Dietary vitamin A supplementary effects on performance and immuno-competence of broiler chickens

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SUMMARY

This study was carried out to evaluate the effect of varying vitamin A supplementary concentration levels on performance characteristics and immunological response to Newcastle disease (ND) vaccinations of broiler chickens. A feeding trial for a period of 8 weeks was conducted using 120 day old broiler chicks divided into 3 treatment groups with 4 replicates of 10 birds each. Birds in treatment A served as the control group and fed diets containing the National Research Council (NRC) requirement level of vitamin A; birds in treatment 2 and 3 were fed diet supplemented with 100 and 200mg/kg of dietary vitamin A respectively throughout the study. The birds were vaccinated with ND vaccines using a stipulated vaccination regime. The results showed that FBW of birds fed diets containing 100mg/kg (2172.34g) and 200mg/kg (2160.01g) were not significantly different ($P > 0.05$) but were significantly ($P < 0.05$) lower than those fed the control diet (2633.33g). The birds in the control group also best utilized their diets having an FCR of 1.56 compared with 1.84 and 1.85 of those fed 100mg/kg and 200mg/kg of vitamin A supplementation respectively. The immunological responses after vaccination with ND vaccine (Hitchner B1 strain) revealed that chickens fed either 100 or 200mg/kg of vitamin A recorded the highest antibody titre ($\log_2 7$) while those fed the control diet had the lowest mean titre ($\log_2 6$). Administration of ND vaccine (LaSota strain) elicited the highest antibody titre ($\log_2 9$) in birds fed 200mg/kg vitamin A which was significantly different ($P < 0.05$) from that of birds fed the control diet ($\log_2 7$). The haematological parameters had the MCV, MCH and lymphocyte values significantly ($P < 0.05$) influenced by the varying diets. It was concluded that immune response of birds to ND vaccinations due to vitamin A activity improved with higher supplementary levels while FBW and FCR were best for birds in the control group.

Keywords: Antibody, broilers, growth, Newcastle disease, vitamin A:

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INTRODUCTION

Due to intensive husbandry of poultry in commercial conditions, infectious diseases can spread quickly throughout a broiler or a turkey flock. The efficacy of the immune system, which is the primary line of defence, is dependent on adequate nutrient supply for the development of its key organs, for the rapid expansion of effector cells and for the subsequent synthesis of antibodies. Among the micronutrients, vitamins A, D, and E were demonstrated to have a direct modulating activity on the immune system (Klasing, 1998). Through research into the biological mechanisms of vitamin action, it has now been established that substantially higher intake of some vitamins may significantly influence the immune process in chickens (Muhammad, 2003). The recommended dietary inclusion of vitamins is aimed at preventing clinical deficiencies and until recently most of studies overlooked the potential role of vitamins in optimizing immune response in the chicks, particularly in response to infections from bacteria and viruses. An effective disease prevention program may be provided by proper vaccination and suitable supplementation with a given vitamin to provide optimum immune response. In commercial poultry, a number of therapeutic substances are being used in combating various pathogens. The ultimate success of therapeutics is based not only on the direct effect on a pathogen, but also efficiency of the immune response. Therefore vitamin supplementation may be used as an adjunct to both therapeutic and prophylactic treatments (Muhammad, 2003).

Vitamin A is essential for the integrity of epithelial tissues, which represent a major defense against the entry of pathogens (Kjolhede and Beisel, 1995). Epidemiologic studies, clinical trials, and experimental studies in animal models have firmly established that vitamin A deficiency is a nutritionally acquired immunodeficiency disorder that is characterized by widespread immune alterations and increased infectious diseases and mortality (Semba, 1994). Reviews of randomized, controlled epidemiological studies have led to the conclusion that vitamin A supplementation (to correct a deficiency) can reduce the severity of some infections, including diarrheal diseases (Beaton, 1996; Kirkwood, 1996).

Newcastle disease (ND) caused by ND virus which is an *avulavirus* is one of the most important avian viral diseases because of its high economic impact on the poultry industry. In many tropical and subtropical countries virulent strains of Newcastle disease virus (NDV) are endemic. In Nigeria, Newcastle disease is considered one of the most important constraints for the development of profitable poultry farm and poultry-fish integrated farms in urban and rural areas (Adene, 2004). Newcastle disease is preventable through good husbandry practices and vaccination.

Vaccination for protecting chickens from Newcastle disease is routinely practiced throughout the world (Al-Zubeidi, 2009).

Hence, this study was proposed to investigate the effect of supplementing varying concentration levels of vitamin A in diet of broiler chickens on immunological response to Newcastle disease vaccinations.

MATERIAL AND METHODS

Experimental site

This study was approved by the Research Committee of the Department of Animal Production and Health, The Federal University of Technology, Akure (FUTA) Nigeria. The feeding trial was conducted at the Poultry unit of the Teaching and Research Farm of FUTA, Nigeria. The laboratory analyses were done at the Central Research Laboratory, FUTA and Microbiology Laboratory of the Department of Animal Production and Health, FUTA.

Table 1 Gross composition of experimental diets

Ingredients %	Starter diet	Finisher diet
Maize	58.50	50.50
Soy bean meal	18.50	17.00
Wheat offal	-	13.00
Groundnut cake	12.50	11.00
Fish meal	4.00	1.00
Bone meal	2.75	2.75
Oyster shell	1.00	1.00
Methionine	0.15	0.15
Lysine	0.10	0.10
Premix *	0.20	0.20
Salt	0.30	0.30
Vegetable oil	2.00	3.00
Total	100	100
Calculated		
Crude protein (%)	23.09	19.90
Metabolisable energy (MJ/kg)	13.13	12.66
Calcium (%)	1.73	1.54
Available phosphorus (%)	0.77	0.72
Lysine (%)	1.12	1.01
Methionine (%)	0.49	0.44

* 2.5kg of premix contains the following: Vitamin A (i.u)- 12,000,000; Vitamin D3 (i.u)- 2,500,000; Vitamin E (i.u)- 30,000; Vitamin K (mg)- 2,000; Vitamin B1 (mg)- 2,250, Vitamin B2 (mg)- 6,000; Vitamin B6 (mg)- 4,500; Vitamin B12 (mcg)- 15; Niacin (mg)- 40,000; Pantothenic acid (mg)- 15,000; Folic acid (mg)- 1,500; Biotin (mcg)- 50; Choline chloride (mg)- 300,000; Manganese (mg)- 80,000; Zinc (mg)- 50,000; Iron (mg)- 20,000; Copper (mg)- 5,000; Iodine (mg)- 1,000; Selenium (mg)- 200; Cobalt (mg)- 500; Anti-oxidant (mg)- 125,000.

Experimental diets

Two basal diets (starter and finisher diets) were used in this study and the gross composition is presented in Table 1. They were formulated based on the National Research Council (1994) table of feedstuffs that met the requirements of broiler chickens. The starter diet was mixed in one batch and sub-divided into three equal portions. One portion served as the control (diet 1) and the other two portions were designated diets 2 and 3. Thereafter dietary vitamin A was added to the experimental diets at 0, 100mg/kg and 200mg/kg respectively then thoroughly mixed. The finisher diets were also prepared using the same procedure.

Experimental design and animal management

A total of one hundred and twenty (120) day-old broiler chicks of the Abor acre breed purchased from a reputable hatchery in Akure, Ondo State, Nigeria were used for the study. Brooding was done in a conventional manner with temperature ranging from 35^{o}C at day old to 29^{o}C at 3 weeks of age and then kept stable at 25^{o}C thereafter. The chicks were divided into three treatment groups with four replicates of 10 birds each using a completely randomized design. The chicks were reared on deep litter system with wood shavings as beddings. They were fed their respective experimental diets by feeding the starter diets from day 1 to 28 day and the finisher diets from day 29 to 56 day. The birds were given water and feed *ad libitum* and reared using common management practices for broiler chickens as outlined by the Teaching and Research Farm of FUTA. The experimental chickens were vaccinated with Newcastle disease vaccines (NDV) - NDV intra-ocular (Hithner B1 strain) at 3 days old and NDV LaSota via the oral route at 28 days old.

Performance criteria measurement

The weight of birds was measured at day old (initial weight) then on a weekly basis (final weight). Thereafter weight gain for each week over the trial period was measured as the difference between the initial weight and the final weight. The feed consumption was recorded per replicate and the feed conversion ratio calculated as a ratio of feed consumed to weight gain of birds per replicate.

Blood and sera collection

Samples of blood for the purpose of serum analysis were collected from 3 birds per replicate in each treatment group before the trial commenced via the heart to determine baseline maternal antibody titre levels against Newcastle disease. The birds were sedated using chloroform before the bleeding exercise. Thereafter, in each treatment 12 birds (3 per replicate) were randomly selected and blood was collected 14 days after administering each of the ND vaccines through the jugular vein for serological analysis to determine the antibody titre values. At the end of the 8 weeks experimental period blood was also collected for haematological and serum protein biochemistry analysis from 12 birds in each treatment.

Relative organ measurements

At the end of the 8 weeks feeding trial three (3) birds per replicate making a total of twelve (12) birds per treatment were randomly selected, slaughtered, defeathered and eviscerated. The organs were dissected out and measured. The organs measured were liver, heart, spleen, lungs, gizzard, proventriculus, kidney, and bursa of Fabricious and expressed in g/kg body weight.

Laboratory analysis

Haemagglutination and Haemagglutination Inhibition Test (HA/HI Test): serum samples were analysed using beta (β) micro haemagglutination inhibition technique (Thayer and Beard, 1998) to determine the antibody titre levels as a measure of the immunological response elicited in the vaccinated experimental birds.

Haematological parameters: the erythrocyte sedimentation rate (ESR), packed cell volume (PCV), red blood cell count (RBC), haemoglobin concentration (HB) and white blood cell differentials were analysed as described by (Lamb, 1981). The Mean corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and the Mean Corpuscular Volume (MCV) were also calculated accordingly.

Serum protein biochemical analysis: the protein content (albumin, globulin and the total protein), alkaline phosphate (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of serum samples were estimated using commercial diagnostic kits (Randox Laboratories Limited, UK test kits).

Statistical analysis

All data generated on performance characteristics, relative organ weights, haematological variables, immunological responses and serum biochemistry were subjected to one-way analysis of variance (ANOVA). Where significant differences were found, the mean were separated using the Statistical Analysis System 9.2 (SAS) software (2008).

RESULTS

Performance characteristics

The indices used to measure performance criteria of the experimental birds which include final body weight (FBW), total feed intake (TFI), total weight gain (TWG) and feed conversion ratio (FCR) are summarized in Table 2. The different supplemental levels of vitamin A affected the growth performance of experimental broiler chickens. It was observed that FBW of birds fed the control diet (2633.33g) was significantly ($p<0.05$) higher than that of birds fed the rest test diets. The TWG of the experimental chickens were significantly ($p<0.05$) different among the treatment groups with birds fed the control diet (2587.40g) having the highest weight gain compared with birds in other treatment groups. The weight gain of the birds on a daily basis also followed a similar trend. The varying dietary treatments also significantly ($p<0.05$) influenced the FCR with birds fed control diet (1.56) being able to better utilize their diets than birds fed 100mg/kg and 200mg/kg of vitamin A supplemented diets that recorded FCR of 1.84 and 1.85 respectively.

Table 2: Performance characteristics of broiler chickens fed vitamin A supplemented diets

Parameters	Diet 1	Diet 2	Diet 3	±SEM
Initial weight(g/bird)	45.93	45.98	45.90	0.03
Final body weight (g/bird)	2633.33^{a}	2172.34^{b}	2160.01^{b}	0.58
TWG (g/bird)	2587.40^{a}	2126.36^{b}	2114.11^{b}	1.54
TFI (kg/bird)	4036.34	3912.50	3911.10	0.19
Weight gain (g/bird/day)	36.96^{a}	30.38^{b}	30.20^{b}	0.04
Feed intake(g/bird/day)	57.66	55.89	55.87	0.61
Feed conversion ratio	1.56^{a}	1.84^{b}	1.85^{b}	0.34

Means on the same row with different superscripts are statically significant ($P<0.05$); TWG-Total weight gain; TFI- Total feed intake

Table 3: Mean antibody titre values of broiler chickens fed vitamin A supplemented diets in response to ND Vaccinations

Parameters	Diet 1	Diet 2	Diet 3
Baseline titres	$Log_2 4$	$Log_2 4$	$Log_2 4$
Titre After NDV i/o	$Log_2 6$	$Log_2 7$	$Log_2 7$
Titre After NDV Lasota	$Log_2 7^{b}$	$Log_2 8^{ab}$	$Log_2 9^{a}$

Means on the same row with different superscripts are significantly different ($P<0.05$) NDV-Newcastle disease vaccination

HA/HI tests

The results of the HA/HI tests are presented in Table 3 and the antibody titre values did not show significant ($p>0.05$) difference among treatment groups after the first ND vaccination. Thereafter, significant ($p<0.05$) difference was noticed for the second ND vaccination when the birds fed the diet containing 200mg/kg of vitamin A had the highest titre value of $log_2 9$ and birds fed control diet recorded titre value of $log_2 7$ which was the lowest.

Haematological parameters

Table 4 shows the haematological parameters of the experimental chickens and it was observed that only the MCV, MCH and lymphocyte values were significantly ($p<0.05$) influenced by the dietary treatments. The MCV (1.11μ^3) and MCH (3.69 pg) of birds fed 200mg/kg of vitamin A diet were significantly ($p<0.05$) different from those of birds (1.20μ^3 and 4.02 pg) fed 100mg/kg of vitamin A and also birds (1.22μ^3 and 4.08 pg) fed control diets. The lymphocyte value of birds (61.97%) fed 200mg/kg vitamin A supplemented diets was significantly ($p<0.05$) higher than that of birds (58.67%) fed the control diet while it was not significantly ($p>0.05$) different from that of birds (61.00%) fed 100mg/kg of vitamin A supplemented diet.

Table 4: Haematological parameters of broiler chickens fed vitamin A supplemented diets

Parameters	Diet 1	Diet 2	Diet 3	±SEM
ESR (mm/hr)	2.67	2.27	2.52	0.14
PCV (%)	27.67	28.00	27.33	0.38
RBC (x 10^6 mm^3)	2.26	2.32	2.46	0.04
Hb (g/100ml)	9.23	9.33	9.10	0.17
MCHC (%)	33.35	33.32	33.29	0.24
MCH (pg)	4.08^a	4.02^a	3.69^b	2.31
MCV(μ^3)	1.22^a	1.20^a	1.11^b	1.64
Lymphocyte (%)	58.67^b	61.00^a	61.97^a	0.92
Heterophils (%)	25.67	23.67	24.00	0.41
Monocytes (%)	14.33	12.33	13.33	0.82
Basophils (%)	2.57	2.33	2.00	0.06
Eosinophils (%)	0.97	0.93	1.01	0.03

Means on the same row with different superscripts are significantly different ($P<0.05$)
ESR - Erythrocyte sedimentation Rate, PCV - Packed cell volume, RBC- Red Blood cell
Hb- Haemoglobin, MCHC- Mean cell haemoglobin concentration,
MCH- Mean cell Haemoglobin, MCV-Mean cell volume

Table 5: Serum metabolites of broiler chickens fed diets supplemented with varying levels of vitamin A

Parameters	Diet 1	Diet 2	Diet 3	±SEM
Alkaline Phosphate(IU/L)	170.32	171.68	175.53	0.53
Aspartate aminotransferase(IU/L)	119.58	120.36	121.41	0.17
Alanine aminotransferase(IU/L)	3.67	3.48	3.56	0.32
Total Protein (g/dl)	6.58^b	6.39^b	7.87^a	0.69
Albumin (g/dl)	2.14	2.08	2.16	0.40
Globulin (g/dl)	4.44^b	4.31^b	5.71^a	0.21

Means on the same row with different superscripts are significantly different ($P<0.05$)

Serum protein biochemical analysis

In Table 5 the values of the serum biochemical parameters analysed are shown. The birds fed diet 3 had their total protein (7.87g/dl) and globulin (5.71g/dl) values significantly (p<0.05) different from that of birds being fed the other diets. The table further showed that alanine phosphate (ALP) and aspartate aminotransferase (AST) values increased with higher supplementary levels of dietary vitamin A.

Relative organ weights

The weights of organs of experimental birds were not significantly (p>0.05) influenced by the varying dietary treatments as observed in Table 6.

Table 6: Relative organ weights of broiler chickens fed varying levels of Vitamin A supplemented diets (g/kg body weight)

Parameters	Diet 1	Diet 2	Diet 3	±SEM
Liver	22.7	19.4	18.1	1.89
Kidney	3.3	2.9	3.1	0.13
Heart	4.2	4.4	4.1	0.39
Spleen	0.8	1.1	0.9	0.11
Gizzard	18.1	17.8	17.4	2.11
Pancreas	1.6	1.6	1.7	0.28
Proventriculus	4.9	4.5	4.7	0.43

DISCUSSION

Vitamin A supplementation of the diet could prevent inhibition of growth in poultry that would otherwise experience a deficiency of this vitamin (Jianmin *et al.*, 2014). The results of this present study revealed that the weight of experimental birds fed diet with the NRC required dietary vitamin A was the highest and this is in agreement with NRC (1994) that suggests that there is no need for dietary vitamin A supplementation above the recommended level in broiler chickens. It agrees with work done by Uni *et al.*, (2000) where it was reported that the absence of vitamin A interferes with the normal growth rate of chickens by influencing the functionality of the intestine by altering proliferation and maturation of cells in the small intestinal mucosa. This study therefore ascertains the fact that vitamin A is essential for improved growth in broiler chickens though at the recommended level.

This study also showed birds fed diet not supplemented with dietary vitamin A relatively had the highest feed intake than birds on other test diets. This finding is not in line with work done by Raza *et al.,* (1997) where it was reported that dietary vitamin A increased appetite of birds resulting in improved feed consumption and feed conversion ratio and higher final body weight.

Investigations by several workers have shown that vitamin A supplementation in feed of poultry birds caused improvement in immune function in response to infectious diseases and vaccinations. The result of this present study has confirmed this, in that supplementing vitamin A at inclusion rate of 200mg/kg in feed elicited the highest level of antibody titre in response to ND vaccinations compared to that of birds fed diets supplemented with vitamin A at lower inclusion levels. In contrast, in laying hens Coskun *et al.* (1998) investigated dietary levels of up to 24,000 IU vitamin A per kg over one year, but could not record any beneficial effects on the various parameters of immune response. Though reportedly, a high level of dietary vitamin A (12,000 IU/kg) increased the antibody titer against NDV of heat-stressed hens (Lin *et al.*, 2002).

It has also been proven that supplementation of vitamin A either on the day of vaccination or few days afterwards increased antibody titer when it was demonstrated that the optimum HI titer against Newcastle disease was obtained when the feed contained 20,000 I.U. Vitamin A per kg of feed (Muhammad, 2003).

However, previous work showed that excessive vitamin A intake has a detrimental effect on the immune function of birds (Freidman *et al*., 1991) This fact was further ascertained by report of Jianmin *et al*., (2014) which indicated that supplementation of excessive vitamin A was detrimental to humoral immunity.

The haematological parameters of broiler chickens in this current study showed linear increase in percentage of blood lymphocytes as vitamin A levels increased. This resulted in high antibody titre values of birds fed diets supplemented with the highest level of vitamin A since lymphocytes are responsible for antibody production. However, Jianmin *et al*. (2014) suggested that supplemental levels of vitamin A above the tolerable dose could impair T-cell immunity because he observed significant decrease in peripheral blood T lymphocyte-cell proliferation activity in his study with broiler breeder chickens.

Serum metabolites AST and TBIL are usually measured clinically during diagnostic tests for liver function and to determine liver health. The levels of serum AST generally increase with muscle or liver damage (Sohail *et al*., 2011). Signs of toxicity such as reduced egg weight reported by Jianmin *et al*. (2014) caused by excess supplementation of vitamin A as indicated from high concentration levels of AST in broiler breeder hens due to liver malfunction is similar to the findings of this present study where it was seen that AST values of the experimental birds increased with higher vitamin A supplementary levels.

Reportedly, the liver is the major storage site for vitamin A, containing 80% of total body reserves when vitamin A status is normal (Blomhoff *et al*., 1991) However excessive amounts of vitamin A retained in liver may exert a hepatotoxic effect. McCuaig and Motzok (1970) showed that the livers of broilers became light and clay-colored when the birds were fed high doses of 325,000 IU/kg vitamin A. This may be in line with findings of this present study which showed that relative weight of liver of broiler chickens reduced as inclusion level of dietary vitamin A increased.

CONCLUSIONS

In this present study it can be concluded that supplementation of vitamin A above the NRC recommended level in broiler chicken diets did not lead to any significant improvement in performance traits, though it however caused an increase in humoral immune response to ND vaccinations.

REFERENCES

Adene, D.F. 2004. Poultry Health and Production. In: Principles and Practices, 1st Edition, Stirring Holden Publishers, Nigeria, pg 296.

Al-Zubeidi, 2009. Evaluation of two different vaccination programs against Newcastle disease in Ninevah province. Journal of animal and veterinary Advances, 82:228-231.

Beaton, G.H. 1996. Effectiveness of Vitamin A supplementation in the control of young children morbidity and mortatility in developing countries. ACC/SCN State of the Art Nutrition policy discussion, Paper No. 13.

Blomhoff, R., Wake, K. 1991 Perisinusoidal stellate cells of the liver: important roles in retinol metabolism and fibrosis. FASEB J 5: 271–277.

Coskun, B., Inal, F., Celik, I., Erganis, O., Tiftik, A.M., Kurtoglu, F., Kuyucuoglu, Y. and Ok, U. 1998. Effects of dietary levels of vitamin A on the egg yield and immune responses of laying hens. Poultry science, 77:542-546.

Friedman, A., Meidovsky, A., Leitner, G. and Sklan, D. 1991. Decreased resistance and immune response to *Escherichia coli* infection in chicks with low or high intakes of vitamin A. Journal of Nutrition, 121: 395-400.

Jianmin, Y., Abdelfatah, R.R., Yuming, G., Yongwei, W. And Shuangshuang, G. 2014. Effect of dietary vitamin A on reproductive performance and immune response of broiler breeders. PLOS ONE http://dx.doi.org/10.1371/journal.pone.0105677

Kjolhede, C. and Beisel, W.R. 1995. Vitatmin A and the immune function: A Symposium. Journal of Nutrition. Immunology 4: xv-143.

Kirkwood, B.R., Ross, D.A., Arthur, P., Morriss, S.S., Dollimore, N. and Binka, F.N. 1996. Effect of vitamin A supplementation on the growth of Young children in Northern Ghana, American Journal of clinical Nutrition 63(5):773-781.

Klasing, K.C. 1998. Nutritional modulation of resistance to infectious diseases. Poultry Science, 77:1119-1125

Lamb, G.B. 1981. Manual of Veterinary Techniques in Kenya. Published by CIBA-GEGY.127-147.

Lin, H., Wang, L.F., Song, J.L., Xie, Y.M. and Yang, Q.M. 2002. Effect of dietary supplemental levels of vitamin A on egg production and immune responses of heat stressed laying hens. Poultry Science, 81(4): 458-465.

McCuaig, L.W. and Motzok, I. 1970. Excessive dietary vitamin E: its alleviation of hypervitaminosis A and lack of toxicity. Poultry Science, 49: 1050–1052.

Muhammad, S. 2003. Role of Vitamins in Immune Response in Poultry: The Veterinary news & views [weekly] and on World Veterinary Association web site (Oct 23, 2003 - 02:43 PM).

National Research Council, 1994. Nutrient requirement of Poultry. 9th Rev. Ed. National Academy Press, Washington, DC.

Raza, A., Khan, F.K., Saced, M.A. and Bashir, N.I. 1997. Effect of vitamin A on growth traits, immune-regulatory organs and immune response in broiler chickens. Journal of Applied Animal Research, 12:81-88.

SAS Institute 2008 Statistical Analysis System (SAS), users' Guide. Release 9.2 Ed. SAS Institute Inc., Cary, NC.

Semba, R.D. 1994. Vitamin A, immunity and infection. Clinical Infectious Disease, 19:(3) 489-499.

Sohail, M.U., Rahman, Z.U., Ijaz, A., Yousaf, M.S., Ashraf, K., et al. (2011) Single or combined effects of mannan-oligosaccharides and probiotic supplements on the total oxidants, total antioxidants, enzymatic antioxidants, liver enzymes, and serum trace minerals in cyclic heat-stressed broilers. Poultry Science, 90: 2573–2577.

Thayer, S.G. and Beard, C.W. 1998. Serological Procedure In: A Laboratory Manual for the Isolation and Identification of Avian Pathogens, 4th Ed. Amer. Assoc. Avian Pathologist, Pennsylvania, USA, pp: 255-258.

Uni, Z., Zaiger, Gal-Garber, O., Pines, M., Rozenboim, I. and Reifen, R. (2000). Vitamin A deficiency interferes with profiliferation and maturation of cells in the chickens small intestine. British Poultry Science, 41:410-415.