World Applied Sciences Journal 7 (3): 366-370, 2009
ISSN 1818-4952

Effect of Some Vitamins and Electrolytes on Chickens Infected with Infectious Bursal Disease

Babiker Mohamed Alamin, Yahia Ibrahim and Noura K.M. Salih

Department of Internal Medicine, College of Veterinary Science,
University of Bahr El Ghazal, Elshabia, Elmawna Road,
West to Ahmed Gasim Hospital Khartoum North, Sudan

Abstract: To study the effect of adding some vitamins and electrolytes to drinking water of chickens vaccinated and challenged with infectious bursal disease virus (IBDV), two hundred commercial layer chicks in a good health were vaccinated twice using intermediate strain of live attenuated IBD vaccine (D78) at day 15 and 22. Fifteen days latter birds were allocated to two treatments of 100 chicks each. The first treatment was challenged with IBDV and given the basic diet and supplemented with commercial product consisted of potassium citrate 18%, sodium citrate 12%, phenyl butazone 1.6%, vitamin B1 0.03%, vitamin B2 0.015%, Nicotine amide 0.32% Menadione bisulfite 0.11%, vitamin C 1.1%, trihydroxyethyl rutocide 0.12% and stabilizer ad 100% in a dose of 2.5 gm/L of drinking water for 5 days, while the other treatment was challenged with IBDV and only given the basic diet. Addition of these vitamins and electrolytes to the drinking water of chickens vaccinated wih IBD vaccine and challenged with IBDV reduced the mortality rate from 40 to 24%, and increased bursa to body weight $\times 10^{-3}$ ratio from 2.83±0.32 to 3.89±0.55 and lowered histopathological change of bursae tissues grade from 3.4±0.25 to 2.6±0.13. The mean. antibodies titre and sample absorption to positive control absorption ratio (S/P) of the birds given diet supplemented with vitamins and electrolytes was elevated from 13042±1107.42 to 17221±1153.22 and from 4.78±0.37 to 6.33±0.37 respectively.

Key words: Electrolytes · Vitamins chicken IBD · Adverse effect

INTRODUCTION

Infectious bursal disease (IBD) is an acute highly contagious viral disease of young susceptible chickens [1]. It causes mortality of about 10%, but mortality can reach 40-50% [2]. The disease has characteristic clinical signs and gross lesions. Bursa of Fabricius is the primary target organ for IBDV replication. The virus causes lymphoid depletion of the bursa of Fabricius and hence significant depression of humoral antibodies response [3].

Vaccination is the major preventive measure for the control of the disease [4]. Live attenuated vaccines are usually used to control the disease. Although these vaccines failed to prevent chickens from clinical IBD outbreak [5] and from variant strain viruses [6]. No ideal live IBD vaccine in a term that not cause the disease, bursal lesions or immune-suppression.and fully protect chickens from the clinical disease is available.

There is no specific treatment for the disease, but palliative treatment may be undertaken. Passive hyper-immune therapy is given to chicks as an alternative for vaccination [7]. Another alternative is the trial to improve the immune-responsiveness to vaccine and disease resistance by supplementing ascorbic acid [8]. Amakye-Anim *et al.* [9] also reported a beneficial effect of ascorbic acid. Electrolyte preparation containing citric acid, citrates and vitamin B group have vital role in metabolism and body immune response. The present study was a trial to determine whether the supplementation of drinking water with some vitamins and electrolytes improve the performance of chickens infected with infectious bursal disease virus (IBDV) or not.

MATERIALS AND METHODS

Experimental Design: Two hundred day old commercial layer chicks were reared in isolated pens.The chicks were vaccinated twice against infectious bursal disease virus (IBDV) at day 15 (primary) and day 22 (booster) via drinking water route using intermediate strain vaccine

Corresponding Author: Babiker Mohamed Alamin, Department of Internal Medicine, College of Veterinary Science, University of Bahr El Ghazal, Elshabia, Elmawna Road, West to Ahmed Gasim Hospital Khartoum North, Sudan

D78. Birds at 37 days of age were randomly allocated to two treatments of 100 chicks each, Supplementation of the drinking water with vitamins and electrolytes was the main effect. The first treatment was challenged and given the basic diet (Table 1) in addition to supplemention with vitamins and electrolytes, while the other was challenged and given only the basic diet.

Vitamins and Electrolytes Supplements: Vitamins and electrolytes used in the present study :were a commercial product (Table 2).given in a dose of 2.5 gm/L of drinking water for 5 days starting from the day when IBD clinical signs appeared post challenge as prescribed by the manufacturer.

IBDV Inoculums: IBDV inoculum for artificial challenge was prepared and performed as described by Khan *et al.* [9]. Bursa derived STC strainof serotype 1IBDV was used as a challenge virus in a dose of 1×10^{3}•^{2} 50%. bursae of Fabricious were collected from IBDV infected chicks maintained at the faculty of veterinary medicine-university of Khartoum and stored at -20°C till used. A 20%(W/V) bursae homogenate was prepared by blending them in sterile phosphate buffer saline (PH 7.4). The preparation was freeze-thawed thrice and centrifuged at 2500 rpm for 20 minutes. The supernatant was collected and filtered via whatman No 1 filter. The filtrate was centrifuged at 2500 rpm for 20 minutes and the supernatant was collected in sterile bottle. Penicillin 1000 IU/ml, Streptomycin 10 mg/ml and Gentamycin 1 mg/ml were added to prevent bacterial contamination. Virus was tested against known positive anti-sera of IBDV by Agar Gel Precipitation Test (AGPT).

Clinical Signs: Clinical signs of the IBD including anorexia, dullness, birds reluctant to move on drive, in-coordination, watery diarrhea and ruffed feathers [2] was observed 2-3 days post challenge [10].

Postmortem Gross Lesions: Gross lesions of IBD including enlarged or atrophied bursa of Fabricius and covered with yellowish serious fibrious exudates or hemorrhagic with cherry appearance, swollen pale kidneys, dry dehydrated muscles, hemorrhage on the body skeletal muscles surfaces, erosions and hemorrhages of the mucosa of proventriculus, [2] and classified as positive or negative.

Bursa to Body Weight $\times10^{-3}$ ratio: Bursae of Fabricious were removed and their weight were determined using a sensitive balance. The bursa weight was then calculated as a ratio of the body weights multiplied by 10^{-3}. According to Giambrone and Glosser [11] and the bursae were preserved for histopathologic examination.

Table 1: Composition of the basic diet used in the study

Ingredient	Diet (%)
Sorghum grains	54.00
Sesame cake	12.00
Groundnut cake	8.00
Wheat bran	16.00
Lime stone	4.00
Super concentrate (rearing 1)	5.00
Lysine	0.35
Methionine	0.15
Na chloride (common salt)	0.50
Total	100.00

Table 2: Composition of the poultry preparation supplemented in drinking water used in the experiment

Ingredient	% of the preparation
Potassium citrate	18.00
Sodium citrate	12.00
Phenyl butazone	1/6.00
Vitamin B1 (Thiamine)	0.03
Vitamin B2 (Riboflavin)	0.015
Nicotine amine	0.32
Menadyonebisulphate	0.115
Vitamin C (Ascorbic acid)	1.10
Trihydroxyethyl rutoside	0.12
Stabilizer exipient ad	100.00

Histopathological Examination: Bursae of Fabricius tissues were fixed in 10% buffered formalin. Each bursa was trimmed to the thickness of 5 mm in size, dehydrated in a series of alcohol concentrations and imbedded in paraffin wax using automatic tissue processor. Sectioning of tissues was done to a thickness of 5 micrometer in a microtome. The bursa tissues were mounted on glass slides, dewaxed and stained with H&E and examined under the microscope using ×4, ×10 and ×40 objectives and the pathologic changes were subjectively graded in scale from 1 to 4 according to the degree of follicular atrophy and as described by Henrey *et al.* [12].

Serology (ELISA): To determine anti-IBD antibody titre ELISA as described by the manufacturer of the ELISA reader and IBD ELISA kit (Bio-check company-Holland) and described by Blankfard and Silk [13] was performed. Twenty three sera samples were collected and testes from each treatment for anti-IBD antibodiy titre, sample absorption to positive control absorption ratio (S/P), coefficient of variation. among individuals within each treatment and the number of positive, suspicious and negative samples were calculated.

Statistical Analysis: Mean values were analysed using t-student test at 5% of significance ($P<0.05$).

RESULTS

Morbidity and Mortality: The cumulative morbidity in both treatments was 100%. The cumulative mortalatyin

Table 3: Effect of vitamins and minerals supplementation on clinical signs, gross lesions, mortality rate, mean histopathlogical score and mean bursa to body weight×10^{-3} ratio

Treatment	Clinical signs	Gross lesions	Mortality rate	Mean histopathological changes score	Mean ratio B/Wt×10^{-3}
Birds given vitamins and minerals	Positive	Positive	24%	2.60±0.13	3.89±0.55
Birdsnot not given Vitamins and minerals	Positive	positive	40%	3.40±0.25	2.83±0.32

Table 4: Mean ELISA titer to infectious bursal disease virus in chickens before challenge and post challenge in chickens given diet su

Treatment	Mean titer	Min and max titrer	Mean S/P ratio	No of N/S/P samples	CV%
Birds befor challenge	6923±1244.12	2115-9775	3.76±0.17	0/0/23	43
Birds given vitamins and minerals post challenge	17221±1153.22	15177-19411	6.33±0.35	0/0/23	31
Birdsnot not given Vitamins and minerals post challenge	13042±1107.42	10772-16993	5.78±0.37	0/0/23	29

birds given diet not supplemented with vitamins and electrolytes was higher than in those given diet supplemented with vitamins and electrolytes by 10 days post challenge at the end of the experiment (Table 3).

Clinical Signs and Postmortem Gross Lesions: Birds in both treatments exhibited clinical signs that disappeared after 6-7 days and gross lesions of IBD. non of them passed challenge without showing the clinical disease.

Bursa to Body Weight×10^{-3} Ratio: Ten days post challenge birds vaccinated with IBD vaccine and given vitamins and electrolytes had significantly ($P<0.05$) higher bursa to body weight $10x^{-3}$ ratio than chickens vaccinated with intermediate strain of IBD vaccine, challenged and given diet not supplemented with vitamins and electrolytes.

Histopathological Changes: Histopathological changes in bursa of Fabricius tissues score ten days post challenge was significantly ($P<0.05$) lower in chickens fed basic diet supplemented with vitamins and electrolytes than in birds fed basic diet not supplemented with vitamins and electrolytes. Bursae of Fabricius of birds given diet not supplemented with vitamins and minerals showed higher histopathological score. Microscopic examination of bursal lymphoid follicles were depleted and atrophied lymphocytes (60-100% follicular atrophy) compared to 20-70% observed in bursae of Fabricius of birds given basic diet supplemented with vitamins and electrolytes Table 3).

Antibody Titer Against IBD: The mean antibody titre before challenge (6923±1244.12) failed to protect chickens in both treatments from clinical IBD, although this level is higher than the cut off point. Antibodies titre against IBD was significantly ($P<0.05$) higher in birds vaccinated with intermediate strain of IBD, challenged with IBDV and fed diet supplemented with vitamins and electrolytes than those of birds not supplemented (Table 4). No significant ($P<0.05$) difference was detected in Cofficient of variation %(CV%) of titer within each treatment..

DISCUSSION

Viral infection is a stress factor that depletes vitamin C levels in the body tissues and interferes with its biosynthesis [14]. It also causes depletion of leukocyte ascorbate resulting in non specific immue-supression [15]. Supplementation of the diet of IBD infected chickens with vitamins and minerals is beneficial [16]. The present study showed that vitamins and electrolytes supplementation lowered mortality rate but failed to prevent them from the clinical signs and postmortem gross lesions. This findings disagreed with the results reported by Amakye-Anim *et al.* [10] who demonstrated that birds given diet supplemented with vitamin C, vaccinated with IBD vaccine and challenged with IBDV exhibited no mortality or clinical disease. One thousand ppm per day of vitamin C recommended by Amakye-Anim *et al.* [10] was found to be beneficial in this study. It increase antibody production and decrease mortality by providing the required vitamin C level not sufficiently synthesized in the body.

Vitamin C increase the resistance to infectious diseases in chickens by improving immune system [8]. Phagocytosis, the first line of defense against pathogens involves increased consumption of Ascorbic acid and dihydroascorbate [17]. Ascorbic acid can modulate the activity of B cells and hence increase antibody production [18].

Use of poultry electrolyte preparation containing glucose and citric acid may restore solute and volume deficits that resulted from diarrhea and provides energy to anorexic birds [19]. Hirschhorn [20] found that treatment with an oral rehydration solution containing electrolytes alleviate anorexia and stimulate food intake in human infants with diarrhea. That may explain the beneficial effect of electrolytes contained in preparation used in the present study.

Vitamin B group is known to play crucial role in energy metabolism. It helps the body to convert protein, fats, and carbohydrates into viable energy source for other metabolic functions. Vitamin B1 (thiamin) is known to be necessary for the complete and proper oxidation of sugar. Its addition to the drinking water improve appetite, muscular tones particularly in the intestine, general weakness. Vitamin B2 (Riboflavin) converts to coenzyme ; flavin adenine dinucleotide and flavin mononucleotide. These are vital coenzymes required for the production of ATP.Vitamin B2 is also essential for proper immune system functioning, reinforcing national antibody reserves which are the first line of defense against viruses. Nagorna-Stasiak and Wawrzenska [21] found that vitamin B2 stimulates intestinal motility, and chickens intestine is 10 times sensitive to vitamin B2 than that of rabbit intestine.

ACKNOWLEDGMENT

This research was financed by the Administration of Scientific Research, Ministry of High Education and Scientific Research, Sudan.

REFERENCES

1. Parkhurst, R.T., 1964. Pattern of mortality in avian nephrosis Poultry Sci., 43: 788-789.
2. OIE manual of diagnostic tests and vaccines jerrestrial animals 2004. 5th Edn. chapter 1 part 2 Section 2-7.
3. Wood, G.W., J.C. Muskett and D.H. Thornton, 1981. The interaction of live vaccine and maternal antibody on protection against infectious bursal disease. Avian Pathol.,10: 365-373.
4. Zaheer Ahmed, S. Inayat, K. Naeem and S.A. Malk, 2003. Comperative immune response pattern of commercial infectious bursal disease vaccines against field isolates in Pakistan. Inter J. Poultry Sci., 2(6): 449-453.
5. Owoade, A.A., M.N. Mulders, J. Kohnen, W. Ammerlaan and C.P. Muller, 2004. High sequence diversity in infectious bursal disease virus serotype1 in poultry and turkey suggests West-African origin very virulent strain. Arch. Virol., 149 (4): 653-672.
6. Saif, Y.M., 1998. Infectious bursal disease and hemorrhagic enteritis. Poultry Sci., 77: 1186-1189
17. Stankova, L., M.B. Gerberdt, L. Nagel and R.H. Bigley, 1975. Ascorbic acid phagocyte function Infect Immun., 12: 252-255.
7. Muhamead Wasif Malk, Najma Ayub, Irfan Zia Qurashi, 2006. Passive immunization using purified IgYs against infectious bursal disease of chickens in Pakistanj Vet. Sci ., 7 (1): 41-46.
8. Rund, B., 1989. Vitamin C plays role in immunity Poult Dis, 48: 44-55.
9. Khan, K.M., S.A. Shah and M. Afzal, 1988. Observations on Gumboro disease in Pakistan. Rev. Sci. Tech. Int. Epiz., 7: 625-629.
10. Amakye-Anim, J., T.L. Lin, P.Y. Hester, D. Thiagarajan, B.A. Watkins and C.C. Wu, 2000. Ascorbic acid supplementation improved antibody response to infectious bursal diseasevaccination in chickens. Poultry Sci., 79: 680-688.
11. Giambrone, J.L. and L. Glosser, 1990. Efficacy of live vaccines against serologic subtype of infectious bursal disease virus Avian Dis 34:7-11Studies on infectious bursal disease in chickens.
12. Henrey, C.W., R.N. Brewer, S.A. Edgar and B.W. Graw, 1980. Scoring microscopic lesions in the bursa of Fabricius, thymus, spleen and kidney in gnotobiotic and buttery reared White Leghorm experimentally infected with infectious bursal disease virus. Poultry Sci., 59:1006-1017.
13. Blankfard, M. and B.C. Silk, 1989. ELISA software R. Giathersburg Md USA.
14. Gross, W.B., 1988. Effect of environmental stress on the responses of Ascorbic acid- treated chickens to *Escherichia coli* challege infection. Avian. Dis., 32: 432-436.
15. Thomas, R.P. and G. Holt, 1987. Vitamin C and immunity. An assessment of the evidence. Clin. Exp. Immunol., 32: 370-379.
16. McDowell, E.R., 1989. Vitamins in animal nutrition Academic Press New York NY USA.
18. McCorkle, F.R., R. Taylor, E. Stinson and B. Glick, 1980. The effects of megalevels of vitamin C on the immune response of the chickens. Poultry Sci., 59: 1324-1327.

19. El Hadri, L., J.D. Garlich, M.A. Qureshi, P.R. Ferket and N.H. Odefalla, 2004. Glucose and electrolytes supplementation of drinking water improve the immune responses of poult with inanition. Poult. Sci., 83: 803-809.
20. Hirschhorn, N., 1982. Oral rehydration therapy for diarrhea in children. A Basic Primer. Nutr. Rev., 4: 97-104
21. Nagorna-Stasiak, B. and M. Wawrzenska, 1987. Effect of vitamin B1,B2, B6, folic acid and vitamin C on the motor activity of chicken's intestines in chronic experiments and *in vitro*. Pol Arch Weter, 27 (2-3): 89-98.