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ORIGINAL RESEARCH

The effect of a combination of β(1-3) D-Glucan and *Propionibacterium granulosum* on productive performance and immune modulation of immunocompromised and non-immunocompromised broiler chickens

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Abstract

Aim: The effect of a specific combination of a soluble β (1-3) D-Glucan and *Propionibacterium granulosum* (Betamune®) was investigated on productive performance, immune response and immune dysfunction caused by cyclophosphamide (CP) in broiler chickens.

Materials and Methods: Three hundred and sixty one-day-old broiler chicks were randomly allocated into four groups for 5 weeks. Betamune® supplementation of 0.25 ml / L drinking water (presence or absence) for the first 7 days of age and CP (presence or absence) subcutaneous inoculation with 4 mg / chick for the first 3 days of life was done.

Results: Treatment of broiler chicks with Betamune® improved productive performance variables as compared with the blank control birds, where there were 10 points less in cumulative feed conversion ratio and significant increase ($P<0.05$) in final body weight, both intestinal length and diameter, and European production efficiency factor (EPEF). It also modulated the immune response, where there was non-significant improve in haemagglutination inhibition (HI) antibody titers against Newcastle disease (ND) virus vaccine and significant increase ($P<0.05$) in phagocytic % and phagocytic index. The lesion score after ND challenge reached only 70 in β (1-3) D-Glucan group as compared with 80 in blank control group. The histomorphological examination of Betamune® treated chickens at 5 weeks of age revealed lymphoid hyperplasia in bursal follicles, lymphoid cells of cortical portion of thymus glands and lymphoid cells in the white pulps of spleen. CP did affect bird's weight and suppressed immune system. Treatment CP suppressed birds with Betamune® significantly increased ($P<0.05$) final body weight, dressing weight %, giblets weight %, intestinal diameter, improved FCR (28 points less than untreated group), decreased cumulative mortality and improved EPEF. Betamune® counter attacked immune dysfunction caused by CP, where there was significant increase in HI antibody titer against ND vaccine, no significant increase in phagocytic % and phagocytic index and improve in the lesion score after ND challenge (99 as compared to 133). Betamune® supplementation reduced microscopic lesion scores associated with CP immune dysfunction.

Conclusion: It could be concluded that administration of a specific combination of soluble β1.3, D-Glucan and *Propionibacterium granulosum* (Betamune®) to broiler chickens improved chicken zootechnical performance response variables, had a potent immunomodulatory effect (potentiated immune response), evoked their immune response and enhanced their vaccination effectiveness.

Keywords: chickens, cyclophosphamide, *Propionibacterium granulosum*, β (1-3) D-Glucan

Introduction

Many diseases can be modified by direct administration of biological compounds that activate key pathways in the immune system. The term immunosuppression is defined as a state of temporary or permanent dysfunction of the immune response, which insults the immune system leading to increased susceptibility to diseases [1]. It is undoubtedly true that factors contributing to immune-suppression would lead to immune-deficiency.

The latter is a hazard-anticipating causative agent of serious economic impacts in poultry industry all over the world. As a result of the use of antibiotics many pathogenic bacteria have developed resistant strains. This has led to extensive consideration for limiting the use of antibiotics in livestock breeding. Given the demands of both consumers and the policy makers, the development of alternative methods to deal with the problems caused by bacteria in commercial animal production is a high priority for both researchers and farmers. A number of potential immuno–modulators may serve as antibiotic-alternatives for both the promotion of growth and disease resistance in animal production. Huff *et al.* [2] have suggested that yeast β-1, 3/1, 6-glucan may be useful as an alternative to the antibiotics due to its immuno-modulating function.

Immunodeficiency has led to increasing need for the use of immunopotentiators (immunostimulants), which are extrinsic or intrinsic substances that regulate or alter the scope, type, duration or competence of the immune response. Immunopotentiators strengthen the defense and immune mechanisms of the body and currently usable for stimulating the non-specific

immune responsiveness in both the human and veterinary medical practice [3]. Potentiating of normal immune response in poultry occurs by alteration in any step involved in the host's immunologic reaction either in the classic humoral or in the cell-mediated systems. Eventually, it has already known that many diseases / disorders, that have immunomodulated components, can be modified by administration of biological compounds that activate key pathways in the immune system [4].

Beta-glucan (β-glucan) have been examined in several studies as antibiotic alternative, and have been found to up regulate immune response and stimulate growth in swine and poultry [2,5–7]. The immuno –modulating effects of β –glucan might result in an increase in the functional activity of macrophage and heterophil cells [8–10].

This investigation was dedicated in an attempt to investigate the possible effect of a specific combination of a soluble β1.3, D-Glucan and *Propionibacterium granulosum* produced by Kanzy medipharm, Canada, known under the trade name Betamune® on zootechnical performance, chicken immune response and immune dysfunction in broiler chickens.

Materials and Methods

The present study was carried out at the Poultry Research Center, Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt.

Experimental Birds: The experiment was carried out according to the National regulations on animal welfare and Institutional Animal Ethical Committee (IAEC). Three hundred and sixty, one-day old commercial Arbor Acres plus broiler chicks were used in this study. Chicks were housed in semi closed house. The chicks were provided with 24 hours light throughout the first three days, then 23 hours light and 1 hour dark until slaughter time. Chicks were fed a commercial starter diet (23% crude protein and 3000 k cal ME/kg diet) during the first two weeks of age, commercial grower diet (22% crude protein and 3150 kcal ME/kg diet) from 2-4 weeks of age, and then commercial finisher diet (19% crude protein and 3200 kcal ME/kg diet). The diets compositions are indicated in Table-1. Semduramicin was added at a concentration of 25 ppm as a coccidiostat, during the experimental period. Feed and water were available *ad libitum*. All chicks were vaccinated against Newcastle disease vaccine (ND) at 7 and 21 day of age by using live Hitchner B1 and La Sota strain vaccines, respectively. Live infectious bursal disease vaccine (IBD) was administrated at 14 days of age. Drinking water method was used as rout of administration of live vaccines. On the day 10 of age, 0.5 ml inactivated Avian Influenza vaccine (AIV), (H5N1), was injected subcutaneously in the back of neck.

Experimental design: One day-old Arbor Acres plus broiler chicks (n=360) were allotted into 4 equal groups (1-4) of 90 birds each. Each group was divided into 3 subgroups of 30 each. Those of groups 1 and 2 were immunosuppressed [11] by subcutaneous inoculation of cyclophosphamide (CP) in a dose of 4 mg / chick for the first 3 days of life. Birds of groups 1 and 3 received Betamune® in a dose of 0.25 ml/liter drinking water for the first 7 days of age and repeated from 22 to 28 days of age. Each liter of Betamune® contained β1.3, D-Glucan (3%) and *Propionibacterium granulosum* (0.17 gram). Chickens of group 2 were kept as positive immunosuppressed controls while those of group 4 were kept as blank control.

Measured parameters:

Productive performance: Chicken performance response variables were determined [12]. For body weight; all birds were weighed individually at 1st day and weekly for the entire period of the experiment (5 weeks). Feed consumption was calculated, for each subgroup, weekly; investigate the feed conversion ratio (FCR) (g feed / g live body weight). Feed conversion ratio (FCR) was calculated, for each subgroup, as follows: Feed intake for the subgroup (kg) divided by the total body weight gained for the same subgroup (kg). The total body weights included dead birds during the week. Daily mortalities were recorded for each subgroup. The European production efficiency factor (EPEF) was estimated at the end of the experimental period, for each subgroup. The European production efficiency factor was calculated, for each subgroup, as follows:
(Average live body weight (Kg) * Livability (%))*100 / (Marketing age(day)*FCR) The carcass characteristics (dressing without Giblets (%), fleshing (%), giblets Weight (%), and intestine's length and diameter were measured on 10 birds of each subgroup, at 5 weeks of age.

Immune status assessment: To investigate the possible effect of Betamune® on the humoral immunity; an immunoassay was adopted. For this purpose, blood samples were collected from wing vein from 10 randomly selected birds at weekly intervals (1-5 weeks of age) from each group. The serum samples were subjected to haemagglutination inhibition (HI) test for determining antibody titers against ND vaccine employing 8 haemagglutinating (HA) units [13]. To investigate the possible effect of Betamune® on the cell mediated immunity; measurement of phagocytic activity of peripheral blood monocytes using *Candida albicans* was adopted [14]. Ten chickens from each group were challenged with a velogenic viscerotropic strain of ND (vVND) virus on the 35th day of age and kept under close observation for clinical signs and mortality for further 2 weeks. Dead as well as sacrificed birds at the end of observation period (49 days) were subjected to post mortem examination for lesion scoring of ND.

Histopathological assay: Specimens including bursa of Fabricius, thymus gland and spleen were collected from randomly selected 5 sacrificed chickens / group at 14 and 28 days of age and fixed in 15% buffered formalin. Paraffin-embedded sections were routinely

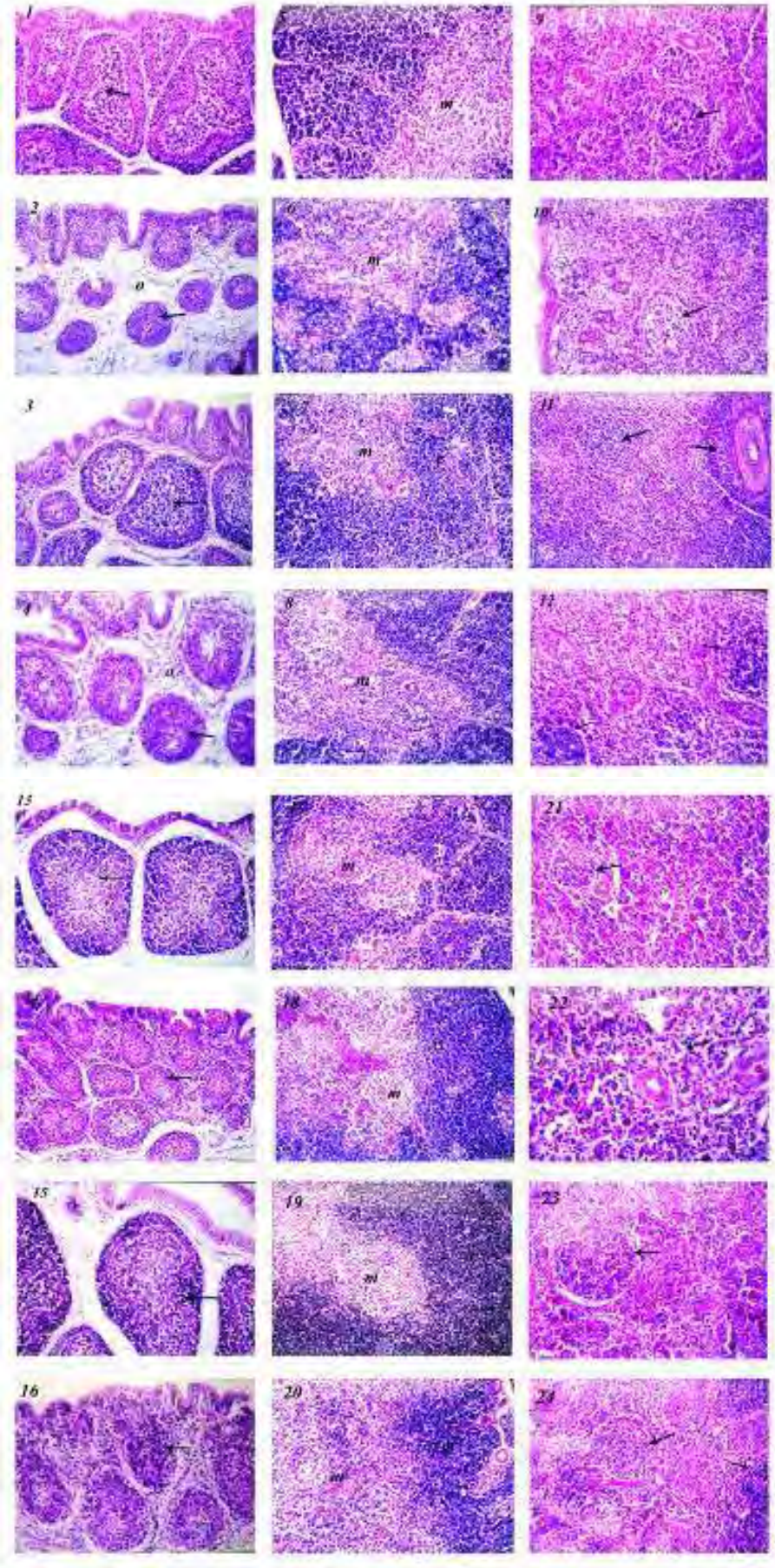

Plate A: Histopathological figures of Bursa of Fabricius, Thymus glands and Spleen of immunocompromised with cyclophosphamide (CP) and non-immunocompromised broiler chickens treated or untreated with Betamune® at 3 weeks of age.

Fig.1: Bursa of Fabricious of control showing the normal histopathological structure of lymphoid follicles in the mucosal layer (H&E X64). Fig.2: Bursa of fabricious of CP group showing lymphoid depletion (arrow) with edema in between (o) in lamina propria (H&E X40). Fig.3: Bursa of Fabricious of Betamune® group showing normal histological structure of the lymphoid follicles (arrow) (H&E X64). Fig.4: Bursa of Fabricious of CP and Betamune® group showing mild lymphoid depletion in the lymphoid follicles (H&E X64). Fig.5: Thymus glands of control group showing normal histopathological structure of cortical (c) and medullary (m) portions (H&E X40). Fig.6: Thymus glands of CP group showing lymphoid depletion in both cortical (c) and medullary (m) portions (H&E X40). Fig.7: Thymus glands of Betamune® treated group showing intact histological structure in both cortical (c) and medullary (m) portions (H&E X40). Fig.8: Thymus glands of CP and Betamune® treated group showing intact histological structure in both cortical (c) and medullary (m) portions (H&E X40). Fig.9: Spleen of control group showing intact histopathological structure of the white (arrow) and red pulps (H&E X40). Fig.10: Spleen of CP group showing lymphoid depletion in white pulps (arrow) (H&E X40). Fig.11: Spleen of Betamune® treated group showing intact histological structure in white and red pulps (arrow) (H&E X40). Fig.12: Spleen of CP and Betamune® treated group showing intact histological structure (H&E X64).

Plate B: Histopathological figures of Bursa of Fabricius, Thymus glands and Spleen of immunocompromised with cyclophosphamide (CP) and non-immunocompromised broiler chickens treated or untreated with Betamune® at 5 weeks of age.

Fig.13: Bursa of Fabricious of control showing normal histological structure (H&E X64). Fig.14: Bursa of Fabricious of CP group showing lymphoid depletion in central portion of the follicles (arrow) (H&E X40). Fig.15: Bursa of Fabricious of Betamune® treated group showing normal histological structure of the lymphoid follicles (arrow) (H&E X64). Fig.16: Bursa of Fabricious of CP and Betamune® treated group showing mild depletion in the lymphoid follicles (H&E X64). Fig.17: Thymus glands of control group showing normal histopathological structure of cortical (c) and medullary (m) portions (H&E X40). Fig.18: Thymus glands of CP group showing lymphoid depletion and hemorrhage in medullary (m) portion (H&E X40). Fig.19: Thymus glands of Betamune® treated group showing lymphoid hyperplasia in the cortical portion (c) (H&E X40). Fig.20: Thymus glands of CP and Betamune® treated group showing mild depletion in both cortical (c) and medllry (m) portions (H&E X40). Fig.21: Spleen of control group showing normal histopathological structure of the lymphoid cells in the white (arrow) (H&E X40). Fig.22: Spleen of CP group showing mild depletion in the white pulps with congestion of red one (arrow) (H&E X64). Fig.23: Spleen of Betamune® treated group showing intact histological structure and hyperplasia of the lymphoid pulps (arrow) with congestion in red pulps (H&E X40). Fig.24: Spleen of CP and Betamune® treated group showing normal intact histological structure H&E X40.

prepared from these fixed organs and stained with Hematoxylin and Eosin [15], and scored for histo-pathological lesions [16].

Statistical analyses: One-way analysis of variance was used using SAS software general liner models procedure [17]. The main factor was immunomodulator

Table-1. Composition of the broilers 3-phase diets (g/kg as fed) and their calculated chemical composition (on as fed basis).

Ingredients	Starter	Grower	Finisher
Yellow corn	524.5	544.2	628.5
Soybean meal 44%	332.4	299.1	221.1
Corn gluten meal 60%	70	70	66.5
Oil	30	43.8	40
Di-calcium phosphate	18	18	18
Lime stone	13	13	13
D.L. Methionine	2.2	2.1	2.3
Lysine hydrochloride	2.9	2.8	3.6
Sodium chloride	4	4	4
Premix*	3	3	3
Calculated analysis:			
Crude protein %	23.0	22.0	19.0
Metabolizable energy (kcal/kg)	3000	3150	3200

*Each gram of mineral mixture contained: vitamin A (trans-retinyl acetate), 9,000 IU; vitamin D3 (cholecalciferol), 2,600 IU; vitamin E (dl-α-tocopheryl acetate), 16 mg; vitamin B1, 1.6 mg; vitamin B2, 6.5 mg; vitamin B6, 2.2 mg; vitamin B12 (cyanocobalamin), 0.015 mg; vitamin K3, 2.5mg; choline (choline chloride), 300 mg; nicotinic acid, 30 mg; pantothenic acid (d-calcium pantothenate), 10 mg; folic acid, 0.6 mg; d-biotin, 0.07 mg; manganese (MnO), 70 mg; zinc (ZnO), 60 mg; iron ($FeSO_4 H_2O$), 40 mg; copper ($CuSO_4 5H_2O$), 7 mg; iodine [$Ca(IO_3)_2$], 0.7 mg; selenium (Na_2SeO_3), 0.3 mg.

Table-2. Effect of Betamune® on body weight of immunocompromised (by cyclophosphamide "CP") and non- immunocompromised broiler chickens

Treatment /Age	0Week	1Week	2Weeks	3Weeks	4Weeks	5Weeks
Control	44.47± 0.3	108.93±1.4^{a*}	301.49±3.7^{a}	635.95±7.1^{a}	1045.45±11.4^{b}	1611.85±20.3^{b}
CP	44.50±0.3	71.90±1.1^{b}	168.19± 5.7^{b}	365.11 ±16.6^{c}	634.12±35.7^{d}	996.15± 62.4^{d}
Betamune®	44.47±0.3	110.32± 1.3^{a}	305.53± 3.7^{a}	652.26±7.1^{a}	1116.46±10.7^{a}	1709.86±14.7^{a}
CP+ Betamune®	45.03±0.3	73.70± 1.2^{b}	179.72± 5.3^{b}	399.51±12.4^{b}	728.23±25.8^{c}	1134.73±38.8^{c}

* Means with different superscripts, within age, are significantly different ($P \leq 0.05$).

Table-3. Effect of Betamune® on Feed Conversion (g feed/g body weight) of immunocompromised (by cyclophosphamide "CP") and non-immunocompromised broiler chickens.

Treatment /Age	1Week	2Weeks	3Weeks	4Weeks	5Weeks	o to 5 week
Control	1.25±0.03	1.70± 0.13^{b*}	1.95± 0.03^{b}	2.10± 0.08	2.10± 0.13	1.95± 0.03
CP	1.40± 0.10	3.25± 0.46^{a}	2.88± 0.51^{a}	2.55± 0.49	2.68± 0.50	2.53± 0.42
Betamune®	1.23± 0.03	1.65± 0.03^{b}	1.93± 0.06^{b}	1.90± 0.04	1.98± 0.03	1.85± 0.03
CP+ Betamune®	1.30± 0.04	3.03± 0.40^{a}	2.53± 0.24^{ab}	2.25± 0.25	2.56± 1.47	2.25± 0.27

* Means with different superscripts, within age, are significantly different ($P \leq 0.05$).

Table-4. Effect of Betamune® on mortality rate (%) of immunocompromised, (by cyclophosphamide "CP") and non-immunocompromised broiler chickens.

Treatment /Age	1Week	2Weeks	3Weeks	4Weeks	5Weeks	Mortality rate
Control	4.38± 1.88^{b*}	3.13±1.57^{b}	0.00±0.00	0.63±0.63^{b}	0.00±0.00^{b}	8.13± 2.58^{c}
CP	23.13± 3.13^{a}	46.25±6.88^{a}	1.88±1.20	5.00±1.44^{a}	3.13±1.20^{a}	79.38± 4.38^{a}
Betamune®	0.63± 0.63^{b}	2.50±1.02^{b}	0.00±0.00	0.00±0.00^{b}	0.00±0.00^{b}	3.13± 1.20^{c}
CP+ Betamune®	19.38± 1.57^{a}	33.13±7.32^{a}	0.00±0.00	2.50±1.02^{ab}	3.13±1.20^{a}	58.13± 9.09^{b}

* Means with different superscripts, within age, are significantly different ($P \leq 0.05$).

(Betamune® or CP) supplementation. Mean values were assessed for significance using Duncan's multiple range test [18] with significance set at $P<0.05$.

Results

Productive Performance: The results presents in Table 2 showed a significant increase ($P<0.05$) in final body weight (at 5 weeks of age) in Betamune® treated group over blank control group. Also, similar result was obtained in cyclophosphamide (CP) + Betamune® treated group over CP suppressed group. There were 10 points less in cumulative FCR in Betamune® treated broiler chickens as compared with the blank control birds (Table 3). On the other hand there was 28 points increase in CP suppressed birds over those CP + Betamune® treated group. The significant decrease in total mortality rate was observed in CP+ Betamune® treated group as compared to CP suppressed group (Table 4). The results clearly showed that EPEF of Betamune® treated group was significantly the highest and following in descending order with control, CP+ Betamune® and then CP suppressed group (Table 5). Finally, Table-6 clearly shows that there were significant increases in dressing %, giblets weight % and intestinal diameter in CP+ Betamune® treated group over CP suppressed one. On the other hand, significant increase in intestinal's length and diameter were recorded in Betamune® treated group over the control one. The fleshing % was significantly higher in both control and Betamune® groups as compared to CP and CP + Betamune® treated groups.

Immune status assessment: The results of humoral immune response are presented in Table (7) which

Table-5. Effect of Betamune® on European production efficiency factor (EPEF) of immunocompromised (by cyclophosphamide "CP"), and non-immunocompromised broiler chickens

Treatment	EPEF
Control	217.20± 6.71[b*]
CP	29.28 ± 12.02[d]
BETAMUNE®	256.13± 6.33[a]
CP+ BETAMUNE®	66.68± 18.37[c]

* Means with different superscripts, within age, are significantly different ($P \leq 0.05$).

Table-6. Effect of BETAMUNE® on carcass characteristics of immunocompromised, (by cyclophosphamide "CP") and non-immunocompromised broiler chickens.

Treatment/Trait	Dressing (%)	Fleshing (%)	Giblets Weight (%)	Intestine Length (cm)	Intestine Diameter(cm)
Control	68.21±1.25 [a*]	30.74±0.56 [a]	5.16± 0.30 [c]	178.40±3.31 [b]	0.990±0.05 [bc]
CP	63.99±1.01[b]	28.48±0.44 [b]	6.93±0.24 [a]	176.80±2.62 [b]	0.830±0.05 [c]
Betamune®	70.43±1.23 [a]	30.89±0.39 [a]	5.43±0.19 [bc]	208.60±6.44 [a]	1.31± 0.08 [a]
CP+ Betamune®	67.25±1.00 [a]	28.81±0.32 [b]	5.99±0.26 [b]	164.40±5.73 [b]	1.18±0.11 [ab]

* Means with different superscripts, within age, are significantly different ($P \leq 0.05$).

Table-7. Effect of Betamune® on haemagglutination inhibition (HI) antibody titer against Newcastle disease vaccine of immunocompromised (by cyclophosphamide "CP") and non-immunocompromised broiler chickens.

Treatment /Age	1Week	2Weeks	3Weeks	4Weeks	5Weeks
Control	5.6±0.40[a*]	3.8±0.20[a]	7.0±0.26[a]	6.2±0.29[ab]	7.1±0.28[a]
CP	2.5±0.22[c]	2.4±0.27[b]	5±0.30[b]	4.8±0.29[c]	5.3±0.26[c]
Betamune®	5.2±0.36[a]	3.8±0.33[a]	7.3±0.37[a]	6.6±0.31[a]	7.4±0.22[a]
CP+ Betamune®	3.7±0.21[b]	3.5±0.27[a]	6.6±0.31[a]	5.7±0.26[b]	6.2±0.33[b]

* Means with different superscripts, within age, are significantly different ($P \leq 0.05$).

Table-8. Effect of Betamune® on Phagocytic percentage and Phagocytic index of immunocompromised (by cyclophosphamide "CP") and non-immunocompromised broiler chickens

Treatment/Trait and age	Phagocytic % (3 Weeks)	Phagocytic % (5 Weeks)	Phagocytic index (3 Weeks)	Phagocytic index (5 Weeks)
Control	50.00±1.96[b*]	53.75±4.97[b]	0.5025±0.10[b]	0.5175±0.13[b]
CP	38.50±3.77[b]	43.50±4.35[b]	0.1800±0.01[c]	0.2250±0.03[c]
Betamune®	69.25±1.49[a]	71.25± 1.49[a]	0.6850±0.02[a]	0.8425±0.04[a]
CP+ Betamune®	50.50±6.54[b]	48.25±3.57[b]	0.3100±0.04[c]	0.4250±0.04b[c]

* Means with different superscripts, within age, are significantly different ($P \leq 0.05$).

indicated a significant increase in CP+ Betamune® treated group over CP suppressed group in the HI titers against ND vaccine at different examined intervals. However, non-significant improve in HI titers were also recorded in Betamune® treated group as compared to blank control one. Cell mediated immunity parameters (phagocytic % and phagocytic index) are reported in Table (8). Treatment with Betamune® showed significant increase ($P<0.05$) in phagocytic % and phagocytic index at 3 and 5 weeks of age as compared to blank control group. Chickens suppressed with CP revealed significant decrease in phagocytic % at 3 and 5 weeks as compared to Betamune® treated group. The same group also revealed significant decrease in phagocytic index at 3 and 5 weeks as compared to both Betamune® and control group. Chickens suppressed with CP and treated with Betamune® showed non-significant increase in phagocytic % and phagocytic index when compared to CP suppressed group at both ages. Results of bioassay (Table 9) showed that the lesion score reached 133 in CP suppressed group as compared to 99 in CP suppressed + Betamune® treated group. While the score reached only 70 in Betamune® group as compared with 80 in control one.

Histopathological assay:

Bursa of Fabricious: In control group, 1st and 2nd samples (at 3 and 5 weeks of age, respectively) showed no histopathological findings and normal histopathological structure of lymphoid follicles in mucosal layer was recorded (Figs.1 and 13 respectively). In CP group lymphoid depletion was observed in the follicles with edema in between at 1st sample (Fig.2). On the 2nd sample, moderate lymphoid depletion was noticed in the central portion of the follicles (Fig.14). In Betamune® group, the 1st sample (3 week-old) revealed no histopathological alteration (Fig.3). However, the second sample showed lymphoid hyperplasia in the follicles (Fig.15). In CP + Betamune® group, the 1st sample showed mild lymphoid depletion in the follicles with edema in between (Fig.4), but only mild lymphoid depletion in the follicles was revealed in the 2nd sample (Fig.16).

Thymus glands: In control group, no histopathological findings were observed and the normal histopathological structure of cortical and medullary portions were recorded, in the 1st and 2nd samples (Figs.5 and 17). In CP group lymphoid depletion was observed in cortical and medullary portion at 1st sample (Fig.6). While, the medullary portion showed focal hemorrhage and lymphoid depletion at 2nd sample (Fig.18). In Betamune® treated group, there was no histopathological alteration at 1st sample (Fig.7). On 2nd sample

Table-9. Effect of Betamune® on organ lesion scores and mortality after Newcastle Disease (vVND) challenge of immuno-compromised (by cyclophosphamide "CP"), and non-immunocompromised broiler chickens

Trait	Treatment			
	Control	CP	Betamune®	CP+ Betamune®
Muscle hemorrhage	0	25	0	17
Proventriculitis	1	10	2	13
Nephritis	4	19	5	18
Rectum hemorrhage	7	12	6	10
Caecal tonsils hemorrhage	20	4	13	11
Payers patches hemorrhage	18	11	16	4
Enteritis	18	19	16	7
Tracheitis	5	19	2	12
Pneumonia	7	14	10	7
Sum lesion score	80	133	70	99
Mortality %	0	100	0	100

Table-10. Effect of Betamune® on histopathological lesion scores of major immune organs of immunocompromised (by cyclophosphamide "CP") and non-immunocompromised broiler chickens.

Examined immune organ	Treatment and age							
	Control		CP		Betamune®		CP+ Betamune®	
	3weeks	5 weeks	3 weeks	5 weeks	3 weeks	5 weeks	3 weeks	5 weeks
Bursa of Fabricious	0*	0	4+	2+	0	0	2+	1+
Thymus glands	0	0	3+	2+	0	0	0	1+
Spleen	0	0	2+	1+	0	0	0	0
Sum of lesion score	0	0	9+	5+	0	0	2+	2+

* Lymphoid depletion: Very severe = 4+, Severe = 3+, Moderate = 2+, Mild = 1+, Nil = 0.

hyperplasia was noticed in the lymphoid cells of the cortical portion (Fig.19). In CP + Betamune® treated group there was no histopathological alteration at 1st sample (Fig.8). While, on 2nd sample there was lymphoid hyperplasia in cortical portion (Fig.20).

Spleen: In control group, there was no histopatho-logical findings observed and the normal histological structure of cortical and medullary portion was recorded in samples taken at the two ages (Fig. 9 and 21). In CP group, lymphoid depletion was recorded in the follicles of the white pulps in 1st sample (Fig.10). Second sample showed mild depletion in white pulps while the red ones were congested (Fig. 22). In Betamune® group, 1st sample showed intact histological structure in white and red pulps (Fig. 11). Second sample showed hyperplasia in the lymphoid cells in the white pulps associated with congestion in the red ones at 2nd sample (Fig. 23). In CP + Betamune® treated group, no histopathological alteration in both samples were recorded (Fig.12 and 24). Histopatho-logical lesion scores of major immune organs of immunoco-mpromised and non-immuno-compromised broiler chicken groups treated and untreated with Betamune® are illustrated in Table (10). The lesion score reached 9 and 5 in CP suppressed group at 3rd and 5th week of age vis 2 and 2 in CP Betamune® treated broiler chickens respectively. No histopathological lesion score were detected in Betamune® or in blank control groups.

Discussion

Comparing treated chickens by a combination of β1.3, D-Glucan and *Propionibacterium granulosum* (Betamune®) with their untreated blank controls revealed a significant improve in broiler productive performance, intestinal length and diameter, HI antibody titer against ND vaccine, phagocytic %, and phagocytic index. The lesion score after ND challenge reached 70 as compared with 80 in blank controls. The present results recorded also ten points in cumulative FCR were less than untreated controls (1.85 vis. 1.95). Neither histopathological lesion score could be detected in treated nor in blank control groups. However, lymphoid hyperplasia in bursa of Fabricius follicles was noticed in Betamune® treated group. Many researchers agree with our results and established the improvement in broiler performance when β-1, 3/1, 6-glucan and *Propionibacterium granulosum* were supplemented in broiler diets [3, 19–23]. Zhang *et al.* [3] interpreted this effect as β-1,3/1,6-glucan may play a role in the initiation of immune modulation; therefore, suitable supplementation would be beneficial to the performance of broiler chickens. In addition, supplementation increases performance by improving the average daily body weight gain and reducing the feed/weight gain ratio. While another researches showed that supplementation of β-1, 3/1, 6-glucan have a physiological effects on intestinal digestive mucosa and increase the villus height of jejunal mucosa of chickens [24–26].

Immunomodulators administered simultaneously with antigens might potentiate specific immune response, particularly to vaccines. β-Glucan can be efficacious as an oral adjuvant to enhance immuno-globulin production in response to vaccination **[**27**]**. Awaad *et al*. [28] proved by an immunoassay and a bioassay the immunomedulatory effect of vitamin E and a compound containing inactivated *Propiobacterium granulosum* (IM-104® produced by Calier laboratories les Franquses del valles, Barcelona, Espana). The protective effect of β-glucans might be due to the antioxidant capacity, antimicrobial activities as well as

the inhibition of early activation of tissue muscular nuclear factor-KB (NF.KB) and NF-IL6 [29].

Many literatures showed that the immuno-modulating effects of β–glucan might result in an increase in the functional activity of macrophage and heterophil cells [8–10], increased production of cytokines (such as IL-1, TNF-α and IL-6) and immuno-globulins [3].

In vitro, broiler macrophages from a cell line or isolated from normal chickens when exposed to various concentration of β-glucan get activated and produce heightened levels of nitrite (a product of nitric oxide syntheses gene activity) and cytokines such as IL-1. Furthermore, β-Glucan exposure also induced macrophage proliferation [30]. Moreover, *in vivo* feeding trials, the supplemented chickens with β-glucan had improved macrophage phagocytic functions, more persistent T-lymphoproliferative response as measured by PHA-P«««mediated swelling in the toe web, much improved antibody response after boost, and increased incidence of CD4 (T-helper) and CD8 (T-cytotoxic) positive lymphocyte subsets in the intestinal leukocyte population. Furthermore, dietary inclusion of β-glucan significantly improved the growth of both primary and secondary lymphoid organ of chickens.

The bacterial-killing and phagocytosis-stimulating effect of purified yeast β-1, 3/1, 6-glucan in four-day-old male Leghorn chickens were reported [29]. Significant increase in phagocytosis of peripheral blood cells and antibody production induced by oral administration of β-glucans in balb / C mice were also recorded [31]. Macrophages are part of the non-specific first line of defense because of their ability to engulf and degrade invading microorganisms. Macrophages perform a variety of functions other than phagocytosis; they act as secretor cells, produce Nitric oxide that kill intracellular microorganisms, secrete many different proteins such as lysosomal enzymes and cytokines that play a key role in regulating immunity [32].

In the present study; the immune compromising effect of cyclophosphamide (CP) on both cell mediated and humoral immunity has been proven with marked negative alterations in histomorphologic features of major lymphoid organs. CP is an immunosuppressive drug, which is commonly used in immunological experiments [33–35]. The depressor effect of CP on the humoral immune response is already established [36]. On the other hand; CP is primarily a B-cell suppressor, however; it also produces transient T-cell deficiency [37]. The marked alteration in the histomorphologic features of major lymphoid organs (bursa of Fabricius, thymus gland and spleen) after CP administration in the present study are completely accord with that reported by other researches [11,33,35]. Comparing treated CP depressed chickens by Betamune® with their untreated controls revealed significant increase in; HI antibody titer against ND vaccination, final body weight, dressing weight %, front part weight %, giblets weight %, intestinal diameter, feed consumption (at 4th week of age) and EPEF. A significant decrease in total mortality rate and gizzard weight was also obtained. While there was a 28 points decrease in FCR. On the other hand; non significant increase in phagocytic %, phagocytic index and weight gain has been reported. For overall judgment on immunomodulation of the studied preparation in immunosuppressed birds; a bioassay was carried out. Challenge with vVND virus resulted in lowered lesion score in Betamune® treated as compared with untreated group (99 vis. 133). This means that administration of Betamune® could partially counter attacks immunosuppression and decreases lesions attributed to ND challenge as well. The histomorphological changes of major immune organs due to CP suppression (expressed as lesion scores) reached 9 and 5 in CP depressed group, 2 and 2 in their Betamune® treated control group at 3rd and 5th week of age respectively

Conclusion

From the aforementioned results; it could be concluded that administration of a specific combination of soluble β1.3, D-Glucan and *Propionibacterium granulosum* (Betamune®) to broiler chickens improved chicken zootechnical performance response variables, had a potent immunomodulatory effect (potentiated immune response), evoked their immune response and enhanced their vaccination effectiveness. The present study proved that Betamune® played a positive role not only in non-immunocompromised broiler chickens but also in immunocompromised birds by counter attacking their immune dysfunction caused by CP.

Author's contribution

All authors contributed equally. All authors read and approved the final manuscript.

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Competing interest

Authors declare that they have no competing interest.

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