Egypt. J. Anim. Prod. 20, No. 1, pp. 55—63 (1980)

Effect of Dietary Protein on the Immune Response and Growth of Baladi Chickens

O. E. Mohamed, A. A. S. Ahmed, M.S. Saber, M.M. El Guindi and G. A. R. Kamar

Faculty of Veterinary Medicine and Faculty of Agriculture, Cairo University, Cairo, Egypt.

THE SUBJECT of the effect of dietary modifications on the immune response in avian species is one that has attracted the attention of some workers. However, most available investigations have been confined mainly to the influence of vitamins (Ponds and Commbs, 1963, Squibb, 1963). Therefore, work directed towards defining the apparent influence of other dietary ingredients on the immune response of chickens would be of value.

The present investigation was carried out primarily to study the effect of type and level of dietary protein on the immune response of chickens of the Baladi variety to Newcastle Disease (ND) vaccines as measured by haemagglutination inhibitting (HI) antibody levels and resitance to challenge. Besides, the rate of growth was determined.

The achieved results showed that :

1. Normal relatively high or low levels of dietary protein supplemented with animal protein or wholly of vegetable origin have no apparent influence of HI antibody level attained by vaccinated or revaccinated birds.

2. Vaccinated young chickens fed on normal or relatively low levels of protein of All-vegetable origin showed lower resistance than those that received corresponding levels of dietary protein supplemented with animal protein, when exposed to challenge with virulent ND virus. However, the influence of type of dietary protein was not evident when the birds were challenged about the 5th month of age.

3. Normal, relatively low or high protein levels as well as the protein type(wholly vegetable or supplemented with animal protein) have no influence upon average values of total serum protein. Advancement of age was associated with a rise in the total serum protein.

4. Difference in body weight gain between vegetable and animal protein fed chickens proved to be insignificant after the 9*th* week of age, if the vegetable diet is supplemented with the required minerals and vitamins.

5. Animal protein supplements for poultry rations fed to native breeds of chickens under our village conditions are not economic, since the gain in body weight is insignificant in reference to the high cost of animal protein supplements.

The subject of the effect of dietary modifications on the immune response in avian species is one that has attracted the attention of some workers. However, most available investigations have been confined mainly to the influence of vitamins (Ponda and Commbs, 1963, Squibb, 1963). Therefore, work directed towards defining the apparent influence of other dietary ingridients on the immune response of chickens would be of value.

This work was planned to investigate primarily the effect of level and type of dietary protein on the immune response of native chickens of the Baladi variety to vaccination against Newcastle Disease (ND) and resistance to challinge. In addition, the growth rate of this variety was studied.

Material and Methods

Chicks : One hundred and ninety four day-old chicks of both sexes of the white Baladi variety were used in this work. They were provided by the Poultry Experimental Station, Faculty of Agriculture, Giza. The birds were banded and were reared in 4 separate rooms fitted with electric heaters. The start temperature was 95°F $\pm$ 2°F, and was gradually reduced by about 5°F every week till the age of 5 weeks when artificial heating was dispen- sed with.

Feeding system : The chicks received fine clear sand and fresh water as day-old. The sand was allowed only once for about 15 minutes. Crushed corn was given for the first 2 days and normal balanced diet for the subsequent 7 days. During the study an experimental ration was placed in front of the chicks throughout the whole day with frequent mixing.

Nd virus strains : The F (Lot 68) and the Komarov (Lot 16) vaccine strains employed on a national ocale in Egypt were used for immunization ofthe birds, at the second and the 66th day of age respectively, while the field virulent " Frashout " strain in the 11th, egg passage served for challenge. In addition, a suitable ND virus strain was used for the Haemagglutination inhibition tests.

Haemagglutination inhibition (Hi) Test : This was carried out in plastic plates in the usual way, using a haemagglutination units /0.25 ml virus suspension.

Determination of total serum proteins : This was conducted according to the Buiret method (King and Wootton, 1959).

Experimental

The experiment began on the 23-rd of May 1967 and ended on the 9th November 1967.

At the 10th day of age, the chicks were divided at random into 4 nearly equal groups. Each group was placed in one of the aforementioned rooms and was fed throughout the experiment on a diet formulated as shown in the following table :

Ingredient	Group I	Group II	Group III	Group IV
Crushed corn	45 parts	45 parts	65 parts	68 parts
Cotton seed meal	25 „	15 „	5 „	-
Wheat bran	10 „	10 „	10 „	10 „
Crushed beans	17 „	17 „	17 „	12 „
Skimimilk (dried)	-	5 „	-	4 „
Fish meal	-	5 „	-	3 „
Bone meal	1 „	1 „	1 „	1 „
Calcium carbonate	1 „	1 „	1 „	1 „
Common salt	0.5 „	0.5 „	0.5 „	0.5 „
Mineral mixture	0.5 „	0.5 „	0.5 „	0.5 „
Mineral mixture*	0.5 „	0.5 „	0.5 „	0.5 „

Every 100 g, contain: calcium 36 g, sodium chloride 10 g, phosphorus 9.5 g, iron 1.4 g, sulphur 600 mg, manganese 130 mg, copper 20 mg, magnesium 100 mg, iodine 50 mg and cobalt 12 mg.

In all groups, dried yeast (3%) was added as a source of vitamin B complex, and green fodder, in the form of Darawa leaves, was frequently given with the ration (5%).

The above mentioned diets provided the following levels and types of dietary protein which were chosen to simulate nearly our village feeding conditions :

Group I : The diet contained 19.4 % total protein wholly of plant origin.

Group II : The diet provided about 19.9% total protein of which 15.4% of plant and 4.5% of animal origin.

Group III : The diet contained only 13.2% total protein wholly of plant origin.

Group IV : The diet provided only 13.4% total protein of which 10.4% of plant and 3% of animal origin.

For serological response and chemical analysis, individual blood samples were taken from each group, 64 days post vaccination with the "F" strain. Sex representative serum pools were prepared for each group and assayed for HI antibody and total protein content. At the same time, the chickens in all groups were revaccinated intramuscularly (I/M) with the Komarov strain except for 6 random birds from each group. The latter were exposed to 104

LD_{50} of the challenge virus to determine their immune status; half the chickens were injected I/M and the other half intranasally. Challenged groups were kept in isolation rooms and observed for symptoms and for death for 3 weeks.

Twenty-two days postvaccination with the Komarov strain, individual blood samples were similarly collected for both serological response and serum protein determination. Similar blood samples were taken 45 and 57 days postvaccination and examined only for their HI antibody content. On the 91st day postvaccination, individual blood samples were taken for the HI test and at the same time the birds in all groups were challenged I/M in doses of 104 LD_{50}. Two weeks after challenge, blood samples were collected for serological and chemical examination.

For determination of the growth rate, individual birds of each group were weighed at different intervals during the experiment. The data obtained were statistically analysed according to Snedecor (1956).

Results and Discussion

The results of immune response to vaccination and revaccination, as judged by HI test and resistance to challenge, are summarized in Table 1. The determined average values of total serum protein, body weight and body weight gain are given in Tables 2, 3 and 4 respectively.

Two weeks after feeding the experimental diet to the birds of group III and IV, two birds from group III and one from group IV died with symptoms and lesions of subcutaneous oedema throughout the whole body.

From Table 1, it is evident that the NI antibody response to vaccination or revaccination with ND live virus vaccine of the 2 groups of chickens maintained on diet containing relatively low levels of total protein (13.2 and 13.4%) was almost similar to that of the corresponding groups that recieved normal or relatively high levels (19.4 and 19.9%) at all intervals to which the blood was tested by the HI test. These findings coincided with those of Squibb (1964) who reported the except for diets extremely definicient in protein (7.7%), HI antibody rise was not impaired.

Moreover, the data here on (Table 1) showed that even the type of dietary protein had no influence to the HI antibody response of the vaccinates or revaccinates. Nevertheless, exposure to challengs *via* the I/M route at 91 days post vaccination with the Komarov strain revealed that the HI antibody level in the group fed on low protein diet of all vegetable origin (group III) was about three times lower than corresponding groups that received either low protein level supplemented with animal protein (group IV) or relatively high levesl supplemented or unsupplemented with animal protein Group II and I).

TABLE 1. Haemagglutination inhibition-antibody response and challenge results of Baladi chickens fed different levels types of dietary protein.

	Group 1			Group II			Group III			Group IV		
	C.M.	HI.Tit	I/M.	C.M.	HI.Tit.	I/Na	C.M.	HI.Tit.	1/Na	C.M.	HI.Tit	I/NaI/Ma
64 days p.v. with "p" (66 days old	5	3/3	2/3	6	3/3	3/3	9	3/3	2/3	6	3/3	3/3
22 days p.v. with Komarov (88 days lod)	66	—	—	30	—	—	46	—	—	46	—	—
45 days p.v. with Komarov(111 days old)	16	—	—	3	—	—	9	—	—	4	—	—
57 days p.v. with Komarov (123 days old	20	—	—	10	—	—	10	—	—	10	—	—
91 days p.v. with Komarov (155days old)	8	—	—	0	—	—	8	—	—	8	—	—
2 weeks p. challenge 170 days old)	3415	—	2/27	2132	—	3/34	853	—	2/30	2132	—	2/24

a = Survivors/challenged birds.
I/N = Intranasal challenge
p.v. = Postvaccination.
C.M.H.Tit. = Gemeteric mean of HI—Titers
I/M = Intramuscular challenge

TABLE 2. Average values of total serum protein content of Baladi chickens fed on different levels and types of dietary protein in relation to vaccination and challenge,

	Average values of total serum pretein content (g / 100 ml)			
	Group 1	Group 11	Group111	Grup IV
64 days p.v. with	2.997	2.772	2.924	2.941
the "F" (66 days)	±0.19	±0.26	±0.27	±0.31
22 days p.v. with	3.754	4.179	3.891	3.827
Komarov (88 days)	±0.21	±0.18	±0.26	±0.19
2 weeks p. challenge	6.62	7.13	7.40	7.13
(170 days)	±0.15	±0.24	±0.14	±0.12

± = Standard error.

TABLE 3. Average growth rate of Baladi chicks fed on different levels and types of dietary protein.

Age (weeks)	Average weight (grammes)			
	Group I	Group II	Group III	Group IV
2	60.1 ±1.40	60.2 ±1.43	59.4 ±1.23	59.9 ±1.04
4	126.5 ±3.04	128.2 ±3.76	103.8 ±2.69	105.2 ±3.6
6	182.2 ±5.6	220.7@ ±6.32	151.3 ±6.18	155.6 ±7.49
9	402.3 ±13.4	443.5@ ±12.7	320.0 ±16.12	330.2 ±14.18
12	666.2 ±18.78	694.3 ±17.2	484.3 ±27.89	489.3 ±20.83
18	1021.4 ±29.5	1070.0 ±30.3	791.15 ±22.8	80.63 ±11.1
22	1249.4 ±42.94	1299.2 ±44.6	1045.8 ±36.13	1054.67 ±58.67

± = Standard error

Using the "t" test, the average weights of the birds of group I and II are significantly higher than that of group III and IV at all periods from the 4th till 2nd week of age (P 0.01).

@ Significantly higher than group I at p 0.01.
@@ Significantly hgther than group I at p 0.05.

TABLE 4. Average body weight gain (Grames) of Balady chickens fed different levels and types of dietary protein during two periods of age.

Age	Group I	Group II	Group III	Group IV
2-12 weeks	603.65@ ±18.5	629.0@ ±16.9	422.37 ±23.6	429.07 ±20.1
12-22 "	584.81 ±31.1	610.19 ±27.8	548.06 ±24.5	557.69 ±20.1

± Standard error.
@ The averagovody weight gain of group a and 1 was found singificantly higher than ithat of group III and IV (P 0.01).

Some inferences, on the other hand, could be pointed in the magnitude of resistance of the experimental groups to challenge. While representative birds from groups maintained on diets containing normal or relatively low total protein levels supplemented with animal protein proved to be absolutely resistant to the challenge vuirs administered via other the 1/N or 1/M route, 9 weeks postvaccination with the " F " strain, one out of 3 birds corresponding to group I and I.I which received respectively normal and low levels of diterary protein of all vegetable origin developed nervous symptoms typical of the infection, 4 days following their I/M challenge. The higher resistance of animal protein fed chickens may suggest the possible influence of the type of dietary protein on the resistance of vaccinated birds as judged by challenge. Early observations made by Ackert and Beach (1933) as well as Alicata (1938) may support this relation. The former workers showed that diets consisting chiefly of animal proteins and with little or no plant proteins were important in aiding the chickens to build up resistance to infection with ascarids, and that diets consisting chiefly or wholly or vegetable proteins lowered the resistance to ascrid invision.c.ı Alicata, (1938), likewise, observed that birds given a diet consisting principally of animal protein concentrates developed fewer worms than those which were given a diet low in animal protein. Nevertheless, the influence of the type of dietary protein was not evident, when the birds were challenged at about the 5th month of age (13 post-vaccination with the Komarov strain), as nearly similar magnitudes of resistance were determined in all four groups of experimental birds regardless of the level and type of dietary protein.

Serum analysis (Table 2), showed no significant difference in the average values of total serum protein among the 4 groups of birds any interval during the experiment. Moreover, no correlation was found between total serum protein levels and the immune response of the vaccinated group of birds as measured by both HI antibody response and resistance to challenge. However, a direct correlation was noted between the former and the age of the birds in

that, a significant increase in the average values of total serum protein accompanied advancement of age. This finding agreed with that of Brandt *et al.* (1951) who reported that total serum proteins of chickens increase with age. The possible influence of challenge on gama globulin levels and consequently the total protein level could not be determined in this experiment.

From the data given in Table 3, it is evident that the average body weights attained at any period during the experiment by the group of chickens fed on a diet containing 19.9% total protein of which 4.5% of animal origin (Group 11) were relatively higher than those of the group that received about the same level of total protein of all vegetable origin (Group I). Significantly higher body weights, however, were attained by group II than group I at only the 6th and 9th week of age. The relatively lower growth rate of group I may be accounted for the calculated lower lerels of the amino acids lysine, glycine and methionine in the diet fed to these birds (0.75%, 0.92%, and 0.24% respectively) as compared with the diet of group II which covered the requirements of the birds for the mentioned amino acids. Comparable findings were reported by Mahdi (1958) for baladi and Fayoumi chickens. On the other hand, the average body weights attained by both mentioned groups during the period from the 4th to 22nd week of age were found to be significantly higher than group III and IV that received relatively low total protein levels (13.2 and 13.4%) unsupplemented or supplemented with animal protein. This difference may be attributed not only to the relatively lower levels of the protein but also to the the inbalance of the amino acid pattern in the protein fed on the birds of both groups III and IV.

The calculated average body weight gain (Table 4) although significantly higher in group I and II than the other two groups during the period from the 2nd week of age, however, the difference in gain was insiginficant during the second period from 12th 22nd week of age. The insignificant difference in the gain between group I and II and III and IV during the second period may be attributed to the relatively lower protein requirment during this period than the first period.

Form the present investigation, it can be concluded that no apparent influence is exerted on the HI antibody response of ND vaccinated birds fed on diets containing norml relatively high or low total protein levels. Likewise, the type of dietary protein has no influence on HI antibody levels of vaccinated or revaccinated birds, though it may play some role in the magnitude or resistance of these birds when exposed to challenge with a virulent virus. The difference in body weight gain between vegetable and animal protein fed chickens of the Baladi variety proved to be insignificant after the 9th week of age,if the vegetable diet is supplemented with the mineral and vitamin requirements According to Morrison (1959), when plant protein supplements are used as the only or the chief protein supplement in poultry rations, care must be taken to add whatever mineral and vitamin supplements may be needed. Under our village feeding conditions, where plant protein constitutes the chief protein supplement in the diet, such mineral and vitamin supplements may be recommende.

Morevoer, even though relatively higher average body weights and body weight. gain are attained by animal protein fed Baladi chickens than vegetable protein fed ones, yet, the magnitudes of difference, from the economic stand point, do not encourage for supplementing vegetable protein ratios with animal protein owing to the high cost of the latter in reference to the gain in body weight

References

Ackert, J.E. and Beach, T.D. (1933) Trans. *Am. Microbiol.Soc.* **52**, 51.

Alicata, J.E. (1938) *Rep. Hawad Agr. Exptl.* (1937), 93 .

Brandt, L.W., Clegg, R.E., and Andrews, A.C. (1951) *J.Biol. Chem.* **191**, 105.

El-Mahdi, S.M. (1958) *Bull. Fac. Vet. Med.*, Cairo Univ. Vol. S,11,

King, E.J.,an d Wootton, I.D.P. (1959) *Micronalysis in Medical* 3rd. Ed J. and A. Churchill London.

Morrison, F.B. (1959) *Food and Feeding*, 2nd Ed. The Morrison Publishing Company, Clinton, Iows.

Ponda, B.and Commbs. G.F. (1963) *Proc. Soc. Expptl. Biol.* **113**, 350.

Snedecor, G.W. (1956). *Statistical Methods*, 4th Ed. The Iowa State College Press, Ames, Iowa.

Squibb, R.L. (1963). *Poult. Sci.* **42**, 941.

Suqibb, R.L. (1964) *J. Nutr.* **82**, 422.

تاثير البروتين على المناعه والنمو فى الكتاكيت البلدى

اسامه عز الدين محمد ، احمد على سامى ، محمد سامى صابر ، محمود محمد الجندى و محمد جمال الدين قمر

كلية الطب البيطرى وكلية الزراعة ـ جامعة القاهرة

أجريت التجارب على ١٦٤ كتكوتا من النوع البلدى الأبيض عمر يوم واحد وقسمت الى أربعة مجموعات اختلفت علائقها بالنسبة لنوع البروتين (حيوانى ونباتى) وكذلك كميته وقد استمرت التجارب لمدة ٢٢ أسبوعا وقد دلت النتائج على أن نوع وكمية البروتين فى العليقة ليس له تاثير على تكوين الأجسام المضادة (المناعة) ضد مرض النيوكاسل .

كما أن اضافة الأملاح والفيتامينات الى العلائق المحتوية على البروتين النباتى فقط لم يجعل هناك اختلاف ملحوظ فى زيادة وزن الطيور بين المجاميع المختلفة .