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**EFFECT OF VACCINAL DILUENTS AND ENVIRONMENTAL
TEMPERATURE ON THE EFFECIENCY OF VACCINATION IN
POULTRY.**

BY

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The effect of vaccinal diluents and environmental temperature (seasonal variation) on the humeral immune response of broilers vaccinated against Newcastle and Gumboro diseases were studied . Concerning to Newcastle our result indicated that the highest antibody response was recorded in the poultry received the vaccine diluted in distilled water with skim milk , followed by that vaccinated by distilled water and ground water with skim milk , while poultry received the vaccine diluted in tap water recorded the lowest response. The immune response for Newcastle disease virus was higher in summer than winter . In case of Gumboro vaccine and in similarity to Newcastle vaccine the highest antibody titer was recorded in poultry received the vaccine diluted in distilled water with 1% skim milk followed by the birds taken vaccine diluted in distilled water alone . Also tap water recorded the worst titer when used as a vaccinal diluent .The results indicated that the titer of antibody for Gumboro diseases virus was also better in summer than winter.

INTRODUCTION

Poultry industry is considered as a vital economic step on the way to solve the problem of animal protein shortage there for, poultry production jumped from extensive to intensive system of production . However , poultry industry still suffer

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from great losses attributed to many factors of which the high sensitivity of the foreign hybrids of fowl to the environmental conditions and misusing of the vaccines against most common poultry diseases.

Water quality and environmental temperature play an important role in building up the immune response of poultry against vaccines. When potent vaccine is added to fresh untreated water and properly administered to chicks, immune response can be expected. However no immunity would develop if the water contained substance which destroy some or all of the virus. Such a condition could exist when sanitizers are added to the water for control of bacterial and fungal contamination in water (Toylor, 1969).

The influence of environmental temperature on the humoral immune response of poultry have been studied by several authors; Thaxton, (1978) suggested existence of what is called heat induced immune - suppression and cold induced immuno - enhancement to sheep RBCs in New Hampshire chicks.

The present study aimed to verify the existence of a relationship between the immune response of broilers and some hygienic conditions as environmental temperature and quality of water used as a vaccinal diluent.

MATERIAL AND METHODS

Materials :-

1- Experimental chicks:

Four hundred day old A R B O R - A C R E S chicks from cairo poultry company were kept on deep litter and fed on formulated balanced ration obtained from the same company.

2- Newcastle disease virus vaccine:

Hitchner BI vaccinal strain (lote # 330 76 B) produced by Intervet International B . V - Boxmeer (R) Holland.

3- Gumboro disease vaccine:-

Bursal disease vaccine live virus. Bursal BLENTM M(R). Produced by Sanofi (Animal health Inc, over land park , KS 66 210).

4- Vaccinal diluents :

a) Distilled water (used as a diluent in group 1).

b) Tap water (used as a diluent in group 2).

c) Ground water (used as a diluent in group 3).

d) Distilled water + 1% liquid skim milk (in group 4).

e) Tap water + 1% liquid skim milk . (in group 5).

f) Ground water + 1% liquid skim milk . (in group 6).

Grouping :-

Two hundred birds were used for the experiment in summer and another two hundred chicks for the experiment in winter .

In both experiments, summer and winter, all chicks were housed on deep litter system , under brooding temperature of 33 - 35 °C which was decreased by 3 °C weekly.

At eight days old , the chicks were divided into six equal groups each of about 30 chicks.

At age .of 21 day birds were kept under the natural environmental temperature which ranged from (20-40) °C in summer and from (18 - 28) °C in winter.

vaccination program:-

vaccination was carried out according to the following table:-

Age of birds	the vaccine used
Nine days old	HB1
15 days old	IBD
19 days old	HB1
26 days old	IBD+HB1
38 days old	HB1

the same program of vaccination was followed in both winter and summer.

Collection of blood samples:-

Blood samples were collected from the wing vein of the experimental chicks in a clean plastic centrifuge tubes. Samples were collected at 32, 38, and 45 days old. Serum was collected in a special plastic vials and stored at -20 C^o till the microdilution technique of hemagglutination inhibition test and ELISA were carried out.

A) Detection of the immune response to Newcastle disease virus vaccine:-

The serological response to vaccination against Newcastle disease was determined by hemagglutination inhibition (H.I) test as described by Allan and Cough (1974).

B-Detection of humoral immune response to vaccination against Gumboro disease: Enzyme linked immune sorbant assay { ELISA } was carried out according to recommendation of Kaphagy et al.(1990) ELISA test was performed using infectious bursal disease antibody test kit, produced by KIRKEGAARD and PERRY laboratories, 2 cessa court Gaitherburg Maryland 20879.

DISCUSSION

Regarding the effect of vaccinal diluent for ND vaccine, table(1) showed the titers in summer and winter at different ages. In summer highest titer was recorded in group1 (distilled water), followed by the titers obtained from the group received the vaccine diluted in ground water(group3) while the lowest mean titer was recorded when tap water was used (group2).

At the same age, when 1% skim milk was added as stabilizer a greater titers were recorded. The highest mean titer was recorded in the group received the vaccine diluted in distilled water with 1% skim milk(group4), followed by group6(ground water plus 1% skim milk), the lowest mean titer was recorded in group5(tap water plus 1% skim milk). At 38 days(group1) showed the highest mean followed by group3 and the least titer was recorded in group 2 .

In the groups of skim milk the titers were higher, group4 recorded the highest titer followed by group 5 , while group6, in which ground water with skim milk was used as a vaccinal diluent, gave the lowest titer .

Similarly at age of 45 days group1 recorded the highest figure followed by group3, while group2 showed the lowest mean. Regarding addition of 1% skim milk to the vaccinal diluent, group4 recorded the highest mean titer followed by group6, while group5 recorded the lowest mean titer.

From the above results, it is obvious that water quality plays a role in vaccination process, where the use of distilled water as a vaccinal diluent gave the highest antibody titers followed by ground water, while administration of the vaccine diluted in tap water, recorded the worst response, which may be attributed to free chlorine involved.

Regarding skim milk addition, it is clear that the response was enhanced, but the distilled water with skim milk remained the best compared to the other two groups.

In winter, group(1) at 32 days old recorded the best titer followed by group3 lowest value was recorded by group2.

Regarding skim milk addition ,group4, group5 and group6 showed better mean titers than that recorded in groups to which skim milk were not added.

At 38 days group1 showed the highest titers , followed by group3 while the lowest mean titer was recorded by group2, in the groups received the vaccinal diluent with 1% skim milk the mean titers were better than comparable groups.

At age of 45 days the recorded mean titers were much better in groups of distalated water, ground water and tap water consecutively

Regarding skim milk addition, the mean titer of group4 remained the highest followed by group6 while the lowest group was that received the vaccine diluted in the tap water with skim milk (group5).

From the aforementioned results, it is clear that in winter as well as in summer the distilled water gave the best result as a vaccinal diluent if compared to ground and tap water, while tap water was the worst as a vaccinal diluent. When skim milk was added to water used as a vaccinal diluent an increased titers were recorded but the distilled water with skim milk remained the best as a vaccinal diluent.

Also this table showed that vaccinal diluent can affect the immune response measured by HI, this effect may be due to direct effect on the infectivity of the virus strain, which depends on time of exposure, the quantity of the virus, the nature of suspending medium and interaction between treatments(Alexander,1991).

The obtained results coincide with those recorded by Winterfield and seadale(1956); Cherby(1966);Yoshida et al. (1968) ; Taylor (1969);Gentry and Braune (1972); Dsenne, (1973); Ottis (1976); Kim and Spradbrow(1978) and Hasan(1993) who recorded the lowest titer when tap water, with residual chlorine was used as vaccinal diluent and also with Motto and Coleman,(1994) who stated that water must be free from chlorine which kill the virus in the vaccine upon contact.

Also the above results indicates that mean titers increased in groups received the vaccine diluted in drinking water containing 1% skim milk, than those groups received the vaccine in the same vaccinal diluents but without skim milk (Fig. 1&2).

Distilled water with skim milk remained the best as a vaccinal diluent compared to ground water with skim milk and tap water with skim milk which remained the worst in these groups, but better than used without the skim milk.

These results are in agreement with those recorded by Burtsev et al (1970) Gentry and Braune (1972) and Woodward and Tudor (1975), but not with Aller and Allan (1970) who stated that skim milk as 10% of the drinking water did not stabilize the virus significantly at 25-27 °C.

Table (2) shows the effect of vaccine diluent on titer against Gumboro vaccination. In summer, it was obvious that at 38 days of age group 1 (distilled water) showed the highest recorded mean followed by group 3 (ground water as a vaccinal diluent), while group 2 (tap water) showed the worst titers. When 1% skim milk was added to the water the results improved in all groups (group 4); (group 5) and (Group 6).

At 45 days old, the titers increased but the picture remained the same as the highest recorded means were shown in groups 1 followed by group 3 and group 2 respectively. In groups received the vaccine in drinking water containing 1% skim milk, the results were much better (group 4; group 5 and group 6).

From the above mentioned results, it may be suggested that water quality plays a role in achieving good immune response when water is used as a vaccine diluent and the distilled water gave the best results while tap water remained the worst.

Also the same table shows that the mean antibody titers of different groups in winter at age of 38 days were better in group 1 followed by group 3 and group 2 respectively, while at the same age the titers of group 4; group 5 and group 6 showed higher titers than comparable groups (group 1, group 2, group 3).

At age of 45 days the titers increased in group 1, group 2 and group 3 however in the groups of skim milk the results were slightly higher.

The above mentioned results indicated that the highest destructive effect on the titers of different groups was for the group using tap water as a vaccinal diluent and this may be attributed to the effect of free chlorine of tap water on the virus vaccine, these results are in a good agreement with Landgraf (1967); Leukert and Saif, (1991); Hasan (1993) who found that the tap water containing residual chlorine gave the lowest titers when used for administration of Gumboro disease vaccine; and also with Sluis (1991) who stated that 2% chloramine in solution and alkyl dimethyl benzylammonium chloride are suitable disinfectants for IBD virus. The above results also indicated that the addition of skim milk improved the antibody titer against Gumboro (Fig. 3,4).

As indicated previously in figs (3 & 4) it is obvious that results of summer were higher indicating that the virus vaccine can tolerate the high environmental temperature in summer which agrees with the suggestion of both Lukert and Saif (1991) and Mekkes and Wit (1994).

In case of Gumboro, the decreased level of antibodies in winter although is not so much as in Newcastle but the presence of certain factors in winter rather than environmental temperature could be presumed. One of the greatest problems of winter is the increased humidity inside the house and closure of all ventilating flues to warm the house creating a suitable conditions for many diseases especially for respiratory diseases as Newcastle disease and chronic respiratory disease. Under this condition it could not be expected to have good immune response even if proper vaccination program was employed. Our results also coincided with that of Regnier and Kelly, (1981) who stated that B-cell functions were not compromised by chronic thermal stress.

Conclusion: From the present study we conclude the following:

- 1- Distilled water with skim milk was the best vaccinal diluent for both Newcastle disease and Gumboro disease vaccines.
- 2- Addition of skim milk to the drinking tap water used as vaccine diluent decreases the destructive effect of chlorine and as a consequence economic than to spend the vaccine uselessly.
- 3- Environmental temperature plays also a role in the vaccination response of broilers, the immune response either to ND vaccine or Gumboro vaccine was slightly higher in summer than in winter which may be attributed to suppression of ventilating fans and flues in winter to warm the house which internally increased the humidity, the chance for respiratory diseases and subsequently decreases the vaccination response.

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Table (2): Effect of vaccinal diluent on titers against Gumboro vaccine in summer and winter

		Antibody titer in summer (20-40 °C)					Antibody titer in winter (18-28 °C)				
		* Range		Mean±S.E		Range		Mean ±SE			
Age/day	Group	38 days	45 days	38 days	45 days	38 days	45 days	38 days	45 days	38 days	45 days
	Group 1 (distilled water)	1443-5566	2332-5161	2544.2±28.5	3480.6±376.4	664-4605	1226-5068	2220±556.2	3242±630.5		
	Group 2 (Tap water)	0-2424	0-4347	1087±120	2377±171.4	0-2118	0-2428	910±344.1	1498±421.4		
	Group3 (ground water)	910-2717	1892-3717	2223±172.3	2886±455	0-2383	940-3455	2085±213.2	2909±340		
	Group4(dist. water+1% Skim milk)	1690-3622	2450-4940	2587±144.1	3527.4±278.2	1226-3577	1302-5281	2560±415	3343±431.2		
	Group5 (tap water+1% Skim milk)	797-2382	1728-3510	2004±166.8	2925±121.3	530-2935	790-3151	1885±380.5	2413±295		
	Group6 (ground water+1% skim milk)	1892-2950	1931-3924	2410±271.2	3420±271.8	790-2892	1297-4036	2407±278.2	3096±474.1		

*Log base -2 titer

Table (1) : Effect of vaccinal diluent on humoral immune response against Newcastle disease vaccination in summer and winter.

		Antibody titer in summer (20 – 40 °C)					Antibody titer in winter (18 – 28) °C						
		Range		Mean ±S.E		Range		Mean ±SE					
Age/day	Group	32 days	38 days	45 days	32 days	38 days	45 days	32 days	38 days	45 days	32 days	38 days	45 days
	Group 1 (distilled water)	0-4	1-4	1-5	1.7±0.28	2.2±0.83	3.8±0.58	0-2	0-4	1-4	1.4±0.37	2.1±0.57	3.4±0.51
	Group 2 (Tap water)	0-2	0-3	0-4	0.4±0.21	1.5±0.58	2.4±0.31	0-2	0-2	1-3	0.6±0.20	1.2±0.31	2.0±0.45
	Group3 (ground water)	0-3	0-4	0-5	1.6±0.6	2.0±0.61	3.0±0.37	0-2	1-3	1-4	1.0±0.26	1.8±0.37	2.6±0.45
	Group4(dist. water+1% Skim milk)	0-5	1-6	2-5	2.8±0.80	3.4±0.98	3.8±0.49	0-4	0-5	2-7	2.0±0.63	3.0±0.75	3.75±0.25
	Group5 (tap water+1% Skim milk)	0-4	0-5	1-4	1.0±0.23	2.7±0.45	2.8±0.23	0-3	0-4	2-5	1.1±0.26	2.0±0.37	2.5±0.17
	Group6 (ground water+1% skim milk)	0-4	0-5	1-5	2.6±0.37	2.6±0.21	3.2±0.23	0-3	0-5	1-7	1.3±0.21	2.2±0.49	3.0±0.36

*Log base -2 titer

NB: negative control=0 & Positive control =5

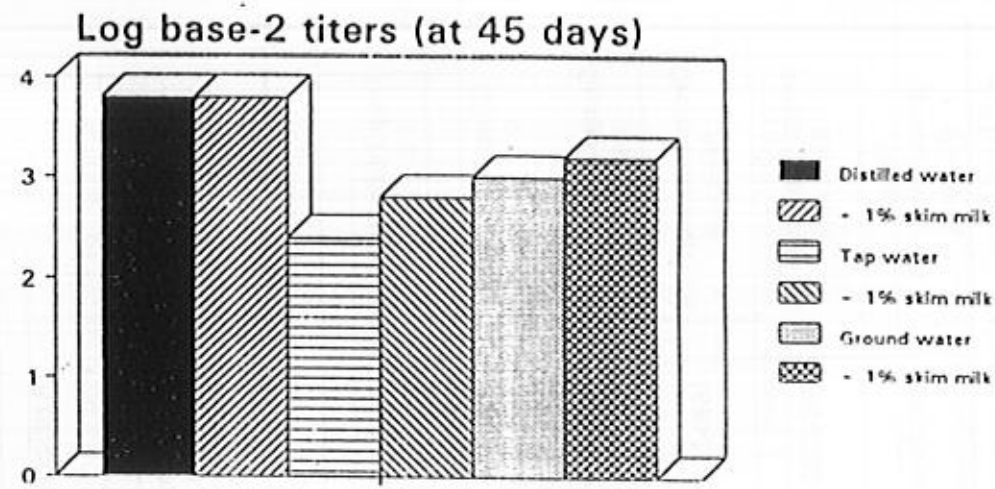


Fig.(1): Effect of skim milk addition on mean titers against ND vaccine (in summer)

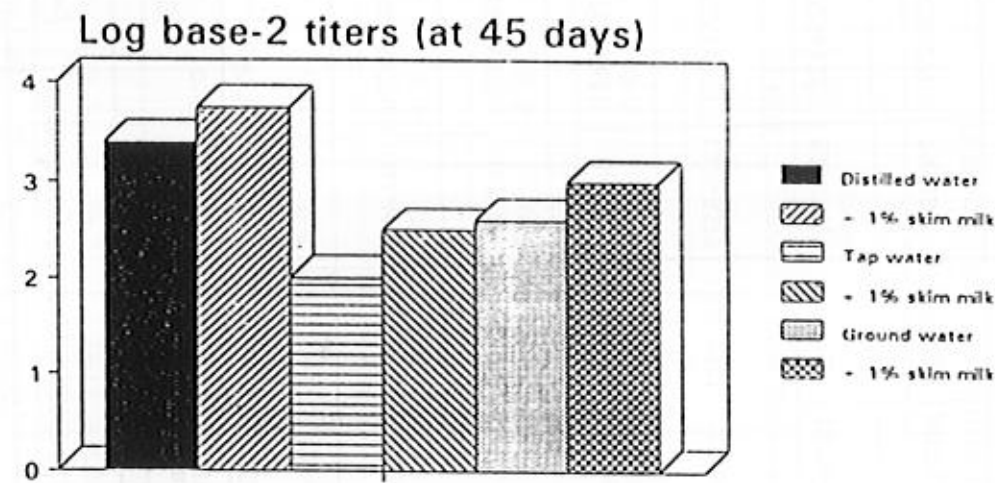


Fig.(2): Effect of skim milk addition on mean titers against ND vaccine (in winter)

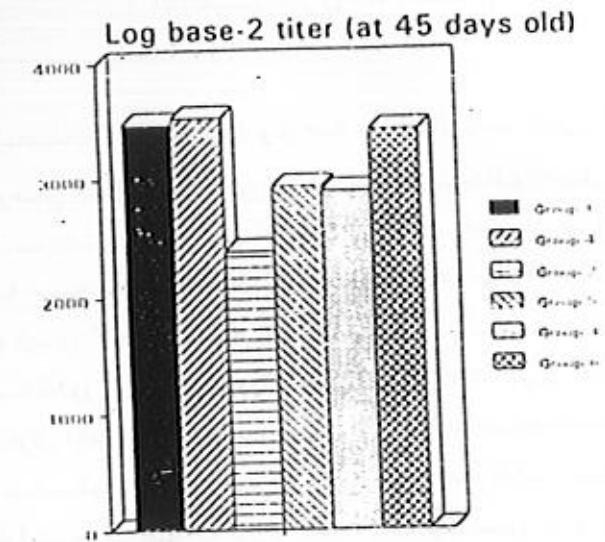


Fig.(3):Effect of skim milk addition on mean titers against Gumboro disease vaccine (in summer).

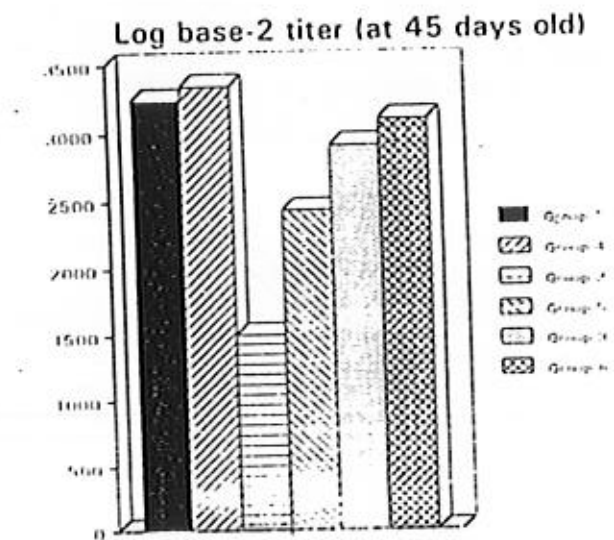


Fig.(4):Effect of skim milk addition on mean titers against Gumboro disease vaccine (in winter).

الملخص العربي
تأثير مخففات اللقاحات و درجة حرارة الجو علي كفاءة
التحصين في الدجاج

تم دراسة تأثير مخففات اللقاحات و درجة حرارة الجو علي الأستجابة المناعية للدجاج اغصن ضد مرضي النيوكاسل و الجمبورو. بالنسبة لتحصين النيوكاسل أظهرت النتائج أن أعلى استجابة مناعية سجلت في الدجاج الذي أعطى اللقاح في ماء مقطر مضافا اليه 1% من اللبن منزوع الدسم يليها استخدام الماء المقطر فقط , واستخدام المياه الجوفية مضافا اليه اللبن بينما يعطى استخدام مياه الصنبور كمخفف للقاح أسوأ النتائج. دراسة تأثير درجة حرارة الجو علي الأستجابة المناعية للطيور أظهرت أن مستوي الأجسام المناعية كانت أعلى في الصيف عنها في الشتاء . أما بالنسبة لتحصين مرض الجمبورو فإنه أيضا سجل أعلى معدلات استجابة مناعية في المجموعه التي استخدمت ماء مقطر مضافا اليه 1% من اللبن منزوع الدسم يليها استخدام الماء المقطر فقط ثم استخدام المياه الجوفية مضافا اليه اللبن و كذلك احتلت مياه الصنبور المركز الأخير من حيث الأستجابة المناعية للطيور في هذه المجموعه . و بدراسة تأثير درجة حرارة الجو وجد أن الأستجابة المناعية للطيور لتحصين مرض الجمبورو كانت أعلى في الصيف أيضا عنها في الشتاء.