# The effects of different sources of commercial rations on the humoral immune response of broilers to Newcastle disease vaccination

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### RÉSUMÉ

OYEJIDE (A.), TEWE (O. O.), OWOADE (A. A.). — Influence de rations alimentaires commerciales de différentes origines sur la réponse immunitaire humorale (RIH) de poulets de chair à la vaccination contre la maladie de Newcastle. Rev. Elev. Méd. vét. Pays trop., 1985, 38 (4): 443-448.

Des rations désignées par les lettres U, A et P, comportant des composés de démarrage et de finition ont été fournies par des fabricants locaux.

Elles ont été distribuées à un total de 300 poulets par ration. Pour chacune d'elles 100 poulets ont été vaccinés contre la maladie de Newcastle avec 2 souches, B1 à un jour et Lasota à 28 jours.

50 animaux, toujours pour chaque ration, n'ont reçu que le vaccin B1 à un jour. Enfin, dans les mêmes conditions, 150 poulets ont été vaccinés à 14 jours contre la bursite infectieuse (maladie de Gumboro).

Une analyse globale des régimes a montré que les taux de matières azotées totales pour les fractions « démarrage » et « finition » étaient respectivement de 24,34 et 20,59 p. 100 avec U, de 19,25 et 20,13 p. 100 avec A et de 23,38 et 20,78 p. 100 avec P.

La réponse immunitaire humorale à la vaccination contre la maladie de Newcastle a été déterminée par l'inhibition de l'hémagglutination. Celle-ci était la plus élevée avec la ration A et la plus basse avec la ration U, ces valeurs étant mesurées à la 4°, 6°, 8° et 9° semaine des essais.

La souche Lasota a accru de façon significative cette réponse immunitaire, comme l'ont généralement mis en évidence les titres plus élevés décelés chez les poulets qui ont reçu cette vaccination par comparaison avec les sujets non vaccinés.

En conclusion, des rations d'origines différentes peuvent jouer un rôle dans la réponse immunitaire à la vaccination contre la maladie de Newcastle.

Mots clés: Poulet - Ration alimentaire - Maladie de Newcastle - Vaccination - Réponse immunitaire - Nigeria.

### **SUMMARY**

OYEJIDE (A.), TEWE (O. O.), OWOADE (A. A.). — The effects of different sources of commercial rations on the humoral immune response of broilers to Newcastle disease vaccination. *Rev. Elev. Méd. vét. Pays trop.*, 1985, 38 (4): 443-448.

Three commercial rations designated U, A and P (with starter and finisher components) obtained from different local manufacturers were fed to a total of 300 broiler chicks each. One hundred of the broilers on each feed received 2 Newcastle disease (ND) vaccinations, B1 at day old, and Lasota at day 28. The remaining 50 birds on each feed received only the day old B1 vaccination. All 150 birds on each feed received infectious bursal disease (IBD) vaccination at day 14.

Proximate analysis of the diets showed that crude protein level in the starter and finisher rations were 24.34 p. 100 and 20.59 p. 100 for diet U, 19.25 p. 100 and 20.13 p. 100 for diet A, 23.38 p. 100 and 20.78 p. 100 for diet P respectively. Humoral immune response to ND vaccination as determined by haemagglutination inhibition (HI) titration was highest on the A diets and lowest on the U diets during the 4th, 6th, 8th and 9th weeks of the experiments. Lasota vaccination significantly boosted this immune response as evidenced by generally higher titres in birds receiving this vaccine when compared to those which did not receive the vaccine.

It is concluded that feeds from different sources have potential to influence immune response to ND vaccination.

Key words: Chick - Diet - Newcastle disease - Vaccination - Immune response - Nigeria.

### INTRODUCTION

Newcastle disease (ND) is a highly contagious viral infection of chickens the world over, ant its control is essential for the

successful development of the poultry industry in any country. HILL, DAVIS and WILDE (7) first described outbreaks of ND in Nigeria. Government efforts to control the disease have since centred around the local production of vaccines at the National Veterinary Research Institute, Vom, for routine vaccination of all birds.

The recent trend by which ND outbreaks have been reported sporadically in vaccinated flocks (11) has however constituted a source of great concern in the country, especially against the background of an ever increasing investment in the poultry industry.

Reasons that could be advanced to explain these « vaccination breaks » may include poor quality of vaccine, poor vaccine handling, wrong vaccination methods, the use of vaccines that are not specific for the virus strains causing the disease outbreaks (3) and the neutralising effect of maternal antibodies (5).

Additionally, scarcity of feed ingredients in the country has reached critical levels in recent times, and there is the belief among field workers that variations in the resistance of ND-vaccinated birds may be associated with low protein levels in the available commercial feeds.

In a bid to test this hypothesis, the following study was designed to examine the effects of commercial rations produced by 3 different feedmillers on the immune response of broiler chickens to routine ND vaccination.

# **MATERIALS AND METHODS**

### Experimental design

A total of 450 Cobb broilers (obtained at day old from Food Commodities Production Group, Agege) were used for this study. These birds were randomly divided into 6 groups as illustrated in table I. Thus, 100 birds on each of 3 different feeds received B1 and Lasota vaccinations at day 0 and day 14 respectively, while 50 birds on each feed received B1 vaccination at day old only.

TABLE NoI-Experimental design

Diet Vaccination	υ	A	Р
B1 + Lasota	100	100	100
B1 only	50	50	50

### Diets

Diet U was compounded according to standard methods (4) at the University of Ibadan Feedmill using yellow corn (obtained from Temperance Enterprises Ltd, Otta). Other ingredients in the rations were purchased from commercial sources. Diet A was obtained from local feed stores and represented the product of a local franchise of one of the largest feedmillers. Diet P was obtained from Livestock Feeds Ltd, Ikeja. Proximate analysis of all feeds was carried out according to establisched methods (1).

Birds were fed starter components of each diet for the first 28 days and finisher components for the rest of the experimental period.

### Vaccinations

Two attenuated ND vaccines containing the B1 or the Lasota virus strain were used in these experiments. The vaccination protocol was as outlined in table I. All birds also received the infectious bursal disease (IBD) vaccine on day 14 as is routinely practised in the field. All three vaccines were obtained from the National Veterinary Research Institute, Vom.

### Preparation of serum samples

Bleeding of chickens was carried out at ages, 4, 6, and 8 weeks through the jugular vein using  $21G \times 1 \frac{1}{2}$ ' sterile disposable needles. Blood was collected into 10 ml plastic tubes from which serum was obtained some 6 hours later, into clean, labelled screw cap plastic containers. The samples were placed in a water bath at 56 °C for 30 min to remove non-specific inhibitors of haemagglutins, and subsequently stored at -20 °C until assayed for ND antibodies.

# The haemagglutination (HA) and haemagglutination inhibition (HI) test procedures

The HA tests was performed on reconstituted Lasota vaccine according to the procedures of ALLAN and GOUGH (2) using the microfitration techniques. The dilution of mixture containing 4 HA50 units of Lasota virus was calculated, and stock vaccine mixture adjusted to this dilution for use in the HI test. The HI test was carried out according to ALLAN and GOUGH (2) using the beta-procedure.

Chicken erythrocytes for the HA and HI tests were collected into Alsever's solution from 6-8 week old isolation-reared chickens free of ND antibodies. The red cells were subsequently

washed in phosphate buffered saline (PBS) pH 7.2, and used in the tests as 0.5 p. 100 suspension in buffer.

# Statistical analysis

The geometric means of the reciprocal titres (GMT) per group and their standard deviations were derived according to standard procedure (10). The GMTs were then compared for significant differences by the students' test.

### RESULTS

# The proximate composition of diets

The proximate compositions of the diets are presented in Table II.

Protein levels in the starter rations were 24.34 p. 100, 23.08 p. 100 and 19.25 p. 100 in the U, P and A rations respectively. Protein levels in the finisher rations were 20.59 p. 100, 20.78 p. 100 and 20.13 p. 100 fort the U, P and A diets respectively. Levels of dry matter, crude fibre, ether extract and nitrogen-free extract are stated in table II.

# Serology

A summary of the humoral immune response of experimental chickens to ND vaccination is presented in table III. A temporal comparison of these responses is presented in Figure 1.

The chicks used in this experiment had maternal antibodies to ND. The geometric mean HI titre (GMT) of the maternal antibodies was  $10.9 \pm 4.5$  (GMT  $\pm$  SD) across groups.

There were significant variations (P < 0.05 in the immune response among birds fed by the

different commercial diets studied. Following B1 vaccination, A-fed birds responded with the highest GMT (68.5  $\pm$  4.7) by the 4th week. Birds on the P diet hat the second best response with a GMT of 48.5  $\pm$  3.5, while those on the U diet had the lowest HI titre (36.7  $\pm$  5.2).

Following Lasota vaccination in the 4th week, all experimental birds demonstrated significantly increased antibody titres 2 weeks later. The earlier trend during the primary immune response was however maintained, i. e. the A-fed chickens responded with the highest GMT (337.8  $\pm$  16.2), the P-fed chickens responded with a GMT of 294.1 ± 19.8, while the U-fed chickens responded with a GMT of  $128.0 \pm 19.8$  (table III). On the other hand, HI titres of birds which had not received Lasota vaccination had almost completely waned by the 6th week. Thus there was a marked difference (P < 0.05) between the GMT of the Lasota-vaccinated and unvaccinated birds (table III).

Subsequently, in weeks 8 and 9, specific immune response to ND in all the chickens declined rapidly, although at a rate apparently commensurate with the earlier trend whereby birds on the A diet had the highest titres, and those on the U diet had the lowest.

In the 8th week of the experiment, the Lasota-unvaccinated birds on the U diet showed a significant leap in HI antibody titre, rising from a GMT of  $3.0 \pm 4.9$  in the 6th week to  $111.4 \pm 13.5$  in the 8th week. This happened apparently as a result of contamination from a ND outbreak which occurred around the 6th and 7th weeks of the experiment on nearby farms. There was no evidence of clinical illness in the study groups.

TABLE Nº II - Proximate analysis of feeds

Treat	ments	Moisture	DM p.100	Total Ash p.100	C.P. p.100	C.F. p.100	E.E. p.100	N.F.E. p.100	Gross Energy (kcal/gm)	Starch p.100	Sugar p.100
U 1 2	1	12.00	88.00	6.50	24.34	4. 91	2.44	49.81	4.74	14.40	11.5
	2	13.00	87.00	8.75	20.59	5.29	2.68	49.69	4.19	22.10	9.5
Α	1	13.00	87.00	6.50	19.25	5.66	2.85	52.74	4.98	18,00	9.0
	2	13.25	86.75	7.75	20.13	10.53	3.93	44.41	4.82	26.55	6.5
P	1	14.00	86.00	6.75	23.08	7.18	3.55	45.44	5.00	19.35	4.5
	2	14.00	86.00	8.00	20.78	8.76	3.42	45.04	5.01	37.35	8.0

U = Ration formulated at the University; A = Ration purchased locally; P = Livestock Feeds ration; 1 = Starter ration; 2 = Finisher ration; DM = Dry matter; CP = Crude protein; CF = Crude fibre;

EE = Ether extract; NFE = Nitrogen-free extract.

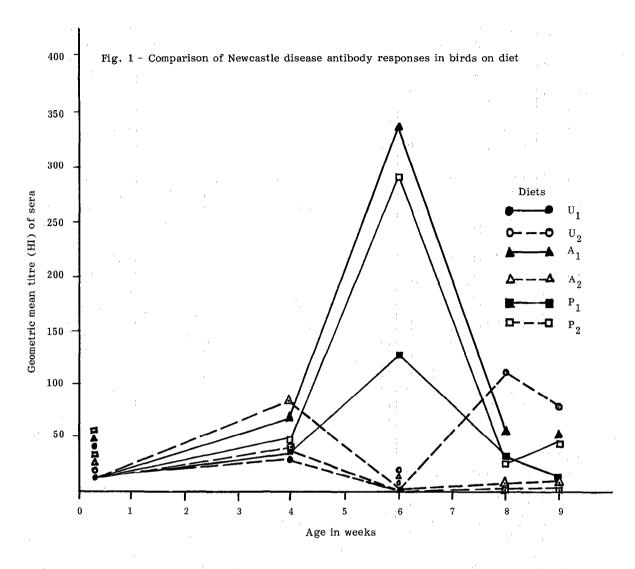


TABLE NºIII - Comparison of the Geometric Mean HI titres (GMT) of sera from broilers fed 3 different diets and vaccinated with B1+ Lasota or B1 only

Groups (vaccinations)	GMT (+ S.D.) of sera collected on							
	Day 3	Week 4	Week 6	Week 8	Week 9			
U1 (B1 + Lasota)	10.9 ± 4.5	36.7 + 5.2ª	128.0 ± 15.5 <sup>a</sup>	34.3 ± 5.5 <sup>a</sup>	13.9 + 9.1 <sup>a</sup>			
A1 (B1 + Lasota)		$68.6 \pm 4.7^{b}$	337.8 ± 16.2 <sup>b</sup>	$59.7 \pm 3.5^{\mathbf{b}}$	52.0 + 7.0 <sup>b</sup>			
P1 (B1 + Lasota)		48.5 <u>+</u> 3.5 <sup>C</sup>	294.1 ± 19.8°	27.9 + 6.3 <sup>a</sup>	48.5 + 5.8 <sup>b</sup>			
U <sub>2</sub> (B1)		31.7 ± 4.8 <sup>a</sup>	3.0 <u>+</u> 4.9 <sup>d</sup>	111.4 ± 13.5 <sup>c</sup>	84.4 + 15.9 <sup>C</sup>			
A <sub>2</sub> (B1)		$84.4 \pm 13.5^{d}$	2.3 ± 6.1 <sup>d</sup>	$8.0 \pm 7.2^{d}$	10.5 ± 9.3 <sup>d</sup>			
P <sub>2</sub> (B1)		$40.6 \pm 5.3^{a}$	$1.7 \pm 5.3^{d}$	1.1 ± 2.7°	1.1 ± 2.7 <sup>e</sup>			

 $\label{eq:continuous} U,A,P\mbox{ - Diets from different sources ; a, b, c, d, e-GMT values with different superscripts are significantly different when compared within columns.}$ 

# **DISCUSSION**

The HI test on the serum of the chicks at day 3 showed that the chicks had maternally-derived antibodies. A similar finding has been

reported for Nigerian chickens (9). Since the level of passively-acquired antibody in the serum of day-old chicks is approximately the same as in the serum of the dam (3) and since vaccination of adult birds is not commonly

practised, it is likely that breeder hens continually have some degree of contact with ND virus during their adult life in this environment.

Maternal antibodies may have marked effects on immune response of vaccinated chickens, interfering with the antibodies formed in response to vaccination at an early age. The interference is mediated through neutralization of the vaccine effect (2, 5, 6). There was no evidence for maternal antibodymediated interference of immune response to B1 vaccination in our study, apparently because levels of this antibody were low.

The effect of secondary vaccination in boosting immunity is illustrated in Figure 1. During the 6th week, 2 weeks after Lasota booster vaccination, the vaccinated birds showed their peak response while the control birds demonstrated very low titres. This is in agreement with the report of GORDON (6) which indicated that peak responses to lentogenic vaccines such as the B1 or Lasota tend to occur 12-14 days after vaccination.

The HI titres of Lasota-unvaccinated birds had fallen below maternal antibody levels by the 6th week, indicating that B1 vaccination alone would probably not protect birds up to this period. Indeed, a spontaneous ND outbreak which swept through nearby farms about this period showed limited activity in the

experimental flocks as evidenced by the leap in HI titres in U-fed, Lasota-unvaccinated birds. This observation confirms the observation of GORDON (6) that virulent field virus which does not kill a bird can give rise to significant rises in HI titres which will however only be recorded when the original immune status of the infected bird is low.

Proximate analysis of the diets revealed only small variations in the proportion of crude proteins in both the starter and the finisher components of the rations. These variations did not correlate with the significant differences noted in the immune response of birds fed on the different diets. It is possible that other nutritional factors such as levels fat (8) of amino-acids and vitamins in the diet may be responsible for some of these differences. This hypothesis would require sophisticated analytical procedures to substantiate.

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#### RESUMEN

OYEJIDE (A.). TEWE (O. O.), OWOADE (A. A.). Influencia de raciones alimenticias comerciales de diferentes origines sobre la respuesta inmunologica humoral de pollos de cría a la vacunación contra la enfermedad de Newcastle. Rev. Elev. Méd. vét. Pays trop., 1985, 38 (4): 443-448.

Se alimentó cada uno de los tres grupos de 300 pollos utilizados con raciones llamadas U, A y P (incluyendo compuestos de comienzo y de acabado), abastecidas por fabricantes locales.

En cada uno de los grupos, se vacunaron 100 pollos contra la enfermedad de Newcastle con dos cepas, B1 a 1 día de edad y Lasota a 28 días. 50 otros animales no recibieron más que la vacuna B1 a 1 día de edad. Se vacunaron los 150 pollos restantes a 14 días de edad contra la enfermedad de Gumboro.

Un análisis global de las raciones mostró que las cantidades de materias nitrogenadas totales para el « comienzo » y el « acabado » eran respectivamente de

24,34 p. 100 y 20,59 p. 100 para U ; de 19,25 p. 100 y 20,13 p. 100 para A y de 23,38 p. 100 y 20,78 p. 100 para P.

Se determinó la respuesta inmunologica humoral para con la vacunación contra la enfermedad de Newcastle por inhibición de hemaglutinación.

Ésta era la más elevada con la ración A y la más baja con la ración U, durante la 4a, 6a, 8a y 9a semana de experiencia.

La cepa Lasota aumentó significativamente dicha respuesta inmunologica como lo evidenciaron generalmente las dosificaciones más elevadas observadas en los pollos vacunados con esta cepa cuando se les comparan con los no vacunados.

En conclusión, raciones de origenes diferentes pueden desempeñar un papel en la respuesta inmunologica a la vacunación contra la enfermedad de Newcastle.

Palabras claves: Pollo de cría - Racíon alimenticia - Enfermedad de Newcastle - Vacunación - Reacción inmunitaria - Nigeria.