
	Male	183.07 ± 0.2	183.14 ± 0.13	183.36 ± 0.14	183.47 ± 0.17	183.24 ± 0.18	> 0.05
MCV (fl)	Female	163.74 ± 0.14	163.52 ± 0.14	163.59 ± 0.14	163.70 ± 0.19	163.76 ± 0.13	> 0.05
	Male	36.01 ± 0.09	36.19 ± 0.08	35.90 ± 0.08	36.00 ± 0.08	36.07 ± 0.07	> 0.05
MCH (ρg)	Female	37.09 ± 0.04	37.12 ± 0.05	37.18 ± 0.04	37.17 ± 0.04	37.11 ± 0.04	> 0.05
	Male	19.79 ± 0.09	19.79 ± 0.06	19.62 ± 0.06	19.73 ± 0.08	19.76 ± 0.07	> 0.05
MCHC (g/dl)	Female	22.72 ± 0.05	22.75 ± 0.04	22.77 ± 0.04	22.80 ± 0.05	22.71 ± 0.05	> 0.05
	Male	9.69 ± 0.05	9.61 ±0.06	9.45 ± 0.05	9.73 ± 0.05	9.6 ± 0.06	> 0.05
Platelets (10^3/mm^3)	Female	9.70 ± 0.05	9.59 ± 0.04	9.57 ± 0.06	9.52 ± 0.06	9.75 ± 0.05	> 0.05
	Male	21.66 ± 0.01	21.57 ± 0.01	21.60 ± 0.01	21.66 ± 0.01	21.59 ±0.01	> 0.05
WBC (10^3/mm^3)	Female	23.44 ±0.05	23.54 ± 0.05	23.53 ± 0.05	23.36 ± 0.05	23.45 ± 0.05	> 0.05
	Male	0.009 ± 0.142	0.01 ± 0.016	0.009 ± 0.010	0.009 ± 0.014	0.009 ± 0.014	> 0.05
Eosinophil (%)	Female	0.01 ± 0.032	0.011 ± 0.013	0.01 ± 0.032	0.01 ± 0.014	0.01 ± 0.017	> 0.05
	Male	0.65 ± 0.07	0.64 ± 0.07	0.64 ±0.06	0.65 ± 0.08	0.63 ± 0.06	> 0.05
Basophil (%)	Female	3.51 ± 0.06	3.49 ± 0.03	3.51 ± 0.03	3.43 ± 0.03	3.48 ± 0.04	> 0.05
	Male	2.01 ± 0.05	1.99 ± 0.04	2.04 ± 0.05	1.98 ± 0.04	2.00 ± 0.04	> 0.05
Monocyte (%)	Female	1.53 ± 0.04	1.55 ± 0.04	1.52 ± 0.03	1.52 ± 0.05	1.50 ± 0.05	> 0.05
	Male	46.36 ± 0.03	46.08 ± 0.03	46.11 ± 0.02	45.96 ± 0.03	46.13 ± 0.03	> 0.05
Heterophil (%)	Female	56.99 ± 0.02	56.97 ± 0.01	57.00 ± 0.01	57.21 ± 0.03	57.40 ± 0.02	> 0.05
	Male	50.96 ± 0.03	51.26 ± 0.02	51.2 ±0.03	51.40 ± 0.04	51.23 ± 0.03	> 0.05
Lymphocyte (%)	Female	37.95 ± 0.03	37.97 ± 0.02	37.94 ± 0.03	37.82 ± 0.03	37.60 ± 0.02	> 0.05
	Male	0.91 ± 0.009	0.89 ± 0.03	0.9 ± 0.007	0.89 ± 0.007	0.9 ± 0.007	> 0.05
H/L ratio	Female	1.51 ± 0.007	1.50 ± 0.008	1.50 ±0.007	1.51 ± 0.008	1.53 ± 0.008	> 0.05

breeding cycles and seasonal changes in captive birds (Moreno et al., 2002).

The results of the analysis of variance showed that leukocyte values did not differ between groups within each week, thereby proved that the variation in crude protein level did not influence the quail's health. The leukocyte counts were reported as an index to measure the health status of the animals (McKenzie et al., 2005). An increase in the number of leukocytes was recorded under conditions of disease, infection or immune system disorder (Makkar et al., 2016).

Effect of dietary crude protein variation on red blood cells

Concerning erythrocytes, the results showed that they were almost identical in males and females. These values were similar as reported by Sturkie and Grimminger (1976); Agina et al. (2017); Lukanov et al. (2018). Although these parameters were within the range of most authors, erythrocytes were lower in this study than those observed by Gabriela et al. (2011) who showed that erythrocytes values was 2.9 ± 0.049 X 106 /mm^3. These differences may be due to the season, as erythrocyte levels drop when the ambient temperature rises. Furthermore, these results showed that the variation in protein levels in the rations had no impact on erythrocyte formation because the quails remained healthy.

The same observations were made by Anna et al. (2018) in chickens. They showed that the variation in crude protein in the 2-week-old chicken had no impact on erythrocytes.

As for hemoglobin, which acts as a buffer reducing excess H+ acid proton according to Lewis et al. (2011), its level remained stable throughout the study. Our results are consistent with those of Mohamed et al. (2012) and Anna et al. (2018). They showed that dietary protein levels did not affect hemoglobin. These results could be because dietary protein supplementation may promote an increase in the globin part of hemoglobin but not the hem part (Shahidullah et al. 2008). The mean hemoglobin levels in this study were found to be similar to those described for the species. However, our values are higher than those found by Mohammad et al. (2019). This difference could be due to the collection site of the blood analyzed. A decrease in hematocrit can be caused by several factors including stress level, environment, nutrition, dehydration and the presence of parasites in the blood (Maheswaran et al. (2008).

Conclusion

This study indicates that the variation in dietary crude protein levels did not impact on the hematological parameters of quails from the growth period to the laying period. On the other hand, levels between 22 and 26% were not likely to alter the health status of Japanese quail. Our study was carried out on quails aged between 21 and 49 days, which represent a narrow age range. Thus, it would be necessary to be interested in quails one day old 49 days old on the one hand.

On the other hand, further work should be conducted to see the effect of amino acids in the feed rations on blood parameters.

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