

# Immune response to Newcastle disease virus in broilers: a useful model for the assessment of detoxification of ervil seeds

E.K. Barbour, M. Kallas & M.T. Farran

Department of Animal Science, Faculty of Agricultural and Food Sciences, American University of Beirut, P.O. Box 11-0236, Riad El Solh Beirut 1107 2020, Beirut, Lebanon

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## Summary

Ervil (*Vicia ervilia*) seeds are produced in the Mediterranean region and used as a source of protein for cattle and poultry. The methods used to assess the toxic effects of legume seeds in the feed of poultry include the observation of one or more parameters, including abnormal signs, weight gain, feed consumption, gall bladder weight, pancreas weight, pancreas proteolytic and amylase activity, haemolysis of red blood cells, liver weight, liver glutathione level, liver and plasma lipid levels, and plasma lipid peroxide levels. The authors describe the use of quantitative determination of the immune response to Newcastle disease virus (NDV) vaccine in broilers as a model that can be used to assess different detoxification treatments of ervil seeds. Broiler chicks fed differently-treated ervil, supplemented as 25% of the diet, at one to four weeks of age and vaccinated intraocularly with live NDV vaccine at eight days of age, showed different immune responses at three weeks post vaccination. Immunosuppression with regards to NDV was apparent in the group of birds raised on untreated ervil supplement, resulting in a mean immune response (titre) of 798.5. Five of the six different treatments of ervil seeds resulted in different degrees of rectification of the immunosuppression, with some broilers reaching a mean NDV immune titre of 2070.6, similar to that obtained in control broilers raised on a basal diet with no ervil seed supplement (mean NDV immune titre of 2333.8;  $P > 0.05$ ). The five successful treatments of ervil (in increasing order of rectification of immunosuppression in broilers, with mean NDV titres in parentheses) were: ground soaked dried ervil (971.6), ground autoclaved dried ervil (1223.1), soaked autoclaved dried ervil (1273.1), soaked dried ervil (1340.0), and ground-soaked autoclaved dried ervil (2070.6).

## Keywords

Broilers – Detoxification – Diet – Ervil seeds – Immunity – Newcastle disease virus.

## Introduction

Throughout the centuries, many countries have produced seed crops that were adapted to their specific environment and were used as a source of protein in feed for animals or poultry (4, 20). However, following the development of basic analytical sciences in the 19th Century and further refinement of

analytical techniques in the 20th Century, scientists recognised the presence of toxicosis in poultry species, due to the use of certain crops in feed (18).

Ervil (*Vicia ervilia*) seeds have been produced for centuries in the Mediterranean region, as a source of protein for cattle and poultry (4). The models used to assess the toxic effects of

legume seeds in the feed of poultry include the quantitative analysis of one or more of the following parameters: signs of toxicity (1), weight gain and feed consumption (14), gall bladder and pancreas weights, proteolytic and amylase activity in the pancreas (18), haemolysis of red blood cells, liver weight, liver glutathione level, liver and plasma lipid levels, and plasma lipid peroxide levels (13).

The authors describe the quantitative determination of the physiological humoral immune response to Newcastle disease virus (NDV) vaccine in broilers, as a model that could be used to assess different detoxification treatments of ervil seeds.

## Materials and methods

### Ervil detoxification and diet preparation

Six different treatments were applied to intact, coarsely ground ervil seeds. An abbreviation was assigned to each treatment, according to the sequence of the steps followed in the procedure for each treatment. The procedures and abbreviations are as follows:

- soaked dried ervil (SD)
- ground soaked dried ervil (GSD)
- autoclaved dried ervil (AD)
- ground autoclaved dried ervil (GAD)
- soaked autoclaved dried ervil (SAD)
- ground soaked autoclaved dried ervil (GSAD).

The soaking step was performed for 24 h in drinking water, with a water to ervil seed ratio equivalent to 5:1 (vol/wt). Seed drying was performed in a dry oven at 50°C for 24 h. Autoclaving was performed at a temperature of 121°C and pressure of 2.72 kg/cm<sup>2</sup> for a period of 4 h. Seeds were ground using a mill. A corn soybean (control) diet and a 25% untreated ervil diet were formulated to be isocaloric and isonitrogenous (Table I). In the other six diets, the 25% untreated ervil was substituted entirely by treated ervil, as described in Table II.

### Bird treatments and sampling

Three hundred male broiler chicks (day-old), obtained from a commercial hatchery, were raised in a battery brooder for a period of seven days, receiving a starter commercial diet and water ad libitum, with 24 h of continuous lighting.

On the eighth day, 168 birds were selected on weight basis (similar weight), individually wing-banded and randomly distributed over eight different treatments, with twenty-one birds (replicates) per treatment. The ninth group, which was fed a basal diet, consisted of only nineteen birds. The diets differed only in the way ervil seeds were treated, as shown in

**Table I**  
**Composition of diets**

Ingredients	Diets	
	Control	Ervil
Yellow corn	50.310%	33.390%
Soybean meal (44%)	41.530%	31.870%
Ervil	–	25.000%
Soybean oil	4.360%	5.840%
Limestone	1.360%	1.390%
Dicalcium	1.600%	1.640%
Salt	0.230%	0.230%
DL-methionine	0.300%	0.390%
Vitamins and minerals <sup>(a)</sup>	0.250%	0.250%
Amprolium <sup>(b)</sup>	0.050%	0.050%
Calculated analysis		
Metabolizable energy (kcal/kg)	3030.00	3030.33
Crude protein	22.50%	22.50%
Total sulphur amino acids	1.00%	1.00%
Methionine	0.65%	0.69%
Lysine	1.28%	1.31%

a) provided per kilogram of diet:  
butylated hydroxytoluene (BHT): 125 mg; biotin: 0.05 mg; cholecalciferol: 2,500 IU; choline: 300 mg; folic acid: 1 mg; niacin: 30 mg; pantothenic acid: 10 mg; riboflavin: 6 mg; thiamine: 2 mg; vitamin A: 12,500 IU (retinyl acetate); vitamin B<sub>6</sub>: 5 mg; vitamin B<sub>12</sub>: 0.02 mg; vitamin C: 100 mg; vitamin E: 30 IU (DL- $\alpha$ -tocopheryl acetate); vitamin K<sub>3</sub>: 3 mg; Co: 0.8 mg; Cu: 8 mg; Fe: 32 mg; I: 2.4 mg; Mn: 96 mg; Se: 0.14 mg; Zn: 80 mg

b) amprolium supplementation as a coccidiostat  
IU : international units

**Table II**  
**Humoral immune response to live Newcastle disease virus (NDV) vaccine in broilers fed differently-treated ervil seeds**

Treatment <sup>(a)</sup>	Mean ELISA titres to NDV <sup>(b)</sup>	
	2 weeks post vaccination	3 weeks post vaccination
CNV	444.0 <sup>(c)</sup>	315.1 <sup>(e)</sup>
CV	455.3 <sup>(c)</sup>	2333.8 <sup>(c)</sup>
U	636.2 <sup>(c)</sup>	798.5 <sup>(d,e)</sup>
SD	719.7 <sup>(c)</sup>	1340.0 <sup>(c,d,e)</sup>
GSD	1129.6 <sup>(c)</sup>	971.6 <sup>(c,d,e)</sup>
AD	626.4 <sup>(c)</sup>	788.6 <sup>(d,e)</sup>
GAD	947.8 <sup>(c)</sup>	1223.1 <sup>(c,d,e)</sup>
SAD	584.2 <sup>(c)</sup>	1273.1 <sup>(c,d,e)</sup>
GSAD	1592.2 <sup>(c)</sup>	2070.6 <sup>(c,d)</sup>
<b>SEM</b>	<b>376.60</b>	<b>454.36</b>

a) the treatments were as follows:  
CNV : control diet, non-vaccinated against NDV  
CV : control diet, vaccinated against NDV  
U : untreated ervil, vaccinated against NDV  
SD : soaked dried ervil, vaccinated against NDV  
GSD : ground soaked dried ervil, vaccinated against NDV  
AD : autoclaved dried ervil, vaccinated against NDV  
GAD : ground autoclaved dried ervil, vaccinated against NDV  
SAD : soaked autoclaved dried ervil, vaccinated against NDV  
GSAD : ground soaked autoclaved dried ervil, vaccinated against NDV

b) mean ELISA titre at day-old in 20 randomly selected birds was 974.2

c-e) means in a column followed by different superscripts are significantly different ( $P < 0.05$ )

ELISA : enzyme-linked immunosorbent assay  
SEM : standard error of the mean

the footnote to Table II. Each diet was supplied ad libitum for a period of four weeks, effective from eight days of age. Birds fed the control or one of the seven ervil diets (Table II) were administered live NDV vaccine intraocularly at the eighth day of age, except one group of birds raised on the control diet.

### Blood sampling and quantitative serology

Blood was sampled from the birds aged one day and then at three and four weeks of age. Serum was collected from clotted blood and stored individually at  $-20^{\circ}\text{C}$ . A commercial enzyme-linked immunosorbent assay (ELISA) was used to quantitate the NDV titre in the collected sera of the birds. Briefly, each serum sample was diluted at 1:500, incubated in the well of a microtitre plate that was coated with NDV antigens. Antibodies in the serum sample that are specific to NDV form a complex with the coated virus antigens. Washing was performed to remove unbound antibodies from the well. A conjugate produced in goats against the light chains of chicken IgG and labelled with horseradish peroxidase was added. The conjugate binds to attached chicken antibodies present in the well. Unbound conjugate was removed by washing, and a substrate of hydrogen peroxide and the chromogen 2,2'-azino-di-(3-ethylbenzothiazoline-6-sulphonate) (ABTS) was added to the well. The absorbance of the developed colour was measured using an ELISA reader at a wavelength of 650 nm. Data were analysed by one-way analysis of variance and means were compared by Tukey's test.

## Results and discussion

The humoral immune responses to live NDV vaccine in broilers fed differently-treated ervil seeds is presented in Table II. Two weeks post intraocular NDV vaccination, the mean ELISA titre to NDV was not significantly different among all treatments ( $P > 0.05$ ). This indicates that a period of two weeks post intraocular vaccination with B1 strain of NDV vaccine in broilers is not sufficient to show differences in systemic humoral immune responses among treatments. However, at three weeks post vaccination, the treatment effect was apparent. Broilers fed a control diet and deprived of NDV vaccination had the lowest mean ELISA titre for NDV, a background of 315.1. The maximum immunosuppression to NDV was apparent in the group of birds raised on untreated ervil (U) or on AD ervil, resulting in respective mean ELISA titres at three weeks post vaccination equivalent to 798.5 and 788.6. Both of these means were significantly lower than that obtained in birds fed the control diet and vaccinated against NDV (CV group), which showed a mean ELISA titre at three weeks post vaccination equivalent to 2333.8 ( $P < 0.05$ ). The data show that autoclaving treatment of dried ervil is not sufficient to have a detoxifying effect on ervil seeds.

The other five treatments of ervil resulted in different degrees of rectification of immunosuppression to NDV in comparison to

the group U which had a mean ELISA titre of 798.5. The five treatments of ervil that resulted in rectification of immunosuppression to NDV vaccination (mean ELISA titre) were: GSD (971.6), GAD (1223.1), SAD (1273.1), SD (1340.0) and GSAD (2070.6). An assessment of the immune responses over a longer period of time is recommended, as this may reveal greater differences between treatments.

The improved ELISA titres to NDV in the five mentioned treatments remained lower, but not significantly different ( $P > 0.05$ ), compared to that obtained in the control vaccinated group of birds raised on the ervil-free diet (CV group; mean ELISA titre of 2333.8). Future investigations will include field trials on a larger scale in an attempt to evaluate the practical applications of the data obtained.

Comparison of the results obtained from each treatment suggests that treating the ervil with a harsher procedure by grinding, followed by soaking in water, then by autoclaving and drying (GSAD) is necessary in detoxification of the ervil. Such a treatment probably assists in freeing the toxins from the ground particles or disintegrating the toxin present in the ervil. The presence of different antinutritional factors in legume seeds has been demonstrated in previous studies. These include canavanine (2), vicine and convicine (11, 17),  $\beta$ -cyanoalanine (5, 19) and vicianin (6, 12).

The destructive effects of these antinutritional factors on different organs of the body have been described elsewhere (3, 9, 10, 16). However, to the knowledge of the authors, none of the previous studies investigated a direct or indirect effect of these factors on the immune response of a host. The available literature describes the damage to other tissues in the body, which could have an indirect negative effect on the immune system; for example, the vicine and convicine in faba beans have been implicated in acute haemolytic anaemia (11), leading to a significant effect on exchange of respiratory gases (3), and in increasing plasma and liver peroxides (13). A further example is  $\beta$ -cyanoalanine, which is implicated in spinal cord degeneration (15) and in cerebral cortical vacuolation, necrosis and morphological alterations (19). The release of hydrogen cyanide from cyanogenic glycosides such as vicianin, resulting in cellular asphyxiation, is documented by Montgomery (12). Future research needs to correlate immunosuppression to other toxic effects of ervil.

The only study in the literature focused on improving the performance and egg quality of commercial layers by using GSAD treatment of *Vicia sativa* seeds included as 25% of the diet is that of Farran *et al.* in the mid-1990s (7). In the same year, Fernandez-Figares *et al.* demonstrated improved ileal amino acid digestibility in broilers fed autoclaved *V. sativa* (8).

In conclusion, the inclusion of untreated ervil in the diet of broilers at 25%, results in significant immunosuppression to NDV vaccination. The use of quantitative determination of the

immune response to NDV vaccine in broilers could be used as a model in the assessment of different detoxification treatments of ervil seed. A treatment of ervil by grinding, soaking, autoclaving and drying (GSAD) resulted in maximum rectification of immunosuppression to NDV vaccination in broilers. The correlation between performance parameters and

changes in the lymphoid elements of the immune system of chickens consuming untreated ervil will be the subject of future investigations.

## Réponse immunitaire au virus de la maladie de Newcastle chez les poulets de chair : un modèle utile pour l'évaluation de la détoxification des graines de vesce

E.K. Barbour, M. Kallas & M.T. Farran

### Résumé

Dans la région méditerranéenne, les graines de vesce (*Vicia ervilia*) sont cultivées et utilisées comme source de protéines pour les bovins et les volailles. Les méthodes permettant d'évaluer les effets toxiques des graines de légumineuses dans l'alimentation des volailles reposent sur l'observation d'un ou de plusieurs paramètres parmi les suivants : signes anormaux, gain de poids, consommation d'aliments, poids de la vésicule biliaire, poids du pancréas, activité protéolytique du pancréas et de l'amylase, hémolyse des globules rouges, poids du foie, niveau de glutathion dans le foie, niveaux de lipides dans le foie et le plasma, et enfin niveaux de peroxydation des lipides dans le plasma. Les auteurs montrent comment la détermination quantitative de la réponse immunitaire au vaccin contre le virus de la maladie de Newcastle chez les poulets de chair pourrait servir de modèle pour évaluer différents traitements de détoxification des graines de vesce. Des poussins ayant reçu, à titre de complément alimentaire pendant les quatre premières semaines de leur vie, des vesces soumises à différents traitements, représentant 25 % de leur ration, et immunisés par inoculation intraoculaire d'un vaccin à virus vivant de la maladie de Newcastle à l'âge de huit jours, ont présenté des réponses immunitaires différentes trois semaines plus tard. Les volailles ayant reçu un complément à base de vesces non traitées ont présenté une réponse immunitaire faible, se traduisant par un titre moyen de 798,5. Sur les six traitements différents qui ont été appliqués aux graines de vesces, cinq ont corrigé à des degrés divers cette immunosuppression, avec des titres moyens d'immunité au regard du virus de la maladie de Newcastle atteignant 2 070,6 chez certains poulets de chair, soit un niveau similaire à celui obtenu chez les poulets de chair du groupe témoin, qui avaient reçu une ration alimentaire sans ajout de graines de vesce (degré d'immunité moyen vis-à-vis du virus de la maladie de Newcastle : 2 333,8 ;  $P > 0,05$ ). Les cinq traitements efficaces (par ordre croissant de correction de l'immunosuppression chez les poulets de chair, avec les degrés moyens d'immunité vis-à-vis du virus de la maladie de Newcastle entre parenthèses) sont les suivants : séchage après broyage et trempage (971,6) ; séchage après broyage et étuvage (1 223,1) ; séchage après trempage et étuvage (1 273,1) ; séchage après trempage (1 340,0) ; séchage après broyage, trempage et étuvage (2 070,6).

### Mots-clés

Détoxification – Graines de vesce – Immunité – Poulets de chair – Régime alimentaire – Virus de la maladie de Newcastle.

## Respuesta inmunitaria al virus de la enfermedad de Newcastle en pollos de engorde: modelo útil para evaluar la detoxificación de semillas de yero

E.K. Barbour, M. Kallas & M.T. Farran

### Resumen

Las semillas del yero (*Vicia ervilia*) se producen en la región del mediterráneo y se usan como fuente de proteínas para el ganado vacuno y las aves de corral. Para determinar el efecto tóxico de las semillas leguminosas presentes en la alimentación de las aves se utiliza un conjunto de parámetros, entre ellos la presencia de signos clínicos anormales, el aumento de peso, el consumo de alimentos, el peso de la vesícula biliar y el páncreas, la actividad proteolítica y amilásica del páncreas, la hemólisis de eritrocitos, el peso del hígado, el nivel de glutatión hepático, el nivel de lípidos hepáticos y plasmáticos y el nivel de peroxidación lipídica en el plasma. Los autores describen la cuantificación de la respuesta inmunitaria de pollos asaderos a la vacuna con el virus de la enfermedad de Newcastle, y su posible utilización como modelo para evaluar la eficacia de distintos procedimientos de detoxificación de las semillas de yero. Se tomó una población de pollos de engorde y a los 8 días de edad se les administró por vía intraocular una vacuna viva de virus de la enfermedad de Newcastle. Entre su primera y su cuarta semana de vida se administró a los polluelos una dieta enriquecida con un 25% de yeros sometidos a distintos tratamientos. Tres semanas después de la vacunación, los animales presentaban respuestas inmunitarias distintas. El grupo de ejemplares cuya dieta contenía yeros no tratados presentaba una ostensible inmunosupresión ante el virus de la enfermedad de Newcastle, reflejada en un título inmunitario medio de 798,5. Por otro lado, cinco de los seis tratamientos a que fueron sometidas las semillas de yero provocaron distintos grados de rectificación de esa inmunosupresión, con pollos que arrojaron en algún caso un título inmunitario medio de 2.070,6, cercano al que se obtuvo con la muestra de pollos de control, alimentados con la dieta no enriquecida (título inmunitario medio de 2.333,8;  $P > 0.05$ ). Los cinco métodos de tratamiento que resultaron positivos fueron (en orden de rectificación creciente de la inmunosupresión, con el título inmunitario medio indicado entre paréntesis): yeros molidos, hidratados y desecados (971,6); yeros molidos, autoclavados y desecados (1.223,1); yeros hidratados, autoclavados y desecados (1.273,1); yeros hidratados y desecados (1.340,0); y yeros molidos, hidratados, autoclavados y por último desecados (2.070,6).

### Palabras clave

Detoxificación – Dieta – Inmunidad – Pollos de engorde – Semillas de yero – Virus de la enfermedad de Newcastle.



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