OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2019.

Research Article
The Effect of Spray-Dried Bovine Plasma and Shrimp Hydrolysate on Components of the Immune System and Zootechnical Parameters in Broiler Chickens

[1]G. Parra, [1]N. Mier, [1]A. Aguirre and [2]R. Riboty

[1]Carrera Ingeniería en Biotecnología, Facultad de Ingeniería y Ciencias Aplicadas, Universidad de las Américas, Quito, Ecuador
[2]Animal Production Graduate Program, Equinoctial Technological University, HQ Santo Domingo, Ecuador

Abstract

Background and Objective: The aim of this study was to evaluate the immunomodulatory effect of spray-dried bovine plasma (SDBP) and shrimp hydrolysate (SH) in components of the immune system and its influence on zootechnical parameters in broiler chickens raised at 2500 m.a.s.l. **Materials and Methods:** One thousand five hundred one-day-old mixed Cobb broilers were divided into four groups with five replicates each in a randomized block design. The animals were raised in a challenging manner, that is with reused and untreated litter and without being vaccinated against *Avian pneumovirus* (APV). A three-phase (1-14, 15-28 and 29-42 days) restricted mash feeding program was used. Birds of the five experimental units were fed for 42 days, with one of the following treatments: a corn and soybean basal diet (Control), SDBP or SH reformulated diets under two dosing strategies (S1 and S2). Inclusion levels were 5, 2.5,1.5% in S1 and 2.5%, 1.5%, 0.5% in S2 on the three-phase feeding program. **Results:** In the first week, the SDBP S2 avoided a drastic drop in maternal antibodies against APV and produced better white blood cell percentages. In week 6, similar results in white blood cell percentages were observed with SDBP S1, SDBP S2 and SH S1. The SDBP S2 generated a better performance than the control group throughout the fattening cycle and the SH S2 produced the same effect as the control group. The treatments produced no significant effect on the immune organ indices and the mortality rate. **Conclusion:** The inclusion of SDBP S2 in broiler chickens' diet has a more evident immunomodulatory effect in white blood cells and maternal antibodies without vaccination and has a positive effect on the animals' performance.

Key words: Blood parameters, broiler chickens, immune organ indices, immunomodulator, performance, shrimp hydrolysate, spray-dried bovine plasma

Received: Accepted: Published:

Citation: Gisella Parra, N. Mier, A. Aguirre and R. Riboty, 2019. The effect of spray-dried bovine plasma and shrimp hydrolysate on components of the immune system and zootechnical parameters in broiler chickens. Int. J. Poult. Sci., CC: CC-CC.

Corresponding Author: Gisella Parra, Carrera Ingeniería en Biotecnología, Facultad de Ingeniería y Ciencias Aplicadas, Universidad de las Américas, Quito, Ecuador
Nataly Mier, Carrera Ingeniería en Biotecnología, Facultad de Ingeniería y Ciencias Aplicadas, Universidad de las Américas, Quito, Ecuador

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The poultry industry is one of the most developed and relevant economic sectors in Ecuador. In recent years, production has increased by 80%[1]. Despite the competitiveness of the sector, factors such as intensive production and high exposure to microbial agents contribute to stress in broiler chickens, making the animals more prone to immunosuppression and to diseases, especially infectious ones[2,3,4]. The consequences of infectious diseases are mainly reflected in lower production, particularly in terms of animal mortality and growth rate[5]. For this reason, it is important to apply different strategies to prevent and control the illnesses.

The most commonly employed strategies are biosecurity programs, vaccination and medication schedules and making the most of genetic resistance to diseases[6]. However, there are other safe, effective and inexpensive alternatives including the utilization of food additives with immunomodulatory capacities, defined as substances that stimulate, suppress or modulate the elements of the immune system[7-10]. These products have certain advantages in comparison with other alternatives, specifically, their direct influence on the gastrointestinal immune system and a higher acceptance amongst consumers in contrast to antibiotics[4,11,12].

The spray-dried animal plasma is a commercial by-product rich in functional and digestible proteins, growth factors and biologically active peptides[13-16]. It is reported that the inclusion of spray-dried plasma in the diet of pigs, cows, turkeys and broiler chickens improves the animals' performance, especially those reared in non-sanitary environments. Other studies showed that cattle and pigs immunologically challenged with enteric bacteria and fed with spray-dried animal plasma were less susceptible to developing a disease related to the challenge[14,17-20]. Although, the action mechanism has been studied, the way in which the product accomplishes those results is unclear and the studies were carried out mainly in pigs[17,20]. The immunoglobulin G and the glycoproteins of the plasma interact with antigens to decrease the overstimulation of the immune system, preventing energy distribution being inclined towards other non-productive activities. Furthermore, the product maintains the integrity of the intestinal mucosa, enhances its morphology and helps the digestive enzymatic activity[17,19,21].

Shrimp hydrolysate, obtained from by-products of the aquaculture industry, contains proteins, lipids, carotenoids, chitin and chitosan[22,23]. The principal studies focussing on its effect on animals have concentrated on the field of aquafarming. It has been demonstrated that amino acids and small peptides of the hydrolysate are responsible for the improvement of animal performance, due to the easy and fast absorption and digestion of those components from an early age[24]. It has been reported that peptides in fish and chitin and chitosan in other animals, enhance the activity of some elements of the immune system such as macrophages and lysozyme[23-25]. Thereby, the shrimp hydrolysate favors protection against infectious diseases. However, in investigations with *Pagrus major* in the presence of a challenge, no significant effect was demonstrated on the prevention of the Edwardsielosis disease[24].

The aim of this study was Z to evaluate the immunomodulatory effect of spray-dried bovine plasma and shrimp hydrolysate in components of the immune system and its influence on zootechnical parameters in broiler chickens.

MATERIALS AND METHODS

Bird management: Day-old male broiler chickens (Cobb500), unimmunized against *Avian pneumovirus*, were obtained from a commercial hatchery (Grupo Oro, Ecuador), raised for 42 days in a controlled temperature under 180 m^2 experimental shed at 2500 m.a.s.l. Poultry gas heaters kept the temperature at 31°C during the first week and decreased by 2°C each week until it reached 20°C during the last week. Cross-ventilation and light required was achieved behandling curtains and conventional light. The animals were reared in floor pens with 75% reused and untreated litter (broiler breeders 41-week-old) and 25% new litter (new rice hulls). Water was offered *ad libitum* and mash feed was provided according to the feed intake restriction to avoid ascites. This restricted feed intake consisted of around 20% less food than the ration recommended by the broiler's genetic house (Cobb, 2015). All diets were formulated using the *Brill® Formulation* program to fulfill all the nutrient requirements according to the poultry breed, for the initial (0-14 days), growth (15-21 days) and final (22-42 days) phases (Table 1).

Experimental design and diets: The feeding trial used a randomized complete block design with 5 groups, each with 5 replicates and each replicate with 60 broilers. It involved the evaluation of 2 dietary additives, commercial spray-dried bovine plasma (PROLECHON, LICAN) and commercial shrimp hydrolysate (Actipal HP1-s1, Aquativ), in two different dosing strategies: S1 (5, 2.5 and 1.5%) and S2 (2.5, 1.5 and 0.5%) in initial, growth and final phases respectively, compared with a control diet composed primarily of corn and soybean meal. All assessed treatments are shown in Table 2.

Table 1: Ingredients and nutritional composition of experimental diets

	Initial phase (1-14 days)					Growth phase (15-28 days)					Final phase (29-42 days)				
Ingredient (%)	Control	SDBPS1	SDBPS2	SH S1	SH S2	Control	SDBPS1	SDBPS2	SHS1	SH S2	Control	SDBPS1	SDBPS2	SHS1	SHS2
Yellow corn	34.05	35.92	34.39	33.88	33.37	55.50	58.20	57.31	56.77	56.50	64.18	65.13	64.56	65.46	64.51
Soybean meal	34.30	28.30	31.20	29.60	31.90	26.31	22.47	23.80	23.60	24.50	27.70	25.90	27.10	25.80	27.10
Rice	23.40	25.00	25.00	25.00	25.00	10.00	10.00	10.00	10.00	10.00	-	-	-	-	-
Palm oil	3.70	2.10	2.90	2.60	3.10	3.80	2.70	3.10	3.10	3.30	4.30	3.80	4.10	3.60	4.10
Calcium carbonate	1.50	1.50	1.50	1.60	1.50	1.50	1.50	1.50	1.50	1.50	1.30	1.30	1.30	1.30	1.30
Monocalcium phosphate 23%	1.20	1.00	1.10	0.70	0.90	0.90	1.00	1.00	0.70	0.80	0.70	0.80	0.70	0.60	0.70
Vitamins and minerals[a]	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Sodium chloride	0.47	0.11	0.29	0.34	0.40	0.48	0.34	0.40	0.42	0.44	0.54	0.45	0.51	0.50	0.52
DL-Methionine 99%	0.28	0.19	0.24	0.29	0.28	0.26	0.22	0.24	0.26	0.26	0.21	0.19	0.21	0.21	0.21
Lysine sulphate 54,6%	0.17	-	0.01	0.08	0.12	0.29	0.17	0.23	0.22	0.26	0.15	0.06	0.12	0.12	0.14
L-Threonine 98%	0.05	-	-	0.05	0.05	0.08	0.03	0.05	0.07	0.08	0.04	-	0.03	0.04	0.04
Antifungal	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Diclazuril	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Mycotoxin adsorbent	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Growth promoter	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Spray-dried plasma[b]	-	5.00	2.50	-	-	-	2.50	1.50	-	-	-	1.50	0.50	-	-
Shrimp hydrolysate[c]	-	-	-	5.00	2.50	-	-	-	2.50	1.50	-	-	-	1.50	0.50
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analyses															
ME (Kcal kg^{-1})	3055.00	3059.00	3058.00	3056.00	3056.00	3141.00	3138.00	3139.00	3143.00	3141.00	3182.00	3178.00	3181.00	3180.00	3182.00
Crude protein (%)	20.98	22.14	21.45	21.43	21.22	18.00	18.23	18.06	18.45	18.21	18.31	18.58	18.42	18.51	18.37
Digestible protein (%)	18.57	19.82	19.07	19.09	18.82	16.06	16.36	16.16	16.54	16.29	16.51	16.83	16.63	16.74	16.59
Lys dig (%)	1.14	1.22	1.14	1.14	1.14	1.00	1.00	1.00	1.00	1.00	0.95	0.95	0.95	0.95	0.95
Met dig (%)	0.58	0.51	0.54	0.59	0.59	0.51	0.48	0.50	0.52	0.52	0.47	0.45	0.47	0.48	0.48
TSAA dig (%)	0.86	0.86	0.86	0.86	0.86	0.77	0.77	0.77	0.77	0.77	0.74	0.74	0.74	0.74	0.74
Thre dig (%)	0.75	0.82	0.75	0.75	0.75	0.67	0.67	0.67	0.67	0.67	0.65	0.65	0.65	0.65	0.65
Tryp dig (%)	0.24	0.27	0.25	0.22	0.23	0.19	0.20	0.20	0.19	0.19	0.19	0.20	0.20	0.19	0.19
Fat (%)	6.11	4.34	5.30	5.19	5.58	6.77	5.73	6.10	6.20	6.35	7.64	7.15	7.45	7.04	7.47
Linoleic acid (%)	1.36	1.18	1.27	1.21	1.27	1.71	1.62	1.65	1.64	1.66	1.92	1.87	1.90	1.85	1.90
Crude fiber (%)	2.93	2.76	2.82	2.73	2.81	3.17	3.11	3.13	3.09	3.12	3.37	3.33	3.37	3.33	3.36
Calcium (%)	1.00	1.00	1.00	1.00	0.98	0.95	0.96	0.96	0.93	0.94	0.85	0.86	0.85	0.84	0.85
Non-phytate P (%)	0.51	0.49	0.50	0.50	0.49	0.44	0.45	0.46	0.45	0.45	0.41	0.43	0.41	0.42	0.42
Sodium (%)	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.20	0.20	0.20	0.20	0.20

[a]Vitamin and mineral premix contained the following ingredients kg^{-1}, Vitamin A: 7.3MIU, Vitamin D3: 3.7MIU, Vitamin E: 36.6KIU, Vitamin K: 2.3 g, Thiamin: 2.3 g, Riboflavin: 5.5 g, Pyridoxine: 2.7 g, Niacin: 38 g, Folic acid: 1.3 g, Cyanocobalamine: 10.7 mg, Pantothenic acid: 10 g, Biotin: 86.7 mg, Cu: 9 g, I: 0.67 g, Fe: 33.5g, Mn: 60 g, Se: 0.22 g, Zn: 60 g and csp: 1 kg. [b]Spray-dried bovine plasma (Prolechon®). [c]Shrimphydrolysate (HP1S1®). SDBPS1: Spray-dried bovine in dosing strategy 1, SDBPS2: Spray-dried bovine in dosing strategy 2, SHS1: Shrimp hydrolysate in dosing strategy 1 and SHS2: Shrimp hydrolysate in dosing strategy 2

Table 2: Treatments in the study

Experimental system	Descriptive details
Control	Basal diet without feed additive
SDBP S1	Basal diet with added spray-dried bovine plasma in dosing strategy 1 (S1)
SDBP S2	Basal diet with added spray-dried bovine plasma in dosing strategy 2 (S2)
SH S1	Basal diet with added shrimp hydrolysate in dosing strategy 1 (S1)
SH S2	Basal diet with added shrimp hydrolysate in dosing strategy 2 (S2)

Sampling and measurements

Blood parameters: On day 1, 2 mL blood samples from the jugular vein of 25 birds were collected in EDTA tubes and the same blood samples from 20 birds were collected in tubes without anticoagulant, in line with Abdel-Fattah *et al*.[26].

On days 7 and 14, 20 broilers per treatment (4 per repetition) with the same weight were selected for blood extraction (from the jugular vein). The samples were divided into tubes with or without anticoagulant. Over the subsequent weeks, until the 6th week, the same procedure was executed but the samples were taken from the wing vein.

Five blood samples per treatment collected into EDTA tubes were used to count blood cells (lymphocytes, monocytes, heterophils and heterophils: lymphocytes ratio) under a microscope, according to Wang *et al*.[27].

The samples in tubes without anticoagulant were stored at a tilted angle at 4°C for 2 days. Then the plasma was harvested and stored at -18°C for subsequent analysis of antibody titers of APV by indirect ELISA. The analysis was performed using the commercial IDEXX APV Ab test kit according to the manufacturer's recommendations (IDEXX, Maine, USA).

Immune organ indices: Every 7th day, 5 birds per treatment, previously used in the blood sampling, were selected at random and weighed. They were stunned by low electrical voltage, slaughtered and bled. The spleen, thymus and bursa of Fabricius were immediately removed and weighed. The relative weights of each lymphoid organ and the ratio between the different lymphoid organ weights were determined.

Performance parameters: On a daily basis, we recorded the mortality, culling and feed intake for each repetition. Also, the birds with 12 h of fasting were weighed weekly. The recollected data was used to calculate the body weight gain, feed intake and feed conversion ratio for the whole period of rearing and for each of the 3 development phases (initial, growth and final). Additionally, a weekly survival rate was determined.

Statistical analysis: The normality and homogeneity of the studied variables were analyzed using the Shapiro Wilk test. When necessary, before analysis, the data were transformed to log or square root. An appropriate statistical analysis of variance was applied for a randomized complete block design (two-way ANOVA) with the pens' positions within the experimental shed as the blocking criterion. A Tukey test was used for the determination of significant differences amongst treatments. $p<0.05$ was considered statistically significant.

The data that did not adjust to the model assumptions of normality and homogeneity of variance were analyzed using the Kruskal Wallis test and Dunn Bonferroni test in order to determine significant differences between treatments. Mortality was assessed via the Log Rank test in order to compare survival amongst treatments. All the statistical analyses were performed with SPSS 23.0.

RESULTS

Blood parameters: As shown in Table 3, the APV antibody titers of birds in the control group and all treatments decreased pronouncedly until day 14. The highest antibody titers during the decrease, precisely on days 7 and 21, were found in the SDBP S2 group. The result of this treatment for both days was significantly different in relation to the control group ($p<0.05$). In addition, on day 7, the antibody titers of the SH S1 were significantly different to the control ($p<0.05$).

The effects of SDBP and SH on the studied hematological parameters of broilers are presented in Table 4 and 5. In certain periods of the experiment, the measured parameters were influenced by the immunomodulators. Between 1-7 days, SDBP S2 showed a significant increase ($p<0.05$) in lymphocytes and a significant decrease ($p<0.05$) in heterophils and H:L ratio. Over the same period of time, the monocytes decreased significantly in SH S2. Between 7-14 days, SDBP S2 manifested a significant decrease and SH S2 a significant increment ($p<0.05$) in lymphocytes. At day 42, the lymphocytes, heterophils and H:L ratio presented significant differences ($p<0.05$) in SDBP S1, SDBP S2 and SH S1respect to the control. Specifically, from day 28 to day 42 those 3 parameters showed significant increases in the treatments mentioned above, with the exception of SH S1 in lymphocytes and SDBP S2 in H:L, in which they decreased significantly.

Table 3: Antibody titers for APV of each treatment

	Antibody titer					
Time (days)	Control	SDBP S1	SDBP S2	SH S1	SH S2	p-value
1	14203.00	14203.00	14203.00	14203.00	14203.00	NS
7	18.72	5.50	4635.77	1741.98	760.66	1.87E-08
14	27.41	20.48	6.14	8.47	3.73	NS
21	1.00	1.32	4.51	1.26	1.00	2.14E-03
28	1.21	1.59	1.00	1.00	1.00	NS
35	1.24	1.00	1.66	1.00	1.67	NS
42	1.54	1.00	1.00	1.00	1.54	NS

Values are geometric means. NS: No significance at p<0.05

Table 4: Percentages of lymphocytes, monocytes and heterophils in each treatment

Treatment/time (days)	1	7	14	21	28	35	42
Lymphocytes (%)							
Control	34.60	39.20^{b}	32.400^{b}	68.00	64.20	60.60	49.80^{b}
SDBP S1	29.60	54.00^{ab}	51.400^{ab}	64.60	70.20	58.60	58.80^{a}
SDBP S2	33.80	63.40^{a}	53.000^{a}	59.40	66.00	58.20	59.60^{a}
SH S1	36.40	51.60^{ab}	48.000^{ab}	51.20	70.00	61.20	58.80^{a}
SH S2	36.60	49.80^{ab}	63.400^{a}	68.00	68.40	62.40	54.60^{ab}
p-value	NS	0.02	0.005	NS	NS	NS	0.02
Monocytes (%)							
Control	3.40	5.40^{a}	6.20	3.75	4.40	1.50	3.80
SDBP S1	5.20	5.80^{a}	3.20	3.00	3.80	4.25	2.40
SDBP S2	3.80	3.80^{ab}	4.40	2.25	2.75	4.80	3.75
SH S1	4.20	2.60^{ab}	2.20	3.25	4.40	2.20	3.80
SH S2	2.80	2.25^{b}	2.00	2.00	2.25	3.50	2.50
p-value	NS	0.01	NS	NS	NS	NS	NS
Heterophils (%)							
Control	61.60	54.00^{a}	40.00	28.80	29.40	36.60	45.80^{a}
SDBP S1	65.00	39.00^{ab}	45.40	32.60	25.60	37.00	37.60^{b}
SDBP S2	60.00	31.80^{b}	41.00	38.60	30.60	36.60	37.00^{b}
SH S1	58.40	44.40^{ab}	49.80	31.75	24.00	36.40	36.80^{b}
SH S2	57.80	47.40^{ab}	34.40	29.00	27.80	36.80	42.60^{ab}
p-value	NS	0.04	NS	NS	NS	NS	0.001

Values are means. Mean values with different letters in the same column differ significantly at p<0.05. NS: no significance at p<0.05

Table 5: Heterophils:lymphocytes ratio in each treatment

Treatment/time (days)	1	7	14	21	28	35	42
Heterophils: lymphocytes ratio							
Control	1.78	1.38^{a}	1.23	0.42	0.46	0.60	0.920^{a}
SDBP S1	2.20	0.72^{ab}	0.88	0.50	0.36	0.63	0.640^{b}
SDBP S2	1.78	0.50^{b}	0.77	0.65	0.46	0.63	0.620^{b}
SH S1	1.60	0.86^{ab}	1.04	0.62	0.34	0.59	0.630^{b}
SH S2	1.58	0.95^{ab}	0.54	0.43	0.41	0.59	0.780^{ab}
p-value	NS	0.034	NS	NS	NS	NS	0.000

Values are means. Mean values with different letters in the same column differ significantly at p<0.05. NS: No significance at p<0.05

Immune organ indices: The relative lymphoid organ weights and the ratio between the different lymphoid organ weights were not affected by any dietary supplementations (p<0.05), with the exception of the relative weight of the thymus in SDBP S1 on day 35, where it showed a significant increment (p<0.05) as compared to the control (Table 6 and 7).

Performance parameters: The effects of SDBP and SH on the performance parameters of broiler chickens are presented in Table 8. During the initial phase, the feed intake was significantly higher (p<0.05) by 1.42 % in broilers fed with SH S2 as compared to the control group. Contrarily, the feed intake in broilers fed with SDBP S1 and SH S1 was significantly lower (p<0.05). The largest difference was found in SH S1 with a decrease of 2.21%. Additionally, for weight gain, significant differences were not found between the experimental groups and the control (p<0.05). Although, the SDBP S2 diet group manifested a higher weight gain than the control, it was not

Table 6: Lymphoid organ relative weights (g g^{-1}) in each treatment

Treatment/time (days)	7	14	21	28	35	42
Bursa of fabricius						
Control	1.87	2.19	1.93	1.82	1.890	1.22
SDBP S1	1.77	1.96	2.03	1.98	1.930	1.47
SDBP S2	1.58	1.94	2.10	1.50	2.370	1.84
SH S1	2.02	2.01	2.43	2.02	1.660	1.84
SH S2	1.46	2.43	2.22	2.06	2.220	1.44
p-value	NS	NS	NS	NS	NS	NS
Thymus						
Control	5.40	4.09	5.14	5.65	3.590^{b}	4.99
SDBP S1	4.35	4.75	4.72	5.61	5.370^{a}	4.91
SDBP S2	5.18	4.98	6.38	3.72	5.030^{ab}	4.72
SH S1	4.65	5.44	4.90	4.60	4.840^{ab}	4.43
SH S2	4.24	5.08	6.61	5.02	4.950^{ab}	5.49
p-value	NS	NS	NS	NS	0.005	NS
Spleen						
Control	1.17	0.82	0.81	0.93	0.990	1.17
SDBP S1	1.03	0.61	0.75	0.89	0.910	0.97
SDBP S2	0.73	0.84	0.75	0.96	0.970	1.53
SH S1	0.59	0.71	0.90	1.32	0.970	1.13
SH S2	0.83	0.75	0.75	1.13	1.120	1.09
p-value	NS	NS	NS	NS	NS	NS

Values are means. Mean values with different letters in the same column differ significantly at p<0.05. NS: No significance at p<0.05

Table 7: Lymphoid organ weights ratio (g g^{-1}) in each treatment

Treatment/time (days)	7	14	21	28	35	42
Bursa: Thymus						
Control	0.36	0.54	0.40	0.32	0.54	0.24
SDBP S1	0.42	0.43	0.46	0.36	0.36	0.30
SDBP S2	0.36	0.42	0.33	0.49	0.45	0.39
SH S1	0.49	0.38	0.50	0.45	0.34	0.37
SH S2	0.36	0.50	0.38	0.44	0.45	0.26
p-value	NS	NS	NS	NS	NS	NS
Bursa: Spleen						
Control	1.09	2.80	2.55	2.08	2.03	1.25
SDBP S1	2.30	3.50	2.82	2.33	2.25	1.55
SDBP S2	3.00	2.42	2.90	1.63	2.49	1.26
SH S1	3.80	3.20	2.83	1.75	1.71	1.63
SH S2	2.57	3.37	2.93	2.08	2.00	1.42
p-value	NS	NS	NS	NS	NS	NS
Thymus: Spleen						
Control	5.97	5.37	6.62	6.51	3.98	4.84
SDBP S1	5.60	8.10	6.54	6.40	6.31	5.12
SDBP S2	8.80	6.25	9.06	4.00	5.56	3.27
SH S1	9.00	9.13	5.57	3.86	5.01	4.00
SH S2	6.97	6.83	8.82	4.92	4.40	5.50
p-value	NS	NS	NS	NS	NS	NS

Values are means. Mean values with different letters in the same column differ significantly at p<0.05. NS: No significance at p<0.05

significantly different. The experimental diets did not affect (p<0.05) the feed conversion ratio but the SDBP S2 group showed the lowest value on this parameter among the other groups.

In the growth phase and over the whole period of rearing, the feed intake was significantly lower (p<0.05) in groups fed the SH S1 and SDBP S1 diets compared with the control and SDBP S2 treatment. The largest difference was found in SH S1 with a decrease of 1.99% during the growth phase and a decrease of 1.49% over the whole period of rearing, as compared to the control. Although, the SDBP S2 group did not present a significant difference (p<0.05), its feed intake value was the highest of all the groups. Additionally, the broilers fed with SH S1 showed a significantly lower (p<0.05) weight gain in comparison with the control. No significant differences in the feed conversion ratio were found (p<0.05) in comparison with the control. Even though the SDBP S1 group had the lowest feed conversion ratio during the growth phase and SDBP S2 over the whole period of rearing. The only significant difference (p<0.05) for this parameter was found between treatments, SDBP S1 and SH S1.

Table 8: Body weight gain, feed intake and feed conversion ratio of each treatment during all phases and over the whole period

Treatment/time	Initial phase	Growth phase	Final phase	Whole period
Feed intake (g)				
Control	427.960^{b}	1370.960^{a}	2352.810^{a}	4151.730^{a}
SDBP S1	418.840^{c}	1343.690^{b}	2333.370^{ab}	4095.890^{b}
SDBP S2	425.700^{b}	1379.910^{a}	2355.830^{a}	4161.450^{a}
SH S1	418.510^{c}	1343.660^{b}	2327.720^{b}	4089.890^{b}
SH S2	434.020^{a}	1359.970^{ab}	2349.400^{ab}	4143.390^{a}
p-value	0.000	0.001	0.007	0.000
Body weight gain (g)				
Control	327.570^{ab}	886.230^{a}	1327.110^{b}	2540.910^{ab}
SDBP S1	321.890^{ab}	875.680^{ab}	1326.230^{b}	2523.810^{b}
SDBP S2	331.870^{a}	885.470^{a}	1381.140^{a}	2598.480^{a}
SH S1	318.480^{b}	845.210^{b}	1283.290^{b}	2446.980^{c}
SH S2	330.280^{a}	866.360^{ab}	1336.570^{b}	2533.200^{ab}
p-value	0.005	0.005	0.000	0.000
Feed conversion ratio				
Control	1.310	1.550^{ab}	1.770^{a}	1.630^{ab}
SDBP S1	1.300	1.530^{b}	1.760^{ab}	1.620^{b}
SDBP S2	1.280	1.56^{0ab}	1.710^{b}	1.600^{b}
SH S1	1.310	1.590^{a}	1.820^{a}	1.670^{a}
SH S2	1.310	1.570^{ab}	1.760^{ab}	1.640^{ab}
p-value	NS	0.011	0.002	0.003

Values are means. Mean values with different letters in the same column differ significantly at p<0.05. NS: No significance at p<0.05

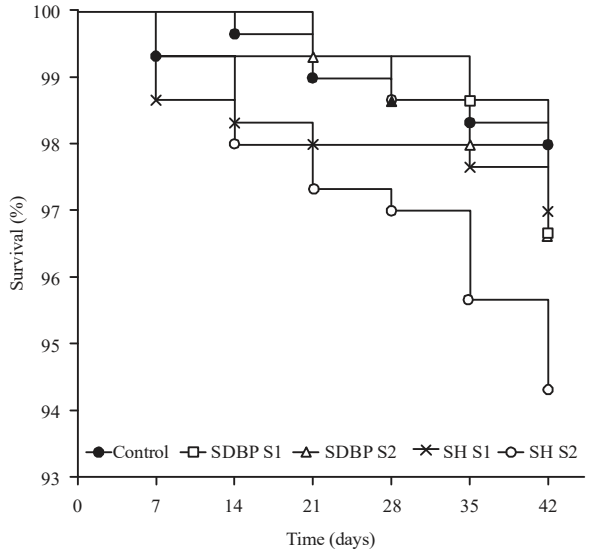

Fig. 1: Kaplan-Meier survival curves that show the effect of the treatments on the survival percentage of broilers

In the final phase, broiler chickens fed a SH S1 diet showed a significantly lower (p<0.05) feed intake by 1.09%, as compared to the control and SDBP S2. Furthermore, the weight gain and the feed conversion ratio was significantly higher and lower (p<0.05), respectively, in broilers fed with SDBP S2.

The survival ratio was not significantly dependent on the experimental diets (Log Rank p<0.05). However, as shown in Fig. 1, the group of animals fed with SDBP S2 presented a higher survival percentage during the first three weeks of rearing. At the end of the experiment, the broilers fed with SH S2 diet had the lowest survival ratio and the control group the highest.

DISCUSSION

It is well established that without vaccination, the initial antibody titers and maternal antibodies decrease in the first 7-10 days of the chick's life until the titers decline below the protection level between 15-20 days[28-30]. In this study, that tendency was evident for the APV antibody titers in the broiler chicken groups studied, due to the absence of a vaccination program for the virus. However, the maternal antibody titers in the first week for the groups of broilers fed with SDBP S2 and SH S1 had less of a significant decrease than the other groups, which might demonstrate a higher stability of the antibodies, probably as a result of the action of the immunomodulators. Selegean *et al.*[31] found a similar effect when studying the influence of a polysaccharide extract from *Pleurotus ostreatus* against the infectious bursal disease virus in unvaccinated broilers[31].

The measurement of immune organ weight is a well-known method for determining the chicken's immune status[32]. Here, the inclusion of SDBP and SH in the diet of the broilers had no significant effect on the relative lymphoid organ weights and the ratios between the different lymphoid organ weights. These results are in accordance with the

findings of Lei and Kim[32] and Zhou *et al.*[33] who studied natural supplements in broilers with a comparable composition to SDBP and SH (supplements such as egg powder rich in immunoglobulin G similar to the composition of SDBP and chitooligosaccharides that are the result from the hydrolysis of chitosan, the main component of SH).

Even though the ratios between the different lymphoid organ weights showed no significant differences, the values of the bursa: spleen ratio were proof that the experimental flock presented adequate immunocompetence, because according to Perozo-Marin *et al.*[34], a value greater than 2 for the ratio bursa:spleen is a sign of the immune system's competence.

The impact of environmental, nutritional and pathological stresses are commonly assessed through changes in hematological parameters[35]. In this study, the differences found in the percentages of immune cells during the whole period of rearing in comparison to the daily established percentages for Cobb broilers[36], the reversal of the normal tendency of the number of lymphocytes and heterophils on days 7 and 28 and the increment of the H:L ratio on the same days are signals of the presence of an inflammatory process. It is estimated that the stimulation of this kind of process is the result of an adaptation to stress. In this case, the stress is an immune challenge originating from rearing the animals in reused litter[37]. Adamu *et al.*[38] demonstrated that in broilers, reused and untreated litter generates a higher white cell count than reused litter treated with a disinfectant.

Considering the hematological parameters in the different study groups, statistical differences were found on days 7 and 42 for lymphocytes, heterophils and H:L and on day 14 for lymphocytes and monocytes. These significant differences indicate that in the first week, SDBP S2 generated better results in terms of percentages of white cells in the presence of a challenge. The same happened in the sixth week, not only with SDBP S2 but also with SDBP S1 and SH S1. McWilliams[39] determined that a constant level of lymphocytes under conditions of stress, in this case a smaller decrease of such cells, is an indicator that the birds are less susceptible to the presence of a stressor. However, our results differ from what Jamroz *et al.*[40] obtained. These authors did not find significant differences in the percentage of lymphocytes and monocytes when evaluating the effect of porcine plasma on the elements of the immune system of broilers reared under controlled conditions.

Both the inclusion of SDBP and SH with the lowest concentration in the whole cycle and in the initial phase, respectively, showed a significant increase in the feed intake, as seen in the results of Okoye *et al.*[41] In the initial phase of rearing broilers, they found a significant increment of feed intake when shrimp waste meal was added to their diet. Additionally, Henn *et al.*[42] determined that a diet including spray-dried porcine plasma in the same animals increased their feed intake only in the final phase. However, Campbell *et al.*[13] and Mahata *et al.*[43] disagree with these results. They found that the inclusion of both products, separately, in broilers' diets didn't significantly affect their feed intake.

The reason for the increment of feed intake is not totally elucidated. Pierce *et al.*[44] studied animal plasma in pigs and stated that the specific mechanism that influences this parameter is not clear. Various authors attribute the increase to different factors such as bird management, feed organoleptic characteristics and the environment[45-48]. In this study, there is not enough evidence to determine the reason for the increment. The management, environment and feed organoleptic characteristics were similar in the control and in all the treatments.

SDBP S2 treatment showed better values for body weight gain and feed conversion ratio in the whole cycle of rearing and in all the development phases, with the exception of the growth phase. In the latter, the control had the best body weight gain and SDBP S1 had the best feed conversion ratio. SH S1 was the least effective treatment in both parameters during all the experimental time period.

Previous studies have described that including animal plasma in the diet of different animals (poultry, pigs, calves etc.) improves their performance, especially in non-sanitary or challenging environments where its action is enhanced[13,49]. Campbell *et al.*[19] confirmed these results when studying the effect of porcine plasma on the performance of broilers. They observed that the immunomodulator improved the body weight gain and feed conversion ratio, which agrees with what was obtained in our experiment. In the aforementioned study, the authors postulated that this improvement is related to the effect of the immunomodulator on the level of stimulation of the immune system[19]. Stimulating the immune system activates processes that require energy consumption, so the availability of energy is reduced for productive functions such as growth[50]. Torrallardona[17] concluded that activating the immune system increases proinflammatory cytokines that inhibit food consumption and animal growth.

Other studies stated that the use of animal plasma improves animal health and hence performance due to its composition[17]. Immunoglobulins provide antimicrobial protection and reduce the activation of the intestinal immune

system in pigs and rats[17]. Glycoproteins prevent antigenic binding at the moment in which pathogens enter the body[17]. Moreover, bovine plasma enhances intestinal health and morphology, keeping pathogens from crossing the intestinal barrier[17,51]. Therefore, the overstimulation of the immune system is minimized and energy expenditure is decreased[19,42].

Shrimp hydrolysate has been described as having an important effect on body weight gain and feed conversion ratio in broilers[43]. These authors observed that despite the significant differences in these parameters, this product, used up to a certain concentration, generates an effect in animal performance similar to the control treatment; these results correspond with our findings. However, Bui *et al.*[24] observed a significant improvement in animal growth, proving the effect of the shrimp hydrolysate on the performance of the red sea bream. The authors attributed this improvement to the nature of the hydrolysate. The peptides of low molecular weight in composition are easily assimilated, allowing for an improvement in the absorption of protein.

Therefore, the effect of the bovine plasma in animal performance is demonstrated by the way in which it reinforces animal immunity and intestinal morphology. While the effect of shrimp hydrolysate is more linked to its ability to enhance the absorption of the intestine. It should be noted that bovine plasma generates better results in the presence of an immune challenge, specifically in the case of this research, the presence of reused litter[49]. It appears that the presence of these challenging conditions does not modify the effect of shrimp hydrolysate on animal performance. The results obtained in this study do not differ from those reviewed in the literature in the absence of an immunological challenge.

Even though not all the comparisons between S1 and S2 of both immunomodulators were significantly different in all phases of rearing, the treatments with lower concentration showed better results for all the performance parameters. Other authors have found different results when studying various concentrations of spray-dried bovine plasma in broilers. Campbell *et al.*[13] arrived at the conclusion that better performance results are reached with a higher concentration of bovine plasma (1.25%). Their work was performed under an adequate adjustment of the diet in order to maintain a similar amino acid content in all treatments[13]. A high concentration of animal plasma can generate nutritional imbalance, especially of amino acids and therefore decrease the productive responses[17]. In all probability, a protein imbalance of the diet in the initial phase of our study occurred when working with the high concentration of bovine plasma. The performance variables might have been affected by the amino acid imbalance. The animal, in order to adequately fulfil physiological functions and manifest suitable performance, has to be fed with a diet adapted to the ideal protein concept to reach a nutritional balance[42-53].

Campbell *et al.*[19] studied the effect of porcine plasma on the performance of broilers and observed an increase in animal growth only in the initial phase. It is suggested that the antibody portion of the animal plasma compensates for the immature state of GALT and enhances protection against pathogens to avoid the suppression of the performance. These results do not agree with our research. However, it is known that when porcine or bovine plasma is included in animals' diets from early stages, the development of the immune and intestinal systems improves and the performance enhances in the subsequent phases of rearing[19].

Our experiment proved that the addition of SH and SDBP in the diet of broilers does not improve their survival. Other authors found the same results when studying the effect of spray-dried bovine and porcine plasma and flour made with shrimp by-products in broilers and the effect of SH in tilapia fish[7,42,46,54].

The studies in which different concentrations of shrimp hydrolysate or shrimp waste meal were used in the diet of broilers have suggested that up to a certain concentration, the effect on animal performance is comparable to that of the control group. At a higher concentration, a decrease in performance parameters is observed[43,54,55]. Similar results were found in our experimental work, where the strategy of lower concentration (S2) yielded better performance. However, it is noted that the aforementioned investigations do not coincide with the maximum concentration at which the product can be used (8-25%). Mahata *et al.*[43] established that it is possible to use the immunomodulator up to a concentration of 8%. Ingweye *et al.*[54] and Okonkwo *et al.*[55] recommended applying the shrimp waste meal up to a concentration of 5 and 25%, respectively, considering that the product was used as a macro-ingredient in the diet, different from the hydrolysate that is a micro-ingredient. According to Leal *et al.*[46], the different results in these investigations could be attributed to the variation in the products' composition, which depends on the method in which they were obtained and their conditions. The high amount of fiber, ash and chitin in shrimp meal influences the digestibility and absorption of nutrients because they can become a physical barrier between digestive enzymes and lipids or proteins, thus hindering the shrimp meal's proper use[43,46].

The immunomodulatory effect of bovine plasma and shrimp hydrolysate was more evident on the percentage of

white cells studied than on the rest of the immunologicals parameters analyzed, especially when there was evidence of stress caused by an immune challenge. During the initial weeks, SDBP S2 showed better immune cell percentages than the other groups and during the final weeks, the treatments with the best percentages were SDBP S2, SDBP S1 and SH S2. The addition of shrimp hydrolysate to the diet of broilers does not generate a positive effect on animal performance. The lower concentration (S2) acts in a similar way to the control and the higher concentration (S1) shows a negative effect on performance. Bovine plasma in a lower dosing strategy (S2) produces a better performance throughout the fattening cycle as compared to the control. The mortality of the flock was not affected by the inclusion of either of the two products.

ACKNOWLEDGMENTS

Authors would like to thank Integracion Avicola Oro S.A. for funding and Biolaboratory Immunological for technical support.

REFERENCES

1. Orellana, J., 2015. Indicadores del sector avicola del Ecuador. Avicultura Ecuatoriana, 213: 4-4.
2. Herrera, H.J.M., 2007. Laboratorio, herramienta clave en la industria avicola. Procesadora Nacional de Alimentos (PRONACA), Quito, Ecuador. http://amevea-ecuador.org/web_antigua/datos/LaboratorioDR%5B1%5D._HECTOR_MONTILLA_.PDF
3. Kinung'hi, S.M., G. Tilahun, H.M. Hafez, M. Woldemeskel, M. Kyule, M. Grainer and M.P.O. Baumann, 2004. Assessment of economic impact caused by poultry coccidiosis in small and large scale poultry farms in Debre Zeit, Ethiopia. Int. J. Poult. Sci., 3: 715-718.
4. Gatne, M.M., R. Patil, K. Ravikanth, M. Shivi and D.S. Rekhe, 2010. Evaluation of immunodulatory effect of stresroak premix in broiler chick. Vet. World, 3: 122-125.
5. Butcher, G. and M. Yegani, 2010. Bioseguridad para la industria avicola. http://www.elsitioavicola.com/articles/1825/bioseguridad-para-la-industria-avacola/
6. Bagust, T.J., 2012. Salud de las aves de corral y control de enfermedades en los paises en desarrollo: Bioseguridad de los centros de produccion y estrategias de apoyo para la prevencion y control de enfermedades. FAO Revision del Desarrollo Avicola. http://www.fao.org/ docrep/016/al733s/al733s00.pdf
7. Abeysekera, A.M., 1998. Immunomodulators from medicinal plants used in Sri Lanka. Vidyodaya J. Sci., 7: 69-80.
8. Mukherjee, P.K., N.K. Nema, S. Bhadra, D. Mukherjee, F.C. Braga and M.G. Matsabisa, 2014. Immunomodulatory leads from medicinal plants. Indian J. Tradit. Knowl., 13: 235-256.
9. El Enshasy, H.A. and R. Hatti-Kaul, 2013. Mushroom immunomodulators: Unique molecules with unlimited applications. Trends Biotechnol., 31: 668-677.
10. Lebish, I.J. and R.M. Moraski, 1987. Mechanisms of immunomodulation by drugs. Toxicol. Pathol., 15: 338-345.
11. Van Eys, J., 2016. Influence of nutrition on immune system. Nutriad International. http://nutriad.com/2015/01/influence-of-nutrition-on-immune-system/.
12. Donald, J., M. Eckman and G. Simpson, 2002. The impact of management on infectious diseases in broilers. Alabama Poult. Eng. Econ. Newslett., 16: 1-4.
13. Campbell, J.M., J.D. Quigley III, L.E. Russell and M.T. Kidd, 2003. Effect of spray-dried bovine serum on intake, health and growth of broilers housed in different environments. J. Anim. Sci., 81: 2776-2782.
14. Campbell, J.M., J.D. Quigley III and L.E. Russell, 2004. Impact of spray-dried bovine serum and environment on turkey performance. Poult. Sci., 83: 1683-1687.
15. Leeson, S., 2015. Spray-dried plasma assessed. Feedstuffs, August 3, 2015, pp: 16-17. https://redmidia.com/microsites/apc/imgs/APC%202%20Leeson%20article%20Feedstuffs%20August_2015.pdf
16. Beski, S.S.M., R.A. Swick and P.A. Iji, 2015. Specialized protein products in broiler chicken nutrition: A review. Anim. Nutr., 1: 47-53.
17. Torrallardona, D., 2010. Spray dried animal plasma as an alternative to antibiotics in weanling pigs-a review. Asian-Australasian J. Anim. Sci., 23: 131-148.
18. Quigley, III J.D. and M.D. Drew, 2000. Effects of oral antibiotics or bovine plasma on survival, health and growth in dairy calves challenged with *Escherichia coli*. Food Agric. Immunol., 12: 311-318.
19. Campbell, J.M., L.E. Russell, J.D. Crenshaw and H.J. Koehnk, 2006. Effect of spray-dried plasma form and duration of feeding on broiler performance during natural necrotic enteritis exposure. J. Applied Poult. Res., 15: 584-591.
20. Gallois, M. and I.P. Oswald, 2008. Immunomodulators as efficient alternatives to in-feed antimicrobials in pig production? Arch. Zootech., 11: 15-32.
21. Campbell, J.M., J.D. Crenshaw, L.E. Russell and S.K. Hayes, 2008. Influence of dietary plasma proteins on supporting animal immunity systems. Proceedings of the 19th Annual Florida Ruminant Nutrition Symposium, January 29-30, 2008, Florida, USA., pp: 79-88.
22. Bueno-Solano, C., J. Lopez-Cervantes, O.N. Campas-Baypoli, R. Lauterio-Garcia, N.P. Adan-Bante and D.I. Sanchez-Machado, 2009. Chemical and biological characteristics of protein hydrolysates from fermented shrimp by-products. Food Chem., 112: 671-675.

23. Khosravi, S., H.T.D. Bui, S. Rahimnejad, M. Herault, V. Fournier, J.B. Jeong and K.J. Lee, 2015. Effect of dietary hydrolysate supplementation on growth performance, non-specific immune response and disease resistance of olive flounder (*Paralichthys olivaceus*) challenged with *Edwardsiella tarda*. Aquacult. Nutr., 21: 321-331.
24. Bui, H.T.D., S. Khosravi, V. Fournier, M. Heraulta and K.J. Lee, 2014. Growth performance, feed utilization, innate immunity, digestibility and disease resistance of juvenile red seabream (*Pagrus major*) fed diets supplemented with protein hydrolysates. Aquaculture, 418-419: 11-16.
25. Wu, G.J. and G.J. Tsai, 2004. Cellulase degradation of shrimp chitosan for the preparation of a water-soluble hydrolysate with immunoactivity. Fish. Sci., 70: 1113-1120.
26. Abdel-Fattah, S.A., M.H. El-Sanhoury, N.M. El-Mednay and F. Abdel-Azeem, 2008. Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. Int. J. Poult. Sci., 7: 215-222.
27. Wang, X., Q. Zhou, J. Shen, J. Yao and X. Yang, 2015. Effect of difference doses of Newcastle disease vaccine immunization on growth performance, plasma variables and immune response of broilers. J. Anim. Sci. Biotechnol., Vol. 6, No. 1. 10.1186/s40104-015-0019-y
28. Faulkner, O.B., C. Estevez, Q. Yu and D.L. Suarez, 2013. Passive antibody transfer in chickens to model maternal antibody after avian influenza vaccination. Vet. Immunol. Immunopathol., 152: 341-347.
29. Leeson, S. and J.D. Summers, 2010. Broiler Breeder Production. Nottingham University Press, Nottingham, UK., ISBN-13: 9781904761792, Pages: 338.
30. Peres, M.F., A.S. Carrijo, J.A. Higa and J.M. de Oliveira, 2006. [Serological evidence of avian pneumovirus infections in broiler flocks in counties of Mato Grosso do Sul]. Pesq. Vet. Bras., 26: 254-258, (In Portuguese).
31. Selegean, M., M.V. Putz and T. Rugea, 2009. Effect of the polysaccharide extract from the edible mushroom *Pleurotus ostreatus* against infectious bursal disease virus. Int. J. Mol. Sci., 10: 3616-3634.
32. Lei, Y. and I.H. Kim, 2013. Effect of whole egg powder on growth performance, blood cell counts, nutrient digestibility, relative organ weights and meat quality in broiler chickens. Livest. Sci., 158: 124-128.
33. Zhou, T.X., Y.J. Chen, J.S. Yoo, Y. Huang and J.H. Lee *et al*., 2009. Effects of chitooligosaccharide supplementation on performance, blood characteristics, relative organ weight and meat quality in broiler chickens. Poult. Sci., 88: 593-600.
34. Perozo-Marin, F., J. Nava, Y. Mavarez, E. Arenas, P. Serje and M. Briceno, 2004. Caracterizacion morfometrica de los organos linfoides en pollos de engorde de la linea Ross criados bajo condiciones de campo en el estado Zulia, Venezuela. Rev. Cient. Fac. Cien. Vet., 14: 217-226.
35. Okuneye, O.J., A.T. Adeoye, N.O. Oloso, O.F. Adekunle and O.G. Fasanmi, 2016. Performance and physiological responses of *Salmonella enteritidis* challenged broilers fed diets containing antibiotic, probiotic and aromabiotic. J. Dairy Vet. Anim. Res., 3: 106-110.
36. Talebi, A., S. Asri-Rezaei, R. Rozeh-Chai and R. Sahraei, 2005. Comparative studies on haematological values of broiler strains (Ross, Cobb, Arbor-acres and Arian). Int. J. Poult. Sci., 4: 573-579.
37. Shanmugasundaram, R., M.S. Lilburn and R.K. Selvaraj, 2012. Effect of recycled litter on immune cells in the cecal tonsils of chickens. Poult. Sci., 91: 95-100.
38. Adamu, Y.A., A. Usman, U.M. Mera, M.B. Abubakar, A. Bello and M.A. Umaru, 2015. Haematological values of broilers managed on titanium dioxide treated litter. Int. Invention J. Med. Med. Sci., 2: 139-143.
39. McWilliams, L.H., 2008. Physiological impact of hematocrit level during stress in broilers. Ph.D. Thesis, Department of Poultry Science, Mississippi State University, USA.
40. Jamroz, D., A. Wiliczkiewicz, J. Orda, J. Kuryszko and T. Stefaniak, 2012. Use of spray-dried porcine blood by-products in diets for young chickens. J. Anim. Physiol. Anim. Nutr., 96: 319-333.
41. Okoye, F.C., G.S. Ojewola and K. Njoku-Onu, 2005. Evaluation of shrimp waste meal as a probable animal protein source for broiler chickens. Int. J. Poult. Sci., 4: 458-461.
42. Henn, J.D., L. Bockor, M.S. Vieira, A.M.L. Ribeiro and A.M. Kessler *et al*., 2013. Inclusion of porcine spray-dried plasma in broiler diets. J. Applied Poult. Res., 22: 229-237.
43. Mahata, M.E., A. Dharma, H.I. Ryanto and Y. Rizal, 2008. Effect of substituting shrimp waste hydrolysate of *Penaeus merguensis* for fish meal in broiler performance. Pak. J. Nutr., 7: 806-810.
44. Pierce, J.L., G.L. Cromwell, M.D. Lindemann, L.E. Russell and E.M. Weaver, 2005. Effects of spray-dried animal plasma and immunoglobulins on performance of early weaned pigs. J. Anim. Sci., 83: 2876-2885.
45. Tucker, J.L., V.D. Naranjo, T.D. Bidner and L.L. Southern, 2011. Effect of salmon protein hydrolysate and spray-dried plasma protein on growth performance of weanling pigs. J. Anim. Sci., 89: 1466-1473.
46. Leal, A.L.G., P.F. de Castro, J.P.V. de Lima, E. de Souza Correia and R. de Souza Bezerra, 2010. Use of shrimp protein hydrolysate in Nile tilapia (*Oreochromis niloticus* L.) feeds. Aquacult. Int., 18: 635-646.
47. Ferket, P.R. and A.G. Gernat, 2006. Factors that affect feed intake of meat birds: A review. Int. J. Poult. Sci., 5: 905-911.
48. Applegate, T.J., 2012. Factors affecting feed intake-what do we know? Proceedings of the Arkansas Nutrition Conference, September 4-6, 2012, Rogers, AR., USA., pp: 1-8.

49. SBregendahl, K., D.U. Ahn, D.W. Trampel and J.M. Campbell, 2005. Effects of dietary spray-dried bovine plasma protein on broiler growth performance and breast-meat yield. J. Applied Poult. Res., 14: 560-568.
50. Campbell, J.M., J.D. Quigley III, L.E. Russell and L.D. Koehnk, 2004. Efficacy of spray-dried bovine serum on health and performance of turkeys challenged with *Pasteurella multocida*. J. Applied Poult. Res., 13: 388-393.
51. Sun, Z., Q. Ma, Z. Li and C. Ji, 2009. Effect of partial substitution of dietary spray-dried porcine plasma or fishmeal with soybean and shrimp protein hydrolysate on growth performance, nutrient digestibility and serum biochemical parameters of weanling piglets. Asian-Australasian J. Anim. Sci., 22: 1032-1037.
52. Wijtten, P.J.A., R. Prak, A. Lemme and D.J. Langhout, 2004. Effect of different dietary ideal protein concentrations on broiler performance. Br. Poult. Sci., 45: 504-511.
53. Campos, A., S. Salguero, L. Albino and H. Rostagno, 2008. Aminoacidos en la nutricion de pollos de engorde: Proteina ideal. Anais do III CLANA-Congresso do Colegio Latino-Americano de Nutricao Animal, November 18-21, 2008, Cancun, Mexico.
54. Ingweye, J.N., B.I. Okon, J.A. Ubua and A.I. Essien, 2008. Performance of broiler chickens fed fish and shrimp wastes. Asian J. Anim. Sci., 2: 58-63.
55. Okonkwo, A.C., I.P. Akpan and L.J. Isaac, 2012. Performance and carcass characteristics of finisher broilers fed shrimps waste meal. Agric. J., 7: 270-272.