Vitamin E in Viral Inactivated Vaccines

A. FRANCHINI, S. BERTUZZI, C. TOSARELLI, and G. MANFREDA

Institute of Zooculture, University of Bologna, Via S. Giacomo 9, 40126 Bologna, Italy

ABSTRACT This research aimed at verifying whether vitamin E added to inactivated and emulsified vaccines enhances the immune response to viral antigens in chicken. Three hundred and twenty broilers (males and females) and 16 types of vaccines, varying in viral antigen [Newcastle disease virus, egg drop syndrome 1976 virus (EDS76V), and infectious bursal disease virus] and vitamin E amount (replacing 10, 20, and 30% of mineral oil) were used. Results show that vaccines with vitamin E, especially when it replaces 20 or 30% of mineral oil, induces a more rapid and higher antibody response than control vaccines. An adjuvant effect of vitamin E was also present in viral vaccine lacking bacterial antigens. Apart from vitamin E content, the Newcastle disease virus and infectious bursal disease virus monovalent vaccines induced higher titers of specific circulating antibodies in birds than did trivalent vaccines. (*Key words*: vitamin E, adjuvant, emulsified vaccine, immune response, broiler)

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INTRODUCTION

The influence of vitamin E on the development and functional activity of the immune system in broilers (Marsh *et al.*, 1981; Franchini *et al.*, 1986) and their resistance to infectious diseases (Heinzerling *et al.*, 1974; Tengerdy and Brown, 1977) is well-known. Vitamin E increases humoral immunity in chickens (Tengerdy and Brown, 1977; Tengerdy *et al.*, 1981), turkeys (Franchini *et al.*, 1990), and mammals and increases phagocytosis, probably by regulating the biosynthesis of prostaglandins (Panganamala and Cornwell, 1982) and their effect on the functional activity and proliferative capacity of immune system cells such as B and T lymphocytes, macrophages, and polymorphonucleated, dendritic, and plasma cells.

The effect of vitamin E on the functional and proliferative activity of immune cells has been exploited to evaluate its influence when it is added to emulsified vaccines for chicks (Franchini *et al.*, 1986, 1988, 1991; Tengerdy *et al.*, 1990) and mammals (Afzal *et al.*, 1984; Tengerdy *et al.*, 1991). Previous research (Franchini *et al.*, 1986, 1988, 1991) using different quantities of vitamin E and mineral oils demonstrated the enhancing effect of this vitamin on the immune response to viral and bacterial antigens. The same research also evaluated the different adjuvant effect of bacterial antigens used in increasing the immune response to Newcastle disease virus (NDV) when present in the same vaccine (Franchini *et al.*, 1988, 1991). The aim of the present research was to verify the adjuvant effect of vitamin E in emulsified vaccine prepared with viral antigens lacking bacterial antigens.

MATERIALS AND METHODS

Three hundred and twenty Arbor Acres broilers (males and females), divided into 16 groups with two replicates of 10 birds per group (5 males and 5 females) were raised in cages from the 1st d of life until the end of the trial (80 d). They had *ad libitum* access to commercial feed consisting of a diet (23% crude protein and 3,000 kcal ME/kg) until 21 d of age and a grower diet (21% crude protein and 3,100 kcal ME/kg) until 80 d.

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TABLE 1. Amount of dl-α-tocopheryl acetate (vitamin E) added to vaccines and the amount of vitamin E in each vaccinal dose

Vaccine[1]	Oily components		Vitamin E per dose
	Mineral oil	Vitamin E	
	(%)		(mg)
Mon. NDV + 0% Vit. E	100	0	0
Mon. NDV + 10% Vit. E	90	10	36
Mon. NDV + 20% Vit. E	80	20	74
Mon. NDV + 30% Vit. E	70	30	116
Mon. EDS76V + 0% Vit. E	100	0	0
Mon. EDS76V + 10% Vit. E	90	10	36
Mon. EDS76V + 20% Vit. E	80	20	74
Mon. EDS76V + 30% Vit. E	70	30	116
Mon. IBDV + 0% Vit. E	100	0	0
Mon. IBDV + 10% Vit. E	90	10	36
Mon. IBDV + 20% Vit. E	80	20	74
Mon. IBDV + 30% Vit. E	70	30	116
Triv. + 0% Vit. E	100	0	0
Triv. + 10% Vit. E	90	10	36
Triv. + 20% Vit. E	80	20	74
Triv. + 30% Vit. E	70	30	116

[1]Mon. = monovalent vaccine; Triv. = trivalent (Newcastle disease virus, egg drop syndrome 1976 virus and infectious bursal disease virus) vaccine; NDV = Newcastle disease virus; EDS76V = Egg drop syndrome 1976 virus; IBDV = infectious bursal disease virus; Vit. E = vitamin E.

Sixteen inactivated and emulsified vaccines, differing in viral antigen and amount of light mineral oils and vitamin E, were used (Table 1). The type of emulsion was water in oil in the ratio 1:4 of aqueous phase to oily phase. All antigens were included in the aqueous phase, inactivated with .2% of β-propriolactone (BPL)[1] at 37 C for 2 h, diluted in PBS, and supplemented with 5% of Tween 80[2] as water-soluble surfactant. Four vaccines were prepared with NDV, Texas strain, as allantoic liquid of infected chicken embryonating eggs, containing $10^{8.29}$ embryo-infectious-dose-50% (EID_{50}) per dose. In the control vaccine, the oily phase was composed of light mineral oil (Marcol 52)[3] supplemented with 10% of Arlacel 80[2] as liposoluble surfactant, whereas in the other three vaccines the 10, 20, and 30% of light mineral oil only were replaced by 10, 20, and 30% of vitamin E as dl-α-tocopheryl acetate,[4] pure oily form. Each vaccine had been tested by high-pressure liquid chromatography (Cohen and Lapointe, 1980) to verify the content of vitamin E.

The same oily phase composition was used to prepare four vaccines with egg drop syndrome 1976 virus (EDS76V), 127 strain, as allantoic liquid of infected duck embryonating eggs, containing $10^{6.27}$ $TCID_{50}$ per dose, four with infectious bursal disease virus (IBDV), 1/65/PV strain, as 199 Minimum Essential Media (MEM)[5] of chicken embryo fibroblast culture, containing $10^{6.1}$ $TCID_{50}$ per dose, and four with all previous three antigens together. Both trivalent and monovalent vaccines were prepared with the same amount of antigens but in the monovalent vaccines PBS replaced the amounts of lacking vaccines.

The vaccines were inoculated (.5 mL per bird) subcutaneously at the back of the neck of all experimental animals at the age of 21 d. At vaccination time and at 7, 14, 21, 28, 38, 48, and 58 d postvaccination (pv), blood samples of all chicks were taken to determinate hemagglutination-inhibiting (HI) antibodies for NDV and EDS76V (Anonymous, 1971) and IBDV antibodies using the ELISA method.

[1]Grand Laboratories, Inc., Larchwood, IA 51241.
[2]ICI, U.S. Inc., Wilmington, DE 19801.
[3]EXXON Corp., New York, NY 10001.
[4]F. Hoffmann-La Roche LTD, Basel, Switzerland.
[5]Sigma Chemical Co., St. Louis, MO 63178-9916.

Statistical Analysis

All data were analyzed using the General Linear Models (GLM) procedure of SAS® (SAS Institute, 1985) with the following three-factor model:

$$Y_{ikl} = \mu + A_i + B_k + e_{ikl}$$

where Y_{ikl} = value of the first chick titer; μ = population mean; A_i = effect of the i^{th} vaccine treatment (i = vaccine containing 100% mineral oils, vaccine with 10% vitamin E:90% mineral oils, vaccine with 20% vitamin E:80% mineral oils and vaccine with 30% vitamin E:70% mineral oils); B_k = effect of the k^{th} sex (k = male, female); e_{ikl} = residual error. Means were compared by the Student Newman-Keuls (SNK) test.

RESULTS AND DISCUSSION

The results (Table 2) show how the production of HI antibody to NDV is significantly higher in birds inoculated with monovalent vaccines in which the mineral oils had been partially replaced by vitamin E. The two groups receiving vaccines with 30 and 20% vitamin E showed an overlapping antibody production and, beginning from 28 d pv, both showed a higher ($P < .01$) titer than the other two groups (control and 10% vitamin E vaccines). In previous samplings (7, 14, and 21 d pv), only the group receiving vaccine with 30% vitamin E showed a higher ($P < .01$) immune response than the control group.

Using the trivalent vaccine, vitamin E was shown to ultimately lower antibody response. The groups inoculated with vaccines containing different percentages of the vitamin showed a more rapid immune response and, at 21 d pv, this response was higher ($P < .01$). However, by 58 d pv, control trivalent vaccine had a higher ($P < .05$) titer than vitamin E trivalent vaccines.

Sex influences were observed only at 28 and 58 d pv. Differences among the means were found to significantly favor females, which from 21 d pv showed HI titers higher than those of males. In brief, the effect of vitamin E on the birds' response to NDV mainly occurs when it replaces 20 and 30% of the oily component, and even more so in monovalent than in trivalent vaccines.

Table 3 shows the trend of production of HI antibody to EDS76V. Analysis of the

TABLE 2. *Hemagglutination inhibiting antibodies[1] to Newcastle disease virus*

Treatment[2]	Days postvaccination						
	7	14	21	28	38	48	58
Triv. + 0% Vit. E	1.12^{BC}	5.26^{A}	5.50^{C}	5.91^{B}	5.87^{B}	4.69^{CD}	4.69^{CD}
Triv. + 10% Vit. E	1.29^{BC}	5.62^{A}	6.87^{AB}	6.04^{B}	5.37^{B}	4.66^{CD}	4.08^{DE}
Triv. + 20% Vit. E	1.25^{BC}	5.79^{A}	6.50^{B}	5.82^{B}	5.41^{B}	4.60^{CD}	3.91^{DE}
Triv. + 30% Vit. E	1.62^{B}	5.75^{A}	7.17^{AB}	6.34^{B}	5.40^{B}	4.40^{D}	3.66^{E}
Mon. + 0% Vit. E	1.04^{C}	4.41^{B}	6.62^{B}	6.22^{B}	5.45^{B}	5.25^{BC}	5.33^{BC}
Mon. + 10% Vit. E	1.12^{BC}	5.50^{A}	7.50^{AB}	6.25^{B}	6.12^{B}	5.50^{B}	5.50^{AB}
Mon. + 20% Vit. E	1.25^{BC}	6.00^{A}	7.37^{AB}	7.29^{A}	6.95^{A}	6.34^{A}	6.26^{A}
Mon. + 30% Vit. E	2.16^{A}	6.04^{A}	7.79^{A}	7.34^{A}	7.20^{A}	6.73^{A}	6.12^{AB}
Males	1.42	5.56	6.67	6.26^{b}	5.95	5.25	4.71^{B}
Females	1.29	5.53	7.05	6.54^{a}	6.01	5.30	5.20^{A}
Treatment × sex	**	NS	NS	NS	*	NS	NS
SEM	.329	1.165	1.053	.697	.777	.749	.890

[a,b]Means within columns with no common superscript differ significantly ($P \leq .05$).

[A-E]Means within columns with no common superscript differ significantly ($P \leq .01$).

[1]Mean titer expressed as a logarithm of serum reciprocal dilution ($\log_2$).

[2]Triv. = trivalent vaccine; Mon. = Newcastle disease virus vaccine; Vit. E = vitamin E.

*$P < .05$.

**$P < .01$.

TABLE 3. Hemagglutination inhibiting antibodies[1] to egg drop syndrome 1976 virus

Treatment[2]	Days postvaccination						
	7	14	21	28	38	48	58
Triv. + 0% Vit. E	2.16^{C}	6.12^{ABC}	8.70^{AB}	7.54^{ab}	6.50	6.91	5.82^{a}
Triv. + 10% Vit. E	3.41^{B}	6.41^{AB}	8.37^{AB}	7.58^{ab}	6.37	6.33	5.45^{ab}
Triv. + 20% Vit. E	3.75^{B}	6.91^{A}	8.37^{AB}	8.39^{a}	6.41	6.30	5.62^{ab}
Triv. + 30% Vit. E	5.87^{A}	6.95^{A}	8.43^{AB}	7.73^{ab}	6.18	6.00	5.80^{a}
Mon. + 0% Vit. E	1.00^{D}	4.54^{D}	8.41^{AB}	7.30^{b}	6.08	6.34	5.19^{ab}
Mon. + 10% Vit. E	1.29^{CD}	5.25^{CD}	8.12^{AB}	7.34^{b}	5.91	6.41	4.86^{b}
Mon. + 20% Vit. E	2.12^{C}	5.79^{BC}	7.62^{B}	7.83^{ab}	6.00	6.52	5.79^{a}
Mon. + 30% Vit. E	2.08^{C}	6.13^{ABC}	8.91^{A}	7.56^{ab}	6.21	6.59	5.31^{ab}
Males	2.65	5.92	8.436	7.52	6.12	6.32	5.21^{B}
Females	2.78	6.10	8.260	7.79	6.29	6.52	5.73^{A}
Treatment × sex	NS	NS	NS	***	NS	NS	NS
SEM	1.191	1.255	1.158	1.290	.945	1.026	.954

^{a,b}Means within columns with no common superscript differ significantly ($P \leq .05$).

$^{A-D}$Means within columns with no common superscript differ significantly ($P \leq .01$).

[1]Mean titer expressed as a logarithm of serum reciprocal dilution ($\log_2$).

[2]Triv. = trivalent vaccine; Mon. = Egg drop syndrome 1976 virus vaccine; Vit. E = vitamin E.

***$P \leq .001$.

response to monovalent vaccines shows that, in the first two weeks after vaccination (7 and 14 d pv), 20 and 30% vitamin E strongly influence the production of HI antibody and that differences compared with the titers of the other groups were significant. Later, intergroup differences were not significant, although the titers induced by vaccines with 30% vitamin E, in particular, are higher than in controls.

The response to EDS76V using trivalent vaccines indicated that they induce a higher production of antibodies than did monovalent vaccines at 7 and 14 d pv only. In this phase, vaccines with vitamin E induced a higher level of specific antibodies than was observed in controls. For EDS76V, the influence of sex did not seem to be very important. Only at 58 d pv were the HI titers of females greater than those of males.

Overall analysis of the response to EDS76V shows that trivalent vaccines are more effective than monovalent vaccines. Vitamin E, particularly at concentrations of 20 and 30%, induced a greater production of specific antibodies against EDS76V in both mono- and trivalent vaccines for 2 wk pv.

In contrast, trends of the response to IBDV (Table 4) show a higher immune response induced by mono- rather than trivalent vaccines. This is more obvious in the first few weeks pv. In this case, replacement of mineral oils by vitamin E seemed to have a greater effect on the production of circulating antibodies. In particular, monovalent vaccines with 20% vitamin E induced at 14, 21, and 28 d pv clearly higher antibody responses than those found in the control; it showed ELISA titers similar to those of the other groups from 38 d pv. The influence of vitamin E on the production of IBDV antibodies was lower in trivalent than in monovalent vaccines. The production of specific IBDV antibodies was not influenced by sex.

In general, the presence of vitamin E in the emulsified mono- and trivalent vaccines used in this research induces a higher overall production of specific antibodies, although not always significant, compared with controls. Comparison with results of previous research using vaccines against NDV, associated with *Pasteurella anatipestifer* (Franchini *et al.*, 1988) or *Escherichia coli* (Franchini *et al.*, 1991), reveals that the presence of the bacterial antigen did not mask the effect of vitamin E on the cells of the immune system involved in the response to inoculated

TABLE 4. Mean ELISA titers of antibodies[1] to infectious bursal disease virus

Treatment[2]	Days postvaccination						
	7	14	21	28	38	48	58
Triv. + 0% Vit. E	.00	.15^{C}	.99^{CD}	1.11^{AB}	1.16^{C}	1.02^{b}	1.24^{AB}
Triv. + 10% Vit. E	.04	.24^{BC}	1.13^{BC}	1.18^{AB}	1.37^{ABC}	1.03^{b}	1.50^{AB}
Triv. + 20% Vit. E	.00	.17^{C}	1.08^{BC}	1.12^{AB}	1.57^{ABC}	1.25^{ab}	1.23^{AB}
Triv. + 30% Vit. E	.09	.43^{AB}	1.00^{CD}	1.25^{AB}	1.31^{BC}	1.23^{ab}	.89^{B}
Mon. + 0% Vit. E	.02	.14^{C}	.63^{D}	1.07^{B}	1.70^{AB}	1.35^{a}	1.38^{AB}
Mon. + 10% Vit. E	.00	.38^{AB}	.84^{CD}	1.15^{AB}	1.41^{ABC}	1.12^{ab}	1.13^{AB}
Mon. + 20% Vit. E	.00	.52^{A}	1.77^{A}	1.54^{A}	1.79^{A}	1.25^{ab}	1.65^{A}
Mon. + 30% Vit. E	.02	.24^{BC}	1.41^{B}	1.41^{AB}	1.67^{AB}	1.33^{ab}	.90^{B}
Males	.02	.29	1.08	1.21	1.49	1.13	1.16
Females	.02	.28	1.13	1.25	1.5	1.26	1.32
Treatment × sex	NS	NS	NS	**	***	***	NS
SEM	.002	.045	.193	.195	.219	.131	.380

^{a,b}Means within columns with no common superscript differ significantly ($P \leq .05$).

$^{A-D}$Means within columns with no common superscript differ significantly ($P \leq .01$).

[1]Arithmetical mean expressed as optical density.

[2]Triv. = trivalent vaccine; Mon. = infectious bursal disease virus vaccine; Vit. E = vitamin E.

**$P < .01$.

***$P < .001$.

vaccines. However, it should be noted that, when *P. anatipestifer* is present in the emulsion, the production of antibodies against NDV is higher in both control and vitamin E vaccines (Franchini *et al.*, 1991).

The present study on the adjuvant effect of vitamin E confirmed our previous papers (Franchini *et al.*, 1988, 1991). In particular, the enhancing effect on the chicken humoral immune response to NDV, EDS76V, and IBDV was observed when 20 to 30% of light mineral oil was replaced with vitamin E. This effect probably was connected with a good balance between the inflammatory effect of mineral oil and the influence of vitamin E on the activity of immune cells (lymphocytes, macrophages, and plasma cells) at the point of vaccine inoculation.

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