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Influence of Magmeal supplementation on haematological parameters in Japanese quails

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Abstract

A study was conducted on 240 Japanese quail birds (*Coturnix coturnix japonica*) from day old to six weeks of age to evaluate the effect of magmeal supplementation on haematological parameters at Madras Veterinary College, Chennai. The birds were divided into 4 groups with 20 birds each in each group in 3 replicates and fed with diet replacing fish meal with magmeal at varying proportion - G₁ (Control group with 7%), G₂ (50%), G₃ (75%) and G₄ (100%). The effect of magmeal inclusion on hematological parameters suggested that there was a significant decrease in hemoglobin concentration in G₂ and G₃ when compared G₁ and then significantly increased ($P < 0.01$) in G₄. There was no significant difference in hemoglobin concentration between the groups on the inclusion of magmeal at six weeks of age. But there was a significantly high increase ($P < 0.01$) in hemoglobin concentration at six weeks than at three weeks of age of birds in control and experimental groups of Japanese quails. The highest hemoglobin concentration observed was 14.98 ± 0.10 g/dL in G₃ at six weeks of age. There was a significant increase ($P < 0.01$) in total erythrocyte count as the percentage of magmeal inclusion increased within the groups at three and six weeks of age. There was no significant difference observed in total leucocyte count between the control and experimental groups and between the three and six weeks upon the inclusion of magmeal at different levels. There was no significant difference in differential count between the three weeks and six weeks of age in control and experimental groups of Japanese quails. Thus it was concluded the inclusion of magmeal can possibly lead to increase in poultry production and consequent economic affordability to the much needed animal protein.

Keywords: Magmeal, Maggot meal, Japanese quail, Haematology

Introduction

Protein sources are included in the diet of all organisms and fish meal containing about 60% protein is the good protein source in the quail diet. However, due to overexploitation and burgeoning human population, fish availability is declining and there is a cut-throat competition for the same between man, animal and bird. This increase in demand has led to increased price of fish meal for poultry feed. In addition, the high feed costs can be attributed to scarcity and high cost of feed ingredients particularly animal protein supplements. The price of fish meal, the most guaranteed animal protein source has become prohibitive^[1]. Hence it becomes imperative to replace fish meal with other animal protein supplements. Magmeal is a core product consisting of dried defatted larvae that is ground into a high protein larvae meal. Maggot meal, popularly magmeal is a potential alternative for fish replacement in the diet of quails. It has high protein content. The high crude lipid acts as protein sparer. It has a dark rich texture with a slightly nutty flavour. It is a rich source of animal protein and limiting essential amino acids - arginine and methionine, that can be fed to poultry, pig and fish. Hence it is very crucial to incorporate magmeal as an alternative to reduce the feeding cost. Blood profiling is used to detect subclinical, clinical, metabolic conditions, incorrect feeding and managemental practices and also the welfare of animals and birds^[2]. Thus the effect of magmeal inclusion on the haematological changes in Japanese quail (*Coturnix coturnix japonica*) was studied.

Materials and Methods

Preparation of magmeal

Maggots were cultured in the Department of Veterinary Parasitology, Madras Veterinary College, Chennai – 600 007 by the floatation method of harvesting of housefly larvae from manure of laying chickens^[3]. It involved flooding of the manure with excess water, thus making the larvae float for their easy removal, washing them in clean water three times until there was no remnant of manure, after which they were dewatered and later oven-dried at 60°

C for 24 hours, drying the magmeal and incorporating into the diet (Figure 1).

Proximate analysis of magmeal

The proximate analysis of the prepared magmeal was done at Animal Feed Analytical and Quality Assurance Laboratory, Veterinary College and Research Institute, Namakkal – 637 002, Tamil Nadu (Table 1).

Table 1: Proximate Analysis of MagMeal

Component	Percentage
Moisture	10.68
Crude protein	48.73
Crude fat	4.28
Ether extract	26.64
Total ash	4.65
Calcium	0.30
Phosphorus	0.63
Nitrogen free extract	5.02
Gross energy	5643 kcal/kg



Fig 1: A handful of maggots and MagMeal

Feed composition and animal trials

A total of 240 birds from day old to 6 weeks of age were maintained in equicaloric and equinitrogenous dietary regime Poultry Research Station, Madhavaram Milk Colony, Chennai – 600 051. The birds fed with quail brooder and finisher mash with varying proportion of fishmeal and magmeal and water *ad libitum* were divided into four groups as follows (Table 2).

Hematological parameters

One milliliter of blood was used for the estimation of hemoglobin (Hb) concentration by cyanmethaemoglobin method as per Van Kampen and Tijlstra [4], total erythrocyte count (TEC) by using Nambiar's diluting fluid [5], total leucocyte count [6] and differential count (DC) by using modified Leishman – Giemsa stain as per the method described by Bancroft and Marilyn [5].

1. Hemoglobin concentration

The hemoglobin concentration was estimated by cyanmethaemoglobin method [4] using commercially available kit purchased from Agapge Diagnostics Limited.

Procedure

	Blank	Sample
Hemoglobin Reagent	5000 μ l	5000 μ l
Sample	-	20 μ l
Mixed well and incubated at room temperature for five minutes. The absorbance of the sample was measured against reagent blank and that of standard directly against reagent blank at 546 nm wavelength in spectrophotometer.		

$$\text{Hemoglobin concentration (g/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 15$$

2. Total Erythrocyte Count (TEC)

The total erythrocyte count was estimated by means of Neubauer counting chambers by using Nambiar's fluid [5].

Procedure	
Working solution of Nambiar's Fluid	
2 % Sodium citrate	1 ml
Gentian violet in 0.1 % in Ringer's solution	2 ml
Brilliant cresyl blue in 0.1 % in Ringer's solution	1 ml
Neutral formalin	3 drops
Preparation of Ringer's solution	
Sodium chloride	0.7 g
Sodium bicarbonate	0.03 g
Potassium chloride	0.026 g
Calcium chloride	0.003 g
Distilled water	100.00 ml

Well mixed anticoagulant blood was drawn up to the mark of 0.5 in the RBC pipette, followed by Nambiar's fluid drawn up to 101 mark. Erythrocytes in five secondary squares were counted and the total was multiplied by 10,000 and the results expressed in millions per mm^3 .

3. Total Leucocyte Count (TLC)

The total leucocyte count was estimated indirectly from a well prepared blood film [6].

Procedure

The average number of leucocytes per five oil immersion fields was calculated and the total leucocyte count was estimated by using the following formula.

$$\text{Estimated TLC (x } 10^3/\mu\text{l)} = \frac{\text{Average No. of leucocytes/ 5 fields}}{1000} \times 3,500,000$$

where the number 1,000 is the average number of erythrocytes in five oil immersion fields and 3,500,000 is the number of erythrocytes per μ l in birds with a normal packed cell volume.

4. Differential Count (DC)

The differential count was carried out according to the method of Bancroft and Marilyn [5].

Procedure

The blood smears were stained by modified Leishman – Giemsa's stain and after drying under oil immersion for counting.

Preparation of 100 ml modified Leishman – Giemsa's stain and staining techniques

Leishman's powder of 150 mg and 30 mg of Giemsa powder were dissolved in 100 ml of acetone free methanol. The solution was stirred and mixed thoroughly for about 1 hour using a mortar and pestle. The solution was filtered thrice in Whatman's filter paper No. 1. A thin blood smear was made from whole blood and smear was air dried. The smear was stained with modified Leishman – Giemsa stain and held for 2 minutes. Distilled water was added slowly over the stain and allowed for fifteen minutes. The smear was washed, air dried and examined under oil immersion lens of microscope.

Statistical analysis

The data were subjected to general linear model (GLM) with interaction in two - way analysis of variance (ANOVA) and post hoc analysis were carried out using Duncan's test for multiple comparisons using SPSS software version 20 as per Snedecor and Cochran method [7].

Results and discussion

1. Hemoglobin concentration

The mean hemoglobin concentration observed in the present study at three week of age decreased to a value from 14.07 ± 0.12 g/dL to 13.66 ± 0.24 g/dL after supplementation of magmeal at 75 per cent level. The mean hemoglobin concentration then increased to 14.08 ± 0.22 g/dL when the quails were supplemented with 100 per cent magmeal. The hemoglobin concentration in the present study falls within the normal range reported by Ali *et al.* [8]. The mean hemoglobin concentration observed in the present study at six weeks of age in quails showed no significant difference between the groups. This finding was similar to that of Mohamed *et al.* [9] in broilers. The mean hemoglobin concentration observed in the present study at six weeks of age in control and experimental groups of Japanese quails showed an increased concentration when compared to birds at three weeks of age. The increased hemoglobin concentration at six weeks of age may be attributed to age and the increased amount of glycine present in the magmeal. The amino acid glycine is important for the hemoglobin synthesis and hence the increase in hemoglobin concentration. The increase in hemoglobin concentration may also be due to the increase in the globin part of the hemoglobin on supplementation of dietary protein [10].

2. Total Erythrocyte Count (TEC)

The average total erythrocyte count recorded in the present study increased from the control value of $3.80 \pm 0.01 \times 10^6/\mu\text{l}$ to $3.90 \pm 0.05 \times 10^6/\mu\text{l}$ at three weeks of age and from $3.74 \pm 0.16 \times 10^6/\mu\text{l}$ to 4.05 ± 0.02 at six weeks of age on supplementation of magmeal in the diet. The present observations are contradictory to the findings of Egbunike *et al.* [11] and Mohamed *et al.* [9] in broilers. They reported no significant difference in the total erythrocyte count on supplementation of dietary protein in broilers. Tehrani *et al.* [12] also reported that erythrocyte does not increase on supplementation of animal protein. The increase in the total erythrocyte count may be attributed to the increase in thyroid hormones which in turn accelerates the metabolism in birds. This increase in oxidative metabolism causes an increase in the release of erythrocytes into circulation. There was no significant difference in the total erythrocyte count recorded between the groups of birds at three and six weeks of age.

3. Total Leucocyte count (TLC)

The total leucocyte count in this study revealed no significant difference between the groups between three and six weeks of age which is contradictory to that of Tehrani *et al.* [12] who recorded a decreased total leucocyte count on inclusion of brine shrimp (*Artemia Urmiana*) in broiler diet.

4. Differential Count (DC)

The average value of heterophils observed in present study at three week and six week of age in control and experimental group of Japanese quails showed no significant difference. This finding is in accordance with Das and Mandal [13] in quails whereas Tehrani *et al.* [12] reported decreased heterophils in broilers on supplementation of brine shrimp (*Artemia Urmiana*). In the present study, the average value of eosinophilic count showed no significant difference between the control and experimental group of birds at three and six weeks of age. This finding was consistent with Das and Mandal [13] in quails of different dietary groups. Lymphocytes are the second most common leucocyte and a decrease in their numbers is commonly associated with diseases causing increased stress. They occur in a variety of shapes varying from uniformly round or irregular to those that seen to mould around adjacent cells in blood cell or have cytoplasmic budding [6]. In the present study, no significant difference was observed in lymphocyte count in control and experiment groups of birds at three and six weeks of age. The present finding is in accordance with Das and Mandal [13] in quails. On the other hand, Tehrani *et al.* [12] reported an increased lymphocyte count in broilers fed with brine shrimp (*Artemia Urmiana*). In the present study, there was no significant difference in the monocytes in control and experiment groups of birds at three and six weeks of age. This finding is in accordance with Das and Mandal [13] in quails. There were no significant differences in the differential count between different groups at three and six weeks of age. Das and Mandal [13] reported that distribution of leucocytes does not have significant effect when quails were supplemented with grasshopper meal at different dietary levels.

Conclusion

Magmeal had a positive impact on hematological parameters in Japanese quails by improving the hemoglobin concentration at three weeks and total erythrocyte count at both three weeks and six weeks.

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Table 3: Effect of magmeal inclusion on haematological parameters at three and six weeks of age in control and experimental groups of Japanese quails (n = 24, Mean \pm SE)

Group	Hemoglobin concentration (g/dL)		Total erythrocyte count ($\times 10^6/\mu\text{l}$)		Total leucocyte count ($\times 10^3/\mu\text{l}$)	
	Three week	Six week	Three week	Six week	Three week	Six week
G ₁ (Control)	$14.07^c \pm 0.12$	14.44 ± 0.20	$3.80^a \pm 0.01$	$3.74^a \pm 0.16$	21.20 ± 0.37	21.15 ± 0.42
G ₂	$13.93^b \pm 0.16$	14.02 ± 0.15	$3.87^{bc} \pm 0.03$	$3.77^b \pm 0.10$	20.73 ± 0.41	21.06 ± 0.40
G ₃	$13.66^a \pm 0.24$	14.98 ± 0.10	$3.86^b \pm 0.02$	$3.85^{bc} \pm 0.03$	21.55 ± 0.47	22.88 ± 0.29
G ₄	$14.08^d \pm 0.22$	14.94 ± 0.11	$3.90^c \pm 0.05$	$4.05^c \pm 0.02$	20.06 ± 0.35	24.50 ± 0.32
F	9.829*	2.352 ^{NS}	0.950*	2.077*	5.626 ^{NS}	16.739 ^{NS}
F	6.985 **		3.027 ^{NS}		7.770 ^{NS}	

NS- Not significant ($P > 0.05$)

*- Significant ($P < 0.05$)

**- Highly significant ($P < 0.01$)

Mean values having same superscript within a column do not differ significantly.

Table 4: Effect of magmeal inclusion on differential count (%) at three and six weeks of age in control and experimental groups of Japanese quails (n = 24, Mean \pm SE)

Group	Heterophils		Eosinophils		Lymphocytes		Monocytes	
	Three week	Six week						
G ₁ (Control)	53.67 \pm 2.56	53.33 \pm 3.10	12.17 \pm 2.19	11.17 \pm 0.75	34.17 \pm 0.19	35.00 \pm 1.77	1.67 \pm 0.33	1.77 \pm 0.21
G ₂	50.67 \pm 3.60	50.33 \pm 2.62	11.87 \pm 2.38	9.38 \pm 1.02	38.87 \pm 2.18	40.17 \pm 1.28	1.45 \pm 0.14	1.30 \pm 0.04
G ₃	50.17 \pm 5.92	54.61 \pm 2.17	12.64 \pm 1.06	9.27 \pm 1.62	37.64 \pm 1.56	36.67 \pm 1.60	1.33 \pm 0.34	1.43 \pm 0.44
G ₄	50.33 \pm 4.03	55.03 \pm 2.81	11.33 \pm 1.77	11.50 \pm 1.47	37.33 \pm 1.07	33.83 \pm 2.11	1.67 \pm 0.15	1.80 \pm 0.56
F	4.025 ^{NS}	2.598 ^{NS}	2.313 ^{NS}	5.981 ^{NS}	2.313 ^{NS}	4.109 ^{NS}	2.767 ^{NS}	1.807 ^{NS}
F	3.315 ^{NS}		4.097 ^{NS}		3.211 ^{NS}		2.287 ^{NS}	

NS- Not significant ($P>0.05$)

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