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Efficacy evaluation of a supplemental polyherbal immunopotentiator liquid on broiler chicken health

Bhagwat Vishwanath Gopal[1+]
Varun Kumar Krishnappa[2]

[1,2]*Himalaya Wellness Company, Makali, Bengaluru 562 162, Karnataka, India.*
[1]*Email: dr.bhagwat@himalayawellness.com*
[2]*Email: dr.varunkumar.k@himalayawellness.com*

(+ Corresponding author)

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ABSTRACT

A chicken's immune system is crucial for its health and disease resistance, as it directly impacts production performance. Immunomodulators are substances such as vitamins, adjuvants, polysaccharides, probiotics, prebiotics, and botanicals that help regulate the immune system and enhance disease resistance. A study was conducted with Cobb 430Y broilers to evaluate the effect of a liquid multi-herb preparation "Immunopotentiator liquid (IPL)" on immunity, performance, and carcass characteristics. The study involved 126 chickens, randomly divided into six groups of 21 birds: G1, G2, G3, G4, G5 and G6. The G3 and G5 groups were supplemented with Geriforte Vet Liquid (GVL), while the G4 and G6 groups were supplemented with IPL. GVL and IPL supplemented by mixing with daily drinking water at 5 ml/100 birds at 1–2 weeks; 10 ml/100 birds at 2–4 weeks; and 20 ml/100 birds from four weeks onward till the chicks reached the marketable age, i.e., 38 days. Additionally, groups (G2, G5, and G6 groups) were vaccinated against New Castle Disease and Infectious Bursal Disease. The results showed that IPL supplementation improved growth performance parameters like body weight and feed efficiency without affecting carcass characteristics or serum biochemical parameters. Further, the immunity parameters, including ND and IBD titers, were elevated in chicks that received IPL supplementation along with vaccinations. In conclusion, the study showed that supplementing IPL with routine vaccinations for 21 days had a positive effect on the immunity, growth performance, and general health of Cobb 430Y broilers.

Contribution/Originality: This paper provides data on Immunopotentiator liquid (IPL) on immunity, performance, and carcass characteristics of Cobb 430Y broilers. Despite the previous studies on different immunopotentiator liquid effects on broilers, this prepare describes the effect of unique combination of herbal extracts.

1. INTRODUCTION

Poultry meat has emerged as a major source of protein owing to its benefits like superior nutritional value and rapid production [1, 2]. However, maintaining poultry health is crucial. One method of achieving this is to support their immune system, which is the body's natural defense mechanism that neutralizes microorganisms [3]. Stress and other factors can compromise the immune system, making birds more susceptible to diseases [4]. Researchers are examining natural methods to bolster the immune system in poultry, and this study investigated an immunomodulator supplement derived from botanicals.

Immunomodulators are substances that support the immune system. In poultry production, researchers are interested in immunomodulators that can help strengthen the birds' natural defenses. This could potentially reduce reliance on specific medications and enhance the welfare of animals in general [5-8].

Researchers are studying the impact of this botanical-based immunomodulator on the immune system, performance, and other characteristics of the birds. This research can help develop strategies to enhance poultry health and potentially reduce the need for certain medications. This could be beneficial for both the birds and consumers because it contributes to overall animal welfare in poultry production.

2. MATERIAL AND METHODS

2.1. Immunopotentiator Liquid (IPL)

"Enrimune liquid," a polyherbal liquid formulation, is an "Immunopotentiator Liquid" developed by M/s. Himalaya Wellness Company, Bengaluru, India, composed of botanicals, including *Emblica officinalis*, *Ocimum sanctum*, *Glycyrrhiza glabra*, *Tinospora cordifolia*, and *Withania somnifera*. Immunopotentiator liquid strengthens the chickens' innate immune systems, which allows them to maintain their health. It also contains antioxidants that safeguard the birds' cells from damage.

2.2. Experimental Chicks and Ethical Approval

One-day-old chicks were maintained in standard conditions and were provided with et libitum food and water throughout the study. The study adhered to the guidelines of the Committee for the Control and Supervision of Experiments on Animals and was approved by the Institute Animal Ethics Committee under protocol number AHP /P/12/22 (Where AHP-Animal Health Product).

2.3. Inclusion and Exclusion Criteria for Study Birds

Inclusion Criteria: Chicks of the same breed, from the same hatch, who were healthy (free from obvious signs of illness or injury) and had a similar weight to other chicks in their group.

Exclusion Criteria: Chicks exhibiting signs of illness, chicks with physical abnormalities, and chicks with a weight that is significantly different from the others in their group.

2.4. Groupings, Diets, and Management

A total of 126 day-old Cobb 430Y commercial chickens were randomly assigned to six groups, designated as G1 through G6, with each group containing 21 chicks. A diet based on corn and soybean meal was formulated to fulfill the nutritional requirements outlined by the NRC for Cobb 430Y commercial broilers. The nutritional composition of the basal diet is detailed in Table 1.

The chicks were housed in semi-closed facilities, which were partitioned into enclosures, each offering a floor space of 60 square feet. The dimensions of each enclosure were approximately 6 feet in length, 10 feet in width, and 5 feet in height. Brooders, feeders, and drinkers were installed within each enclosure. Each chick was allocated a minimum of one square foot of floor space. The size and layout of the pens were adjusted to accommodate the number of chicks housed, utilizing either polyvinyl or thermocol sheets for modifications. Rice husk served as bedding material, which was replenished with fresh litter on a weekly basis or as required. During the first five days of the chicks' life, a thin layer of newspaper was placed over the litter to prevent the feeders and drinkers from becoming obstructed by rice husk. Fresh and clean drinking water was consistently provided to ensure the chicks remained hydrated throughout the duration of the experiment.

Table 1. Nutrient composition of basal diet.

Particulars	Pre-starter feed	Starter feed	Finisher feed
Protein (%)	22.20	21.30	19.40
Metabolizable Energy (kcal/kg)	2868	2875	3063
Crude Fiber (%)	4.03	4.38	3.87
Ether Extract (%)	3.41	3.42	6.31
Calcium (%)	1.04	1.04	0.80
Available Phosphorus (%)	0.40	0.40	0.34
Methionine (%)	0.61	0.60	0.48
Lysine (%)	1.37	1.31	1.10
Met. + Cyst. (%)	0.95	0.93	0.78
Threonine (%)	0.86	0.83	0.75
Sodium (%)	0.18	0.18	0.18
Chloride (%)	0.27	0.26	0.24
Potassium (%)	0.90	0.90	0.79
Dietary electrolyte balance (mEq/kg)	236	234	214

Three lines of feed, viz., pre-starter, starter, and finisher mash feed, were prepared at a local poultry feed manufacturer, in Bengaluru. The chicks were fed pre-starter feed from the 1st to the 14th day, starter feed from the 15th to the 25th day, and finisher mash feed from the 26th to the 38th day. Additionally, chicks in the G2, G5, and G6 groups were administered vaccinations to protect them from specific diseases. The Newcastle Disease (ND) vaccine was administered on the 7th day and the 22nd day as eye drops (the strain used on the 7th day was the live Lentogenic strain from VHL (Venkateshwara Hatcheries Private Limited), whereas on the 22nd day the live I.P. VH (Venkateshwara Hatcheries) strain from Abic was administered). On the fifteenth day, the invasive intermediate strain B2K from Indovax was administered as a vaccination against Infectious Bursal Disease (IBD). Ambient conditions, including temperature, lighting, and humidity, were maintained under the standard farm management practices for Cobb 430Y broilers. The lighting was provided for 24 hours for the first week and 20 hours until the conclusion of the trial.

2.5. Study Design

On day one of the feeding trial, a total of 126 one-day-old Cobb 430Y broiler chicks that were procured from M/s Sriya Farms and Feeds Pvt. Ltd., Bengaluru, were randomly assigned to six groups, viz. G1, G2, G3, G4, G5, and G6, 21 chicks each. G1 and G2 served as normal control and positive control, respectively. All chicks were raised on conventional commercial feed. Additionally, the feeds of the G3 and G5 groups were supplemented with Geriforte Vet Liquid (GVL), whereas the feeds of the G4 and G6 groups were supplemented with IPL. GVL and IPL were supplemented by mixing them with regular drinking water at a rate of 5 ml/100 birds at 1–2 weeks; 10 ml/100 birds at 2–4 weeks; and 20 ml/100 birds from four weeks onward till the chicks reaches the marketable age, i.e., 38-45 days. ND and IBD vaccinations were administered exclusively to the G2, G5, and G6 groups. Table 2 presents study design as follows:

Table 2. Study design, outlining the various groups, their supplementation, duration of supplementation, number of chicks per group, and feeding status.

Groups	Supplementation	Duration of supplementation	Number of chicks/ group	Feed status
G1: Normal control (NC)	No medication No vaccination	38 days	21	Continuous
G2: Positive control (PC)	No medication Only vaccination	38 days	21	
G3: GVL	GVL only	21 days	21	
G4: IPL	IPL only	21 days	21	
G5: GVL + Vaccination	GVL + Vaccination	21 days	21	
G6: IPL + Vaccination	IPL + Vaccination	21 days	21	

Note: G, Group; GVL, Geriforte Vet liquid; IPL, Immunopotentiator liquid.

2.6. Assessment Parameters

2.6.1. Growth Performance Parameters

Bird mortality was monitored daily. Body weight was recorded on day 1 and then both body weight and food intake on days 7, 14, 21, 28, and 38. Cumulative mortality, FCR (Feed Conversion Ratio), and EPEF (European Production Efficiency Factor) were calculated, was assessed to evaluate the effect of IPL on the growth parameters of Cobb 430 Y broilers.

2.6.2. Carcass Characteristics

At the end of the 38-day experiment, six birds with weights close to the average for each group were selected for carcass characteristics. Birds were euthanized in neck positions and allowed to bleed for 2 min. They were then immersed in warm water (51 °C-55 °C) for 120 seconds to facilitate feathering [9]. The feathers were removed by manual plucking. The eviscerated carcass after being cleaned (without the head, neck, and feet), as well as the liver, spleen, bursa, and thymus, were weighed and their weights recorded.

2.6.3. Blood Collection and Serum Separation

Blood samples, approximately 2 ml in volume, were obtained using standard centrifuge tubes (vacutainer tubes). The serum was isolated through centrifugation at 2000 rpm for a duration of 5 minutes and subsequently stored at -20°C until the assessment of biochemical parameters and the analysis of ND and IBD antibody titers. A blood sample was collected from the brachial vein of six birds per group on the 35th day.

2.6.4. Assay of Serum Biochemical Parameters

The biochemical parameters, including total protein, globulin, albumin, A/G ratio (Albumin/Globulin), blood urea nitrogen (BUN), urea, creatinine, serum glutamic pyruvic transaminase (SGPT), and serum glutamic oxaloacetic transaminase (SGOT), were assessed utilizing standardized kit-based assay techniques on the automated biochemical analyzer Erba Transasia EM 360.

2.6.5. Serum ND and IBD Antibody Titers

The ND and IBD antibody titers in serum samples (n = 16) were measured using a standardized ELISA (Enzyme-Linked Immunosorbent Assay) method as described by the manufacturer of the ELISA kit (Biochek, Holland).

3. STATISTICAL ANALYSIS

Data are presented as mean ± standard error of the mean. Statistical analysis was conducted using one-way ANOVA (Analysis of Variance), followed by Dunnett's multiple comparison test to compare control and supplemented groups. A p-value of ≤ 0.05 was deemed statistically significant. The statistical analysis was performed using SPSS (Statistical Package for Social Sciences) software, version 20 (IBM SPSS Statistics [IBM Corp., published 2011]).

4. RESULTS

On day 38 (after the chicks were sacrificed), the body weight (g) was noted to be increased in all the supplemented groups except for G4, when compared to G1. These findings depicted that the body weight of the chicks increased by 4.33%, 2.95%, 5.75%, and 6.91% in the G2, G3, G5, and G6 groups, respectively. These findings also implied that the G6 group exhibited superior growth performance among the broiler chickens. Compared to G1, the FCR of the G3, G4, G5, and G6 groups improved. These findings suggested that the birds in the G3, G4, G5, and G6 groups consumed 5 g, 13 g, 1 g, and 10 g less feed per kilo gram of body weight gain than the birds in

G1. These findings also revealed that G4 was most effective in terms of improving the effects of FCR, followed by G6. The EPEF increased by 3.32%, 6.34%, 7.25%, 12.08%, and 13.60% in the G2, G3, G4, G5, and G6 groups. These findings implied that the G6 group exhibited the most favorable EPEF improvement effects. In comparison to G1, the cumulative mortality rate in G5 was reduced by 50% (Table 3). Overall, these findings highlighted that supplementation of poultry feed with IPL enhances the growth performance parameters, *viz*., body weight, FCR, and EPEF.

Table 3. Effect of IPL on performance parameters in Cobb 430 Y broiler chickens.

Parameters	Group	Day 1	Day 21	Day 38
*Body Weight, g	G1: Normal control (NC)	50.00 ± 4.47	899.25 ± 49.77	2355.95 ± 158.83
	G2: Positive control (PC)	48.33 ± 4.28	933.00 ± 44.59	2458.06 ± 186.00
	G3: GVL	50.00 ± 5.48	904.00 ± 68.93	2425.53 ± 242.21
	G4: IPL	50.24 ± 4.60	909.76 ± 51.56	2325.95 ± 157.58
	G5: GVL + Vaccination	47.14 ± 5.61	851.95 ± 81.45	2491.44 ± 149.45
	G6: IPL + Vaccination	48.10 ± 6.02	925.00 ± 57.76	2518.68 ± 153.82
FCR	G1: Normal control (NC)		1.29	1.69
	G2: Positive control (PC)		1.26	1.71
	G3: GVL		1.29	1.64
	G4: IPL		1.23	1.56
	G5: GVL + Vaccination		1.45	1.68
	G6: IPL + Vaccination		1.33	1.59
EPEF	G1: Normal Control (NC)			331
	G2: Positive Control (PC)			342
	G3: GVL			352
	G4: IPL			355
	G5: GVL + Vaccination			371
	G6: IPL + Vaccination			376
Cumulative mortality, %	G1: Normal control (NC)			9.52
	G2: Positive control (PC)			9.52
	G3: GVL			9.52
	G4: IPL			9.52
	G5: GVL + Vaccination			4.76
	G6: IPL + Vaccination			9.52

Note: *Values are expressed as mean ± SD; n = 21; p > 0.05 as compared to G1, based one-way ANOVA followed by Dunnett's multiple comparison post-hoc test FCR, Feed conversion ratio; EPEF, European production efficiency factor.

The study findings depict that no significant effect of investigational veterinary feed supplement was observed in carcass traits of commercial broiler chicken products, including *ready-to-cook (*RTC, 65.65%), liver (2.63%), spleen (0.14%), bursa (0.11%), and thymus (0.26%) (Table 4).

Table 4. Effect of IPL on carcass traits in Cobb 430 Y broiler chickens.

Groups	RTC (%)	Liver (%)	Spleen (%)	Bursa (%)	Thymus (%)
G1: Normal control (NC)	67.54 ± 1.68	2.73 ± 0.42	0.14 ± 0.04	0.06 ± 0.02	0.30 ± 0.17
G2: Positive control (PC)	68.45 ± 4.03	2.52 ± 0.32	0.16 ± 0.04	0.06 ± 0.02	0.39 ± 0.15
G3: GVL	66.25 ± 1.53	2.74 ± 0.30	0.15 ± 0.04	0.06 ± 0.02	0.25 ± 0.12
G4: IPL	65.23 ± 1.45	2.48 ± 0.22	0.15 ± 0.06	0.05 ± 0.03	0.22 ± 0.11
G5: GVL + Vaccination	66.46 ± 1.47	2.71 ± 0.37	0.15 ± 0.04	0.11 ± 0.07	0.26 ± 0.11
G6: IPL + Vaccination	65.65 ± 1.05	2.63 ± 0.35	0.14 ± 0.03	0.11 ± 0.04	0.26 ± 0.14

Note: Values are expressed as mean ± SD; n = 16; p > 0.05 as compared to G1, based on one-way ANOVA followed by Dunnett's Multiple Comparison Test RTC, Ready to cook.

The results of serum biochemical parameters showed that urea, BUN, and creatinine concentrations were significantly increased in the G2 group, and serum SGPT levels were significantly increased in the G6 group as compared to the G1 group (Table 4). However, all the biochemical parameters were well within the normal range, indicating that supplementation with investigational veterinary feed supplement did not have any adverse effects.

The geometric mean (GM) of ND titer on day 35 increased non-significantly ($p > 0.05$) in the G2, G5, and G6 groups as compared to the G1 group. However, the vaccination index (VI) of IBD titer on day 35 was non-significantly ($p > 0.05$) increased in all the supplemented groups as compared to G1 (Table 5).

Table 5. Effect of IPL on serum ND and IBD titers in Cobb 430 Y broiler chickens.

Groups	ND Titre		IBD Titre	
	Geometric mean (GM)		Vaccination index (VI)	
	Day 21	Day 35	Day 21	Day 35
G1: Normal control (NC)	5.66	10.08	108.47	73.97
G2: Positive control (PC)	6.73	13.93	119.30	134.87
G3: GVL	3.56	4.00	91.09	74.62
G4: IPL	4.49	4.00	88.86	83.22
G5: GVL + Vaccination	6.96	64.00	121.16	137.77
G6: IPL + Vaccination	10.08	90.51	126.32	132.86

5. DISCUSSION

Broilers are increasingly kept in unfavorable and stressful rearing conditions, which can damage their immune systems. Considering the use of antibiotics in poultry feed worldwide, the role of natural feed additives and antibiotic alternatives in strengthening the immune system of chickens should be considered [9]. Due to widespread bans and restrictions on the use of growth promoters supplemented with antibiotics, the focus of research has shifted to finding alternative supplements that can reasonably prevent disease and microbiome development, regulate and ultimately strengthen the intestinal microbial ecosystem. and maintenance of host health by improving immunity [9]. In a literature review, Phillips et al. noted that feeding broilers with natural compounds from medicinal plants, phytogenic substances, plant compounds extracted from fruits and other by-products can improve their immune system. Several natural compounds are effective alternatives to antibiotics and meet the expectations of poultry farming as feed additives with the lowest risk and environmental pressure. In addition, the literature provides evidence that hematin, hepatoprotective, and immunomodulatory properties are present when certain polygrasses are used as feed additives. Therefore, the present experiment was conducted to investigate the immune and performance-enhancing effects of IPL, a multi-herb liquid formulation, in commercial Cobb 430Y broilers.

The study results revealed that poultry feed supplementation with IPL enhances growth performance parameters such as body weight and feed efficiency without affecting carcass characteristics or serum biochemical parameters. Furthermore, immunity parameters, *viz*., the ND and IBD antibody titers following respective vaccination schedules, were increased after poultry feed supplementation with IPL. These findings could be attributed to the individual herbal ingredients present in IPL.

The improvement of growth parameters after the addition of IPL to poultry feed in our study could be mainly due to the anabolic and antioxidant effects of ascorbic acid, gallic acid and tannic acid in E. officinalis Gouda, et al. [7]. Similar findings were reported by Kumari, et al. [10]; Patil, et al. [11]; Sujatha, et al. [12]; Kumar, et al. [13]. In another study, Sujatha, et al. [12]; Alagawany, et al. [14]; Kumar, et al. [13] and Akotkar, et al. [15] reported an increase in body weight when poly-herb mixtures containing E. officinalis were added to poultry feed. In addition, the growth of poultry was positively affected by the addition of G. glabra to their diet, which caused the development of their organs.

Additionally, digestion and appetite improved in broilers fed with feeds supplemented with 2.5 g/kg *G. glabra* [16]. The enhanced growth performance observed in our study following supplementation with IPL could be attributed to the presence of *W. somnifera* in IPL [17, 18].

The literature review suggests that the improved immunity parameters, specifically ND and IBD titers, observed in our study may largely result from the immunomodulatory effects of W. somnifera. This assertion is

corroborated by the findings of Owais et al., who noted an increase in the survival rate of Salmonella-infected broiler chicks when administered W. somnifera root extract orally [18]. The protective influence of W. somnifera root extract on disease symptoms may stem from the immunomodulatory and antimicrobial properties documented in existing literature [17, 19]. Additionally, the elevated immunity parameters, namely ND and IBD titers, recorded in our study among chicks that received IPL-supplemented poultry feed post-vaccination indicate that IPL has an immunopotentiating effect following vaccination. These results align with earlier research conducted by Hanieh, et al. [20]; Landy, et al. [19]; Uddin, et al. [21] and Ahmad, et al. [22]. The immunomodulatory characteristics of W. somnifera are likely attributed to the presence of active glycol withanolides [23].

T. cordifolia has been reported to enhance cell-mediated immune responses in chicks due to the presence of G1-4A, an arabinogalactan polysaccharide present in the plant. G1-4A activates macrophages through TLR6 signaling and NF-kB translocation, along with the resultant production of cytokines that have a role in hematopoiesis, thereby influencing the lymphoid tissues [24].

Moreover, according to Mishra *et al.*, phytochemical compounds like Cordifolioside A, Cordioside, and Ecdysteron present in T. cordifolia could also be responsible for its immunomodulatory property [25].

According to Das, et al. [24] *O. sanctum* exhibited immunostimulant properties by elevating heterophil and lymphocyte counts, enhancing phagocytic activity and phagocytic index, and diminishing the total bacterial count. Oil from tulsi seed can mediate GABAergic pathways and thus modulate both humoral and cell-mediated immunity [26].

In addition, prior research has shown that the pathophysiologic and hematological alterations in chicks experimentally infected with the chicken infectious anemia virus can be ameliorated, and the live body weights of these chicks can be enhanced [17, 27].

Sadekar, et al. [28] demonstrated that birds infected with the IBD virus and fed O. sanctum leaves exhibited notable effects Sadekar, et al. [28]. Maimes highlighted the role of O. sanctum in enhancing the immune system's defense against bacterial and viral infections [27]. Logambal et al. examined the immune responses elicited by O. holly leaf extract and discovered its ability to stimulate antibody production [29]. The immune parameter results from our study, specifically ND and IBD titers, aligned with these previous findings. Furthermore, Goel et al. identified a stimulating effect of O. sanctum on both humoral and cell-mediated immune responses, noting an increase in antibody titers against the O' antigen of S. enterica serovar Typhimurium and a significant rise in skin thickness as measured by the dinitrochlorobenzene hypersensitivity test [30]. Additionally, Goel, et al. [30] reported that Argemone mexicana also stimulated the humoral immune response while inhibiting cell-mediated immunity [31].

Mediratta et al. explored the effects of O. sacred seed oil on both stressed and non-stressed animals, concluding that O. sanctum modulates both humoral and cell-mediated immune responses, potentially through the GABAergic pathway [32]. Babu et al. indicated that the immunomodulatory mechanism of O. sanctum primarily involves hematopoietic and lymphoid cells [33].

6. CONCLUSIONS

Herbal medicines are still important because herbal and alternative medicine has significant potential in veterinary practice.

This study showed that along with conventional ND and IBD vaccinations, supplementation of poultry birds with IPL for 21 consecutive days showed beneficial effects on immune-related and growth parameters of Cobb 430Y broilers.

Therefore, commercial broilers could be recommended to supplement 21 days of IPL as an immunopotentiator to improve the general health status of broilers and thus improve production efficiency.

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