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Changes in antibody titres and comparative efficacy of some unconventional remedies in mitigating the effects of infectious bursal disease virus infection in pullet chicks

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ABSTRACT

Unconventional remedies are used by farmers against infectious bursal disease (IBD). This study assessed changes in IBD antibody titres and efficacy of some unconventional remedies used by farmers against IBD. Day old ISA brown pullets were assigned into eight groups (A-H) and inoculated with a very virulent IBD virus (vvIBDV) at 21 days of age as follows; group A - administered aqueous leaf extract of Kaya senegalensis (KS); B, C, D, E, and F administered remedies designated as SEV, ENP, ASMS, DLP, and OVX, respectively; G, and H were negative, and positive control, respectively. Changes in IBD antibody (Ab) titres, clinical signs, morbidity and mortality rates, gross lesions, weights and organ ratios of bursal (BF), spleen (S) and thymus (T) were assessed. There were protective levels of Ab in groups of chicks at 2 and 7 days of age. SEV and ASMS potentiated Ab response to vvIBDV; SEV mitigated the severity of clinical signs, morbidity and mortality rates, gross lesions and changes in body and BF weights and SBR; KS clinical signs, morbidity and mortality rates, BF weight and BBR; ENP morbidity rate, spleen and thymus weights and TBR; ASMS thymus weight and TBR; DLP gross lesions, BF weight and BBR; OVX body, spleen weights and SBR. The remedies demonstrated varied actions against VVIBDV infection. SEV and KS leaves could be administered at the onset of IBD outbreak and before IBD vaccination. Processing of KS leaves to reduce concentration of antimetabolites is recommended.

Contribution/Originality: Since infectious bursal disease is a viral disease with no effective treatment, there are speculations on the effectiveness of some remedies by farmers. This study therefore provides the first documentation on the use of some commonly used unconventional remedies against infectious bursal disease.

1. INTRODUCTION

Poultry occupies a pivotal position among Nigerian livestock–based vocations, because of its enormous potential to bring rapid economic growth. The poultry sector mainly provides protein, employment, contributes to

Gross Domestic Product [1], and accounts for 58.2% of the total livestock production [2]. In Nigeria, diseases such as infectious bursal disease (IBD) are major threats limiting poultry production [3-5]. Infectious bursal disease, which is a contagious and viral disease of young chickens, is characterized by high morbidity, mortality, destruction of lymphocytes in the bursa of Fabricius (BF) and immunosuppression [6]. The disease has been reported worldwide, and its socio-economic significance is considerable.

For years, IBD has constituted a serious problem for the poultry industry and emergence of the IBD virus (IBDV) in the form of antigenic variants and very virulent (vv) strains has been the cause of significant losses [7]. Biosecurity sometimes fails to prevent IBD outbreaks as the IBDV is hardy and difficult to eliminate from contaminated poultry facilities [8]. Interference by maternally derived antibodies (MDAs) is also a challenge for preventing IBD through vaccination [9]. Therefore, outbreaks of IBD frequently occur in vaccinated flocks [10-12] causing anxiety to poultry farmers and veterinarians who are concerned of loss in returns on investments. There is no known effective chemotherapeutic agent for the control of IBD [13]. Thus in cases of IBD outbreaks farmers are left without any choice but to manage with medications [14] and plant extracts which they claim are effective. No research has been conducted to validate such claims as the medications may be ineffective or unsafe. The objectives of the study were to determine changes in antibody (Ab) titres and the potentials of some unconventional remedies used in the field to potentiate IBD Ab response and mitigate the severity of IBD following inoculation of pullets with a vvIBDV.

2. MATERIALS AND METHODS

2.1. Ethics Approval

Ethical approval for this study was granted by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/2020/012).

2.2. Experimental Chickens

Two hundred (200) 1-day-old ISA brown pullets were purchased from a reputable hatchery, and feed and water were provided *ad libitum*.

2.3. Unconventional Remedies

Aqueous extract of *Khaya senegalensis* (KS) leaves (identification number: 900181) was prepared [15] administered *ad libitum* as the source of drinking water for 7 days.

SEV (vitamins and minerals) recommended for use as an antioxidant and immune booster was administered at 1 mL per litre of distilled water (DW) for 7 days. ENP (amino acids, vitamins, and plant extracts) recommended for use as an anti-stress and antitoxin was administered at 1 mL per litre of DW for 7 days. ASMS (minerals) recommended for use as an anti-viral agent was administered at 1 g per litre of DW for 7 days. DLP containing an antimicrobial and fungicide was administered at 4 mL per litre of DW for 7 days. OVX (acids, minerals and oxidising agents) recommended for use as a disinfectant and water sanitizer was administered at 1 g per litre of DW for 7 days.

2.4. Groupings of Chickens

Chicks were randomly divided into 8 groups (A-H) of 22 chicks each. Blood was collected from each bird at 2, 7, 14, 21, 24 and 28 days of age, and serum was harvested. At 21 days of age, birds in groups A-F and H were inoculated with vvIBDV (Nigerian isolate) using 0.05 mL of vvIBDV inoculum (containing 16 x $10^{4.6}$ CID_{50}) via conjunctival instillation. At the onset of clinical signs birds in A, B, C, D, E, and F were treated with KS, SEV, ENP, ASMS, DLP, and OVX, respectively while G (negative control) and H (positive control) were not treated.

2.5. Enzyme Linked Immunosorbent Assay

The serum collected was analysed for IBD Ab titre using enzyme linked immunosorbent assay (ELISA) as described by IDEXX Laboratories Incorporation, USA. The Ab titre was calculated automatically using a software [16].

2.6. Observation for Clinical Signs, Mortality Rate and Gross Lesions

From 21 (day 0 post inoculation-PI) to 28 days of age (day 14 PI), clinical signs were scored and mortality recorded. Severity of clinical signs and mortality was calculated [17]. Five birds from each group were euthanized on days 21, 24 and 28 (0, 3, and 7 days post inoculation-DPI) of age and examined for gross lesions which were scored, bursa of Fabricius (BF), spleen (S), and thymus (T) were weighed and their organ body weight ratio was calculated [18].

2.7. Data Analyses

Data obtained were analysed using Statistical Package for Social Sciences (SPSS) version 20.0 and presented in Tables. They were expressed as mean ± SEM and subjected to a one-way Analysis of variance (ANOVA) followed by Tukey's *post hoc* test. Values of $P \leq 0.05$ were considered significant. Performance of each group in relation to each variable measured was ranked from 1 to 8, with 1 being most outstanding.

3. RESULTS

3.1. Changes in IBD ELISA Titre

Mean IBD ELISA Ab titres in all groups decreased with age and were ≥ 396 at 2, 7 and 14 days of age. Titres were highest in groups B and D at 7 DPI. The percentage of pullets with Ab titre of ≥ 39 in all groups decreased with age. At 28 days of age (7 DPI) only group B had chicks with Ab titre ≥ 396 (Table 1).

3.2. Clinical Signs and Mortality Rate

The clinical signs observed were sudden death, depression, somnolence, anorexia, diarrhoea, soiled vent, prostration, huddling, weakness and trembling. The signs appeared from day 2 PI in all groups except C. Signs were absent by day 7 PI in groups A, C and F. Clinical sign was most severe 4 DPI in all groups except D. Severity of signs in all groups peaked on day 4 PI and ceased by day 6 or 7 PI (Table 2).

Daily mortality rate was recorded 5 DPI in all inoculated groups and was highest in group D. Overall mortality rates between groups were statistically significant ($P < 0.05$) (Table 3).

3.3. Gross Lesions Scores

Gross lesion scores (GLS) were higher at 3 than 7 DPI in all groups. The GLS were low in groups D and C at 3 DPI. The % reduction in GLS between 3 and 7 DPI were low in A, D and H (Table 4).

3.4. Changes in Body Weights and Organ-Body Weight Ratios

The heaviest body weights (BDWs) were recorded in groups G, H, B and C at 7 DPI. The BDWs between groups A and B, and between C and E were statistically different (SD) ($P < 0.0$) at 3 DPI (Table 5).

There were increases in mean BF weights in groups B, C, D, E, F and G and decreases in groups A and H at 3 DPI. On day 7 PI, there was SD ($P < 0.05$) in BF weight between groups A and G, and groups D, E, F, H and G (Table 6). There were decreases in mean BBRs in groups A, B, C, G and H at 3 DPI. BBRs were significantly ($P < 0.05$) higher in groups A, B, E and G at 7 DPI (Table 6).

Table 1. Mean ELISA antibody titre of ISA brown pullets before and after inoculation with a very virulent infectious bursal disease virus at 21 days of age and treated with some unconventional remedies.

Age in days (Days post-inoculation)	Group (Treatment)							
	A (KS)	B (SEV)	C (ENP)	D (ASMS)	E (DLP)	F (OVX)	G (Negative control)	H (Positive control)
	Mean ELISA antibody titre (% with titre level of ≥ 396)							
2	2,307 ± 145.1 (100.0)	2,253 ± 167.6 (100.0)	2,376 ± 273.9 (91.67)	2,215 ± 200.7 (100.0)	2,407 ± 190.2 (100.0)	1,962 ± 163.2 (91.67)	2,253 ± 204.1 (91.67)	2,407 ± 196.4 (100.0)
7	1,951 ± 151.6 (100.0)	2,036 ± 189.5 (90.90)	1,652 ± 166.7 (91.67)	1,650 ± 199.9 (87.50)	1,974 ± 219.9 (90.90)	1,641 ± 183.3 (88.89)	1,675 ± 125.8 (100.00)	1,819 ± 163.2 (100.00)
14	586.5 ± 49.54 (0.00)	629.4 ± 98.96 (0.00)	517.8 ± 78.09 (0.00)	540.3 ± 124.1 (16.68)	504.0 ± 94.89 (0.00)	563.3 ± 139.9 (12.50)	619.9 ± 80.82 (15.38)	519.3 ± 91.03 (10.00)
21 (0)	133.0 ± 20.69 (0.00)	255.8 ± 35.25 (0.00)	304.9 ± 39.04 (0.00)	252.5 ± 64.59 (0.00)	255.0 ± 32.06 (0.00)	217.4 ± 50.78 (0.00)	189.8 ± 15.99 (0.00)	227.4 ± 46.46 (0.00)
28 (7)	151.6 ± 23.21 (0.00)	510.0 ± 205.3 (28.57)	131.7 ± 58.38 (0.00)	357.7 ± 47.98 (0.00)	172.8 ± 55.14 (0.00)	218.3 ± 98.68 (0.00)	61.43 ± 9.25 (0.00)	230.1 ± 77.26 (0.00)
Ranking	6	1	7	2	5	4	8	3

Table 2. Daily changes in severity of clinical signs of ISA brown pullets inoculated with a very virulent infectious bursal disease virus at 21 days of age and treated with some unconventional remedies.

Group (Treatment)	Days post-inoculation							Ranking
	2	3	4	5	6	7	Average severity of clinical sign (%)	
	Severity of clinical sign (%)							
A (KS)	6.60	14.30	15.60	13.30	12.20	0.00	12.40	3
B (SEV)	0.60	3.20	24.70	6.90	2.80	0.50	6.45	2
C (ENP)	0.00	9.40	28.60	30.00	3.80	0.00	17.95	7
D (ASMS)	5.13	21.70	49.00	10.30	20.50	2.00	18.11	8
E (DLP)	4.20	9.40	38.40	21.20	14.80	6.00	15.67	5
F (OVX)	4.20	19.30	42.30	15.60	5.30	0.00	17.34	6
G (Negative control)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1
H (Positive control)	4.10	5.50	50.70	12.00	5.30	6.00	13.93	4

Table 3. Daily mortality rates (%) in ISA brown pullets inoculated with very virulent infectious bursal disease virus at 21 days of age and treated with some unconventional remedies.

Group (Treatment)	Days post-inoculation						Ranking
	3	4	5	6	7		
	Number of birds dead (%)					Total (%)	
A (KS)	0 (0.00)	1 (4.50)	3 (13.60)	2 (9.09)	0 (0.00)	6 (27.27)	2
B (SEV)	1 (4.50)	0 (0.00)	4 (18.18)	0 (0.00)	1(4.50)	6 (27.27)	2
C (ENP)	0 (0.00)	7 (31.81)	7 (31.81)	5 (22.27)	0 (0.00)	19 (86.36)	7
D (ASMS)	1 (4.50)	1 (4.50)	10 (45.45)	3 (13.60)	1 (4.50)	16 (72.72)	6
E (DPL)	0 (0.00)	2 (9.09)	4 (18.18)	2 (9.09)	1 (4.50)	9 (40.90)	3
F (OVX)	0 (0.00)	1 (4.50)	8 (36.36)	2 (9.09)	0 (0.00)	11 (50.00)	4
G (Negative control)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1
H (Positive control)	3 (13.60)	0 (0.00)	6 (27.27)	3 (13.60)	1 (4.50)	13 (59.09)	5

Table 4. Gross lesion scores of ISA brown pullets inoculated with a very virulent infectious bursal disease virus at 21 days of age and treated with some unconventional remedies.

Group (Treatment)								
	A (KS)	B (SEV)	C (ENP)	D (ASMS)	E (DLP)	F (OVX)	G (Negative control)	H (Positive control)
Days post-inoculation	Gross lesion score							
3	6.80	9.40	4.60	4.80	6.00	10.40	0	8.00
Ranking	5	7	2	3	4	8	1	6
Gross lesion score (% Lesion score reduction)								
7	5.00 (26.47)	2.00 (78.72)	2.20 (52.17)	3.20 (33.33)	1.20 (80.00)	3.60 (65.39)	0 (0.00)	5.60 (30.00)
Ranking	8	3	5	6	2	4	1	7

Table 5. Mean body weights (g) of ISA brown pullets inoculated with a very virulent infectious bursal disease virus and treated with some unconventional remedies.

Days post-inoculation	Group (Treatment)							
	A (KS)	B (SEV)	C (ENP)	D (ASMS)	E (DLP)	F (OVX)	G (Negative control)	H (Positive control)
	Mean body weight (g)							
0	158.2 ± 4.33^{a}	155.0 ± 4.05^{a}	145.8 ± 4.39^{a}	155.4 ± 15.15^{a}	146.2 ± 3.86^{a}	149.8 ± 8.43^{a}	145.0 ± 7.11^{a}	153.4 ± 8.86^{a}
3	140.0 ± 6.06^{a}	188.8 ± 9.94^{c}	169.8 ± 6.81^{b}	139.8 ± 4.11^{a}	148.8 ± 6.90^{a}	145.0 ± 13.13^{a}	176.4 ±16.79^{bc}	171.6 ± 8.59^{bc}
7	162.8 ± 13.54^{a}	193.6 ± 12.94^{abc}	193.3 ± 7.51^{b}	178.2 ± 9.46^{ab}	178.4 ± 14.46^{ab}	191.2 ± 6.97^{b}	245.2 ± 15.24^{d}	213.0 ± 12.10^{c}
	Body weight gain (g)							
	4.60	38.60	47.50	22.80	32.20	41.40	100.20	59.60
	% Change in body weight within 7 days post inoculation							
	2.83	19.94	14.57	12.79	18.05	21.65	40.86	27.98
Ranking	8	4	6	7	5	3	1	2

Note: Means with different superscript alphabets along the same row are significantly different at $P < 0.05$.

Table 6. Mean bursa of Fabricius weights and bursal body weight ratio of ISA brown pullets inoculated with a very virulent infectious bursal disease virus at 21 days of age and treated with some unconventional remedies.

Days post-inoculation	Group (Treatment)							
	A (KS)	B (SEV)	C (ENP)	D (ASMS)	E (DLP)	F (OVX)	G (Negative control)	H (Positive control)
	Mean bursal weight (g)							
0	1.10 ± 0.05^{a}	1.08 ± 0.09^{a}	1.09 ± 0.15^{a}	1.11 ± 0.10^{a}	0.97 ± 0.11^{a}	1.04 ± 0.12^{a}	1.15 ± 0.08^{a}	1.26 ± 0.11^{a}
3	0.87 ± 0.11^{a}	1.22 ± 0.07^{b}	1.19 ± 0.14^{b}	1.18 ± 0.14^{b}	1.07 ± 0.10^{b}	1.13 ± 0.10^{b}	1.29 ± 0.17^{b}	1.20 ± 0.14^{b}
7	0.85 ± 0.12^{a}	0.96 ± 0.10^{a}	0.92 ± 0.02^{a}	0.85 ± 0.07^{a}	0.89 ± 0.08^{a}	0.79 ± 0.09^{a}	1.43 ± 0.15^{b}	0.78 ± 0.09^{a}
	% Changes in bursal weight within 7 days post-inoculation							
	-7.73	-11.11	-15.60	-23.42	-8.25	-24.04	+19.58	-38.10
Ranking	2	4	5	6	3	7	1	8
	Bursal body weight ratio within 7 days post-inoculation							
0	7.00 ± 0.39^{a}	6.95 ± 0.41^{a}	7.47 ± 0.91^{a}	7.26 ± 0.57^{a}	6.64 ± 0.73^{a}	6.93 ± 0.45^{a}	7.99 ± 0.43^{a}	8.20 ± 0.52^{a}
3	6.18 ± 0.57^{a}	6.57 ± 0.50^{a}	7.09 ± 0.87^{a}	8.57 ± 1.18^{a}	7.18 ± 0.50^{a}	7.96 ± 0.74^{a}	7.38 ± 0.65^{a}	7.00 ± 0.69^{a}
7	5.38 ± 0.85^{bc}	5.05 ± 0.67^{bc}	4.76 ± 0.09^{ab}	4.82 ± 0.41^{ab}	5.24 ± 0.78^{a}	4.11 ± 0.41^{ab}	5.87 ± 0.51^{c}	3.77 ± 0.60^{a}
	% Decrease in bursal body weight ratio within 7 days post-inoculation							
	-23.14	-27.34	-36.78	-33.61	-21.08	-40.69	-26.53	-54.02
Ranking	2	4	6	5	1	7	3	8

Table 7. Mean spleen weights and spleen body weight ratio of ISA brown pullets inoculated with a very virulent infectious bursal disease virus at 21 days of age and treated with some unconventional remedies.

Days post-inoculation	Group (Treatment)							
	A (KS)	B (SEV)	C (ENP)	D (ASMS)	E (DLP)	F (OVX)	G (Negative control)	H (Positive control)
	Mean spleen weight (g)							
0	0.44 ± 0.01^{a}	0.45 ± 0.02^{a}	0.44 ± 0.02^{a}	0.43 ± 0.02^{a}	0.43 ± 0.02^{a}	0.45 ± 0.02^{a}	0.40 ± 0.01^{a}	0.45 ± 0.03^{a}
3	0.65 ± 0.21^{a}	0.51 ± 0.02^{a}	0.57 ± 0.04^{a}	0.47 ± 0.05	0.52 ± 0.02^{a}	0.69 ± 0.19^{a}	0.54 ± 0.06^{a}	0.59 ± 0.07^{a}
7	0.61 ± 0.06^{b}	0.87 ± 0.12^{abc}	0.89 ± 0.09^{b}	0.67 ± 0.09^{a}	0.66 ± 0.09^{a}	1.06 ± 0.19^{c}	0.67 ± 0.04^{a}	0.73 ± 0.08^{a}
	% Increase In spleen weight within 7 days post-inoculation							
	27.87	48.28	50.56	35.82	34.55	57.55	40.30	38.36
Ranking	8	3	2	6	7	1	4	5
	Spleen body weight ratio within 7 days post-inoculation							
0	2.79 ± 0.09^{a}	2.92 ± 0.20^{a}	3.07 ± 0.19^{a}	2.87 ± 0.15^{a}	2.98 ± 0.18^{a}	3.08 ± 0.14^{a}	2.79 ± 0.11^{a}	2.95 ± 0.19^{a}
3	4.64 ± 1.48^{b}	2.76 ± 0.15^{a}	3.39 ± 0.26^{b}	3.41 ± 0.41^{ab}	3.55 ± 0.04^{b}	5.02 ± 1.65^{ab}	3.07 ± 0.17^{ab}	3.53 ± 0.47^{b}
7	3.79 ± 0.18^{ab}	4.50 ± 0.55^{b}	4.60 ± 0.40^{b}	3.82 ± 0.55^{ab}	3.66 ± 0.26^{a}	5.68 ± 1.13^{b}	2.81 ± 0.29^{a}	3.39 ± 0.27^{a}
	% Increase in spleen body weight ratio within 7 days post-inoculation							
	35.84	54.11	49.84	33.10	22.32	84.42	0.72	14.92
Ranking	4	2	3	5	6	1	8	7

Table 8. Mean thymus weights and thymus body weight ratio of ISA brown pullets inoculated with a very virulent infectious bursal disease virus at 21 days of age and treated with some unconventional remedies.

Days post-inoculation	Group (Treatment)							
	A (KS)	B (SEV)	C (ENP)	D (ASMS)	E (DLP)	F (OVX)	G (Negative control)	H (Positive control)
	Mean thymus weight (g)							
0	1.23 ± 0.08^{a}	1.46 ± 0.07^{b}	1.33 ± 0.14^{ab}	1.35 ± 0.22^{ab}	1.09 ± 0.09^{a}	1.41 ± 0.11^{a}	1.60 ± 0.05^{b}	1.39 ± 0.15^{ab}
3	0.73 ± 0.04^{a}	1.11 ± 0.13^{b}	1.56 ± 0.32^{c}	0.87 ± 0.12^{ab}	1.20 ± 0.30^{bc}	0.82 ± 0.12^{ab}	0.96 ± 0.10^{ab}	1.04 ± 0.24^{bc}
7	0.73 ± 0.09^{a}	1.01 ± 0.16^{ab}	1.49 ± 0.30^{b}	1.09 ± 0.18^{ab}	0.81 ± 0.23^{a}	0.98 ± 0.10^{a}	1.32 ± 0.21^{ab}	0.95 ± 0.12^{a}
	% Change in thymus weight within 7 days post-inoculation							
	-58.12	-30.82	+10.74	-19.26	-25.69	-30.40	-17.5	-31.66
Ranking	8	6	1	3	4	5	2	7
	Thymus body weight ratio within 7 days post-inoculation							
0	7.83 ± 0.69^{a}	9.42 ± 0.35^{b}	9.09 ± 0.78^{ab}	8.60 ± 0.74^{ab}	7.45 ± 0.53^{ab}	9.51 ± 0.88^{ab}	11.19 ± 0.72^{c}	9.26 ± 1.25^{abc}
3	5.26 ± 0.10^{a}	5.07 ± 1.43^{ab}	9.37 ± 2.15^{c}	6.31 ± 0.96^{abc}	8.04 ± 1.85^{bc}	5.77 ± 0.74^{ab}	5.70 ± 0.76^{ab}	6.05 ± 1.30^{abc}
7	4.47 ± 0.32^{a}	5.27 ± 0.85^{a}	7.80 ± 1.72^{a}	6.21 ± 1.15^{a}	4.40 ± 0.97^{a}	5.14 ± 0.45^{a}	5.39 ± 0.90^{a}	4.47 ± 0.45^{a}
	% Decrease In thymus body weight ratio within 7 days							
	-42.91	-44.06	-14.24	-27.79	-40.94	-45.95	-51.83	-51.73
Ranking	4	5	1	2	3	6	8	7

Note: Means with different superscript alphabets along the same row are significantly different at $P < 0.05$.

There was SD ($P < 0.05$) in spleen weights between group F and groups A, D, E, G, and H at 7 DPI. % increase in spleen weight at 7 DPI was highest in group F (Table 7). There were increases in mean spleen body weight ratios (SBRs) in groups A, C, D, E, F, G and H at 3 DPI. By day 7 PI there were significant ($P < 0.05$) increases in SBRs in groups B, C, F, D and E (Table 7).

There was SD ($P < 0.05$) in thymus weight between groups C and G, and A, B, D, E, F and H at 7 DPI. % change in thymus weight at 7 DPI was highest in group C (Table 8). There were decreases in mean thymus body weight ratios (TBRs) in groups A, B, D, F, G and H at 3 DPI and decreases in all groups at 7 DPI. The highest TBRs were recorded in groups C, D and G at 7 DPI (Table 8).

4. DISCUSSION

The results of ELISA antibody titre show that most or all of the chicks will be infected if exposed to IBDV between 14 and 21 days of age hence the need for initial IBD vaccination of these chicks before 14 days of age [19]. It also indicates that vaccination against IBD within the first week of life should be avoided to prevent vaccination failures due to possible neutralization of vaccine by maternally derived antibodies (MDA). Therefore, routine seromonitoring of commercial chicks to determine the most suitable age of vaccination is necessary. The group that received SEV produced an appreciable detectable amount of Ab at 28 days of age (7 DPI) and was the only group that had chicks (28.57%) with IBD protective antibody titre. The vitamins and minerals in SEV would have enhanced Ab response of the chicks to vvIBDV and reduced the damage to immune cells.

The severity of clinical signs and mortality rate were lowest in the groups treated with KS and SEV. Severity of clinical signs may be related to the extent of pathology caused by the vvIBDV [20]. The antioxidants and vitamins in SEV; antioxidants, anti-inflammatory and antiviral agents in KS might have reduced tissue damage in vital organs and thus the decreased severity of clinical signs observed in groups administered these two remedies.

The highest mortality rates in all groups were recorded on day 5 PI. Groups A (27.27%) and B (27.27%) had the lowest mortality rates while groups C and D had the highest mortality rates of 86.36% and 72.72%, respectively which were higher than the 59.09% in positive control group. The remedies administered to groups A and B might have mitigated morality rates while those given to C and D might have exacerbated the rates possibly resulting from the nature of constituents in the remedies administered to the groups.

Most of the remedies reduced the severity of gross lesions compared to positive control except those treated with OVX. This product contains oxidizing agents which are known to cause tissue damage [21] and their effects might have exacerbate the tissue damages induced by the vvIBDV [8, 22]. The percentage resolution of GLS was highest in the groups that received SEV (B) and DLP (E). SEV contains antioxidants and vitamins that might have reduced tissue damage and stimulated tissue repair while DLP contains antiviral substances which availability *in vivo* could have neutralized some of the vvIBDV inoculated.

Body weight gain was seen in all groups as the birds grew older but was lower in all treated groups and least in group administered KS. The presence of tannins which are bitter and also combine with digestive enzymes in KS (A) might have decreased protein digestion and consequent decrease in weight gain [23-25].

The initial increase in the weight of the BF in groups C (ENP), D (ASMS), E (DLP) and F (OVX) might be due to swelling and hypertrophy [26, 27]. In other groups however there was a decrease in the weight of the BF. The increase in bursal weight noticed in group G (negative control) was the result of normal growth of the BF while the severe decrease in group H (positive control) was because vvIBDV was inoculated and no treatment was administered [28]. All the remedies administered could not prevent reduction in the weight of the BF caused by the vvIBDV but the reduction was lowest in the groups that received SEV (antioxidant and immune modulator) and DLP (antiviral). The increase in BBR noticed on day 3 PI in some groups indicates enlargement of the BF while in others decrease in BBR indicating atrophy. It was reported that the BF initially enlarge before atrophying in IBD [29]. There was however, severe atrophy of the organ on day 7 PI.

The increases in the weights of the spleen noticed in all groups could be as a result of response to vvIBDV [30]. Following inoculation with vvIBDV there were increases in splenic and thymic weights particularly in the groups that received SEV and ENP, and ENP and ASMS, respectively. Thus ENP seemed to have a mitigative effect on the weights of the spleen and thymus. This may be the result of its mitigative effect on body weight loss due to vvIBDV infection. Except in group B, all groups had increases of SBR 3 DPI indicating that SEV contain substances with anti-inflammatory properties. Increases in TBR in groups C and E indicates enlargement of the thymus on day 3 PI but the increase in TBR on day 7 PI in group B could be an indication of healing. The changes caused by vvIBDV on bursal weight and BBR were less severe in groups that received KS, SEV and DLP; on spleen weight and SBR that received SEV, ENP and OVX, on thymus weight and TBR that received ENP, ASMS and DLP. Thus, KS, SEV and DLP mitigated the effect of vvIBDV on the BF, SEV, ENP and OVX spleen and ENP, ASMS and DLP thymus.

In conclusion, SEV and ASMS modulated Ab response to vvIBDV infection. SEV mitigated the severity of clinical signs, mortality rate, gross lesions, body and bursal weight, and SBR; KS clinical signs, mortality rate, bursal weight and BBR; ENP spleen and thymus weight and TBR; ASMS thymus weight and TBR; DLP gross lesions, bursal weight and BBR; OVX body and spleen weight and SBR due to vvIBDV infection. ENP and ASMS exacerbated mortality rates and KS severely reduced body weight gain PI with a vvIBDV. It was therefore recommended that veterinarians can administer SEV and KS at the onset of IBD outbreak and before vaccination against IBD to stimulate the production of high Ab titre and mitigate the severity of the disease. At the dose rate recommended for use, ENP and ASMS, should not be administered at the onset of IBD outbreak and their safety in pullets should be conducted.

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