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Research Article
Responses of Broiler Chicks to *In ovo* Feeding of a Novel Processed Soy Protein Product

[1,2]Apeh Akwu Omede, [1]Momenuzzaman Bhuiyan, [1]Fakrul Islam and [1]Paul Ade Iji

[1]School of Environmental and Rural Science, University of New England, Armidale, 2351 NSW, Australia
[2]Department of Animal Production, Kogi State University, PMB 1008, Anyigba, Kogi State, Nigeria

Abstract

Background and Objective: In recent times, interest in early nutrition has increased as a method of mitigating negative effects of delayed access to feed in birds. One of the methods used is *in ovo* feeding. This study aimed to investigate the effect of *in ovo* feeding (IOF) of a novel product, Hamlet Protein Avistart (HPA), on hatchability, chick quality and early performance of broiler chicks by using hatching eggs from two strains of broiler breeders. **Materials and Methods:** The IOF solution was prepared by suspending HPA in Milli Q water, serially diluted from 150-18.75 mg mL^{-1}, heated and centrifuged to obtain the supernatants used for IOF on Embryonic Day (ED) 17. Eggs with live embryo were distributed to seven *in ovo* feeding (IOF) groups: (control group (intact eggs), sham group (perforated only, SH) and groups injected with either 1 mL Milli Q water (M Q water), 150 (top HPA, T-HPA), 75 (high HPA), 37.5 (medium HPA, M-HPA) or 18.75 (low HPA, L-HPA) mg mL^{-1} HPA solution in four replicates per strain. Hatched chicks were distributed to the IOF groups and reared till 10 days after measuring hatchability and chick quality. Feed intake, body and visceral organs weight were recorded and samples of jejunum and whole pancreas collected for digestive enzyme activities at 5 and 10 days. Data were analysed separately for each strain by one-way ANOVA using Minitab. **Results:** Hatchability rate was improved in the M-HPA and the SH groups and lowest in the MQ group. At hatch, body weight and weight of bursa were highest in the T-HPA group in Cobb 500. The HPA solution affected ($p<0.05$) the shank-to-toe length only in Ross 308. At 5 day, gross responses in chicks improved with higher HPA concentration in the solution, so also the weight of small intestine and gizzard plus proventriculus in Cobb 500 and Ross 308, respectively. At day 10, body weight gain and feed intake were higher ($p<0.05$) in the SH group and the T-HPA groups, respectively in Cobb 500 broiler chicks. The activity of enzyme was not affected by the IOF treatments. **Conclusion:** This study showed that the product has potential to improve hatching weight of broiler chicks and early post-hatch performance till 10 days.

Key words: Body weight, broiler strains, early nutrition, hatchability, *in ovo* feeding, soy protein

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Corresponding Author: Paul Ade Iji, School of Environmental and Rural Science, University of New England, Armidale, 2351 NSW, Australia
Tel: +6167732082

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

INTRODUCTION

Wide hatching window in commercial hatcheries, industry practices and current standard feeding procedure are major causes of delayed access to feed and water in neo-natal chicks. Such delays for more than 24 h have been implicated for negative effects on both turkeys and broiler chickens[1,2], resulting in more susceptibility to pathogens[3] and body weight (BW) loss[4]. It is reported that in birds suffering delayed access to feed and water, there was constrained development of significant muscles and organs, such as the gastrointestinal tract (GIT), the immune system[3,5] and the pectoral muscle[6]. As a result of these numerous problems associated with delayed access to feed, researchers have been working on developing both technical and nutritional strategies that can enhance early access to feed in neo-natal with indirectly feeding the developing embryo before hatch, i.e., *in ovo* feeding (IOF) as one of the prominent strategies.

The overall aim of IOF is to supply extra nutrients to the developing embryo during the late-incubation period. Researchers have made scientific claims or postulations of the potential of IOF to support different physiological systems and responses within the developing embryo, to help maximize performance posthatch and possibly, till market age. To substantiate these claims, several nutrients, supplements or their combinations[6-8] have been administered *in ovo* during late-term embryonic development. These nutrients are reported to be utilised for energy[9], building tissue or stored as energy in the form of glycogen[10] and can stimulate the intestinal development of hatching chicks, evidenced by an increased villus length and improvement in disaccharide digestion[11]. Akshat *et al.*[12] concluded that humoral or cellular immunity in chicks can be modulated by IOF of iron, whereas, selenium supplementation can modulate adaptive or cellular immunity in broiler chickens, while vitamin C has been used as anti-stress agent[13].

However, the use of IOF nutrients and supplements is limited by inconsistencies in its results, which have slowed the rate of adoption of the technique by the poultry industry. A major limitation is in its effect on hatchability, chick quality and sustained growth responses till market age. For example, hatching time and hatchability traits were impaired by the administration of L-carnitine *in ovo*[8,14]. When carbohydrates were added to a commercial diluent for the *in ovo* injection of broiler hatching eggs, embryonic metabolism[15] and again, hatchability[16,17] were lowered. Kadam *et al.*[18] reported that threonine treatment had no significant effect on pepsin activity in the proventriculus or on amylase and trypsin activities in the pancreas, while Dos Santos *et al.*[19] concluded that the *in ovo* inoculation of nutrients to 18 days old embryos did not influence live performance or carcass traits.

Therefore, the search continues for a supplement that supports optimal production performance of birds without compromising other traits like hatchability. Again, because there are several potential IOF nutrients and supplements that vary in physical properties and chemical composition, resulting in varying observations and conclusions, it becomes difficult to predict the response to any of these nutrients or supplements. Hence, the choice of nutrients, products or compounds to be administered *in ovo* deserves further study[10,19]. This study will provide knowledge in the development and identification a novel product for use in IOF from soy protein to mitigate delayed access to feed and with potential to improve growth and performance in broiler chicks.

The objective of the present study was to investigate the effect of IOF of a novel soy protein product, Hamlet Protein Avistart (HPA), on hatchability, chick quality and post-hatch performance to day 10 of broiler chicks from Ross 308 and Cobb 500 strains.

MATERIALS AND METHODS

Animal ethics: This experiment was approved by the Animal Ethics Committee of the University of New England (Approval No. AEC13-168). Health and animal husbandry practices complied with the code of practice for the use of animals for scientific purposes issued by the Australian Bureau of Animal Health[20].

Egg collection and incubation: Hatching eggs (420 each) were obtained from Ross 308 (51 weeks) and Cobb 500 (35 weeks) broiler breeder flocks for this experiment. The average weight of eggs was recorded and maintained at 60±0.5 g. Eggs were incubated at 37.5°C and relative humidity of 60% until Embryonic Day (ED) 17 after which humidity increased to 75% until hatch. The eggs were candled on ED day 16 to sort out dead and infertile eggs. The remaining eggs were then re-weighed and evenly distributed to the different IOF treatment groups. Sixty fertile eggs (4 replicates of 15 eggs per replicate) from each strain of eggs were evenly distributed to each group.

Preparation of IOF supplements and experimental treatments: A high-quality processed soy protein product, HPA, with known nutrient composition was identified as the

test material for IOF. The product was suspended in Milli Q (MQ) water and serially diluted from 150-9.375 mg mL^{-1} in centrifuge tubes. Samples were heated for 60 min at 80°C in a water bath. Samples were then vibromixed and centrifuged at 3200 rpm (Model-Beckmans Bench top Centrifuge, Allegra™ 6R, USA) for 10 min and the supernatants transferred to new tubes and stored at 4°C until used for *in ovo* administration.

There were seven experimental IOF treatments as follows: A control group with intact eggs, a sham group (perforated but without any injected solution, SH), a group injected with 1 mL deionised water (MQ water), a group injected with 150 mg mL^{-1} HPA solution (top HPA, T-HPA), a group injected with 75 mg mL^{-1} HPA solution (high HPA, H-HPA), a group injected with 37.5 mg mL^{-1} HPA solution (medium HPA, M-HPA) and a group injected with 18.75 mg mL^{-1} HPA solution (low HPA, L-HPA).

***In ovo* feeding administration:** *In ovo* feeding was administered on ED day 17 of incubation. To avoid imposing cold shock/stress on the embryos on administration of the solution, the solutions were placed in the incubator for 3 h at 37.5°C[15] to bring the temperature of the solutions to the prevailing temperature within the incubator before injecting them into the eggs.

To inject the IOF solutions, an 18-gauge×1½ inch hypodermic needle was used to first drill a hole into the air cell from the broad end of the egg to locate the amniotic cavity. After that, a 21-gauge×1½ inch hypodermic needle on a 1 mL syringe was carefully used to inject the solutions, locating the amnion before injecting the solution. This was repeated for all the IOF treated groups. For the sham treatment group, a hole was drilled without injecting any solution. All injected eggs were left open for the rest of the incubation time.

Hatchability, chick quality and chick management: After hatch, hatchability and chick quality (BW at hatch, yolk sac yolk free body mass, chick length (CL), shank-to-toe length, toe length, BW:CL ratio, weight of internal organs) records were taken from one bird per replicate and five (unsexed) were transferred to a controlled environment room and distributed according to treatments during IOF administration (per replicate). The average initial weight of the chicks were 45.12±0.35 and 45.32±0.29 for Cobb 500 and Ross 308, respectively. Chicks were reared in multi-tiered brooder cages (600×420×23 cm). The room temperature was gradually decreased from 33°C on day 1 to 26±1°C at 10 days. Chicks were reared for 10 days, within which period they were provided with a common commercial type starter diet following the Cobb 500 and Ross 308 nutrition specification guides for each strain, respectively[21,22]. Water was provided *ad libitum*.

At 5 and 10 days, feed intake (FI) and live BW were recorded for determination of average FI and BW gain. Feed conversion ratio (FCR; feed intake/weight gain) was calculated for each treatment across the strains. At 5 and 10 days, one bird per replicate was slaughtered and the heart, small intestine (with contents), gizzard plus proventriculus, liver, spleen (day 10 only), pancreas and bursa of Fabricius of sampled birds were recorded. The relative organ weight was calculated as mass per unit live body weight (g/100 g of live body weight).

Tissue protein content and digestive enzyme activities: To evaluate the activity of digestive enzymes and protein concentration, the jejunal tissue was processed according to the method described by Susbilla *et al.*[23]. The pancreas was processed in a similar way, except that the pancreas tissue was entire homogenised. The homogenate was then centrifuged at 30,000×g (Avanti® Centrifuge, Model J-E 369001, Beckman Coulter, Inc., USA) for about 15 min at 4°C. Subsamples of supernatant were then taken in duplicate of 1.5 mL into Eppendorf tubes and stored in a freezer (-20°C) until assayed for enzyme activities.

The specific activities of jejunal maltase and sucrase were assessed by incubation with fixed substrate concentrations as standardized for poultry by Iji *et al.*[24], while aminopeptidase activity was assessed as described by Caviedes Vidal and Karasov[25]. The pancreatic trypsin and chymotrypsin activities were assessed using methods described by Erlanger *et al.*[26] and modified by Caviedes-Vidal and Karasov[25]. The concentration of protein in both the jejunal and pancreatic tissue homogenate were measured using the Coomassie dye-binding procedure described by Bradford[27].

Data analyses: All data collected were analyzed by one-way analysis of variance (ANOVA) using Minitab® 17[28]. Data from Ross 308 broiler breeder strain were analyzed separately from those of Cobb 500 broiler breeder strain. Differences between mean values were determined following comparisons using Fisher's multiple range test at $p \leq 0.05$.

RESULTS

Effect of IOF supplementation of HPA solution on hatchability: The results of the effect of IOF supplementation of HPA solution on hatchability from the strains of broilers are

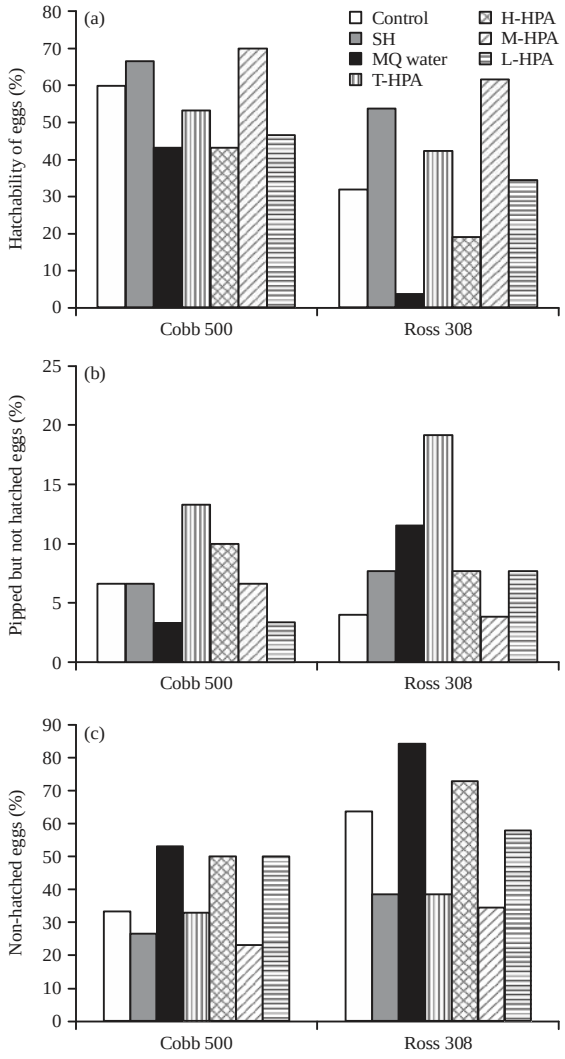

Fig. 1(a-c): Effect of *in ovo* feeding of different concentrations of HPA solutions on hatchability

presented in Fig. 1a-c. Hatchability was mostly improved in both strains in the M-HPA group and the SH group (Fig. 1a). The lowest hatchability was observed in the MQ and H-HPA groups in Cobb 500 and the MQ group (<5% hatchability) in Ross 308. Pipped-but-not-hatched eggs were mostly observed in the T-HPA group in both strains (Fig. 1b). Most unhatched eggs were found in the MQ group in both strains and also in the H-HPA group in Ross 308 (Fig. 1c).

Effect of IOF supplementation of HPA solution on chick quality: In Cobb 500, BW at hatch was highest in the T-HPA group, although this was only significantly ($p<0.05$) higher than the BW of the MQ and L-HPA (Table 1). The control, SH, H-HPA and T-HPA groups were statistically similar ($p>0.05$) in yolk-free body mass but these were significantly ($p<0.05$) higher than the values in the MQ, H-HPA and L-HPA groups. Although not significantly different ($p>0.05$) from those in control, SH and T-HPA groups, chicks from the SH group were the longest ($p<0.05$) while chicks from the MQ group were the shortest. Shank-to-toe and toe lengths were lowest ($p<0.05$) in the H-HPA group. Toe length was shorter ($p<0.05$) in the H-HPA group, while there was no significant ($p>0.05$) difference in the BW:CL of the birds.

In Ross 308, due to low hatchability, data are not available for the MQ and H-HPA solution injected groups. There were no significant ($p>0.05$) differences in body weight at hatch, yolk-free body mass, yolk weight at hatch, chick length, toe length and BW:CL between the treatment groups. Significant ($p<0.05$) difference was only recorded in shank-to-toe length, with the lowest value of this trait observed in the L-HPA group.

Effect of IOF supplementation of HPA solution on chick performance to 10 days posthatch: *In ovo* supplementation of HPA solution had some significant effects on posthatch performance of broiler chicks, assessed on day 5 (Table 2) and day 10 (Fig. 2) posthatch. The day 5 posthatch performance of Cobb 500 showed that there was no significant ($p>0.05$) differences in body weight gain (BWG) of chicks among control group, sham and the *in ovo* treatment groups. However, FI was significantly ($p<0.05$) higher in the MQ group compared to other groups. The least ($p<0.05$) FI was observed in the H-HPA group. Feed conversion ratio (FCR) was mostly improved ($p<0.05$) in the SH group, with the MQ group recording the poorest ($p<0.05$) FCR.

In Ross 308, BWG in the sham and *in ovo* injected groups were significantly ($p<0.05$) higher than in the control group. Feed intake was significantly ($p<0.05$) highest in the SH group, followed by the T-HPA group, while the other injected groups were not significantly ($p>0.05$) different from the control group. Feed conversion ratio followed the same trend as FI, except that the control group was not significantly ($p>0.05$) different from the T-HPA group.

At day 10, there was not enough data to report growth responses for Ross 308. However, in the Cobb 500 broiler chicks, BWG and FI were significantly ($p<0.05$) higher in the SH group and the T-HPA groups, respectively, than in other groups at day 10 (Fig. 2). There was no significant ($p>0.05$) difference between the control group and the T-HPA group in BWG and between the control group and the SH group in FI. The poorest BWG and FI were recorded in the M-HPA group. Feed conversion ratio was significantly ($p<0.05$) affected by treatments, with the SH group being the most efficient in feed conversion, followed by the M-HPA group. The T-HPA group was the least efficient in FCR.

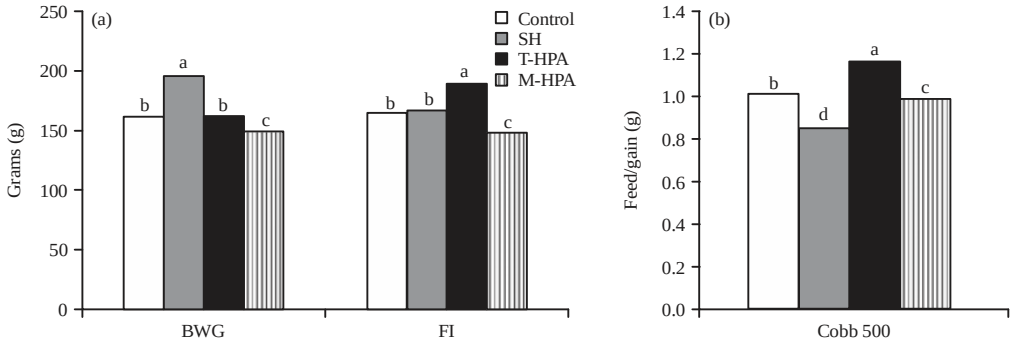

Fig. 2(a-b): Effect of IOF on gross responses of 10 days old Cobb 500 broiler chicks

Table 1: Effect of IOF on quality of neo-natal broiler chicks

				Concentration of HPA (mg mL^{-1})				
Chick quality	Control	SH	MQ water	T-HPA	H-HPA	M-HPA	L-HPA	SEM
Cobb 500								
Body weight* (g)	40.60^{ab}	42.20^{a}	37.10^{c}	43.00^{a}	39.80^{abc}	41.60^{ab}	38.80^{bc}	0.480
Yolk-free body mass (g)	37.10^{a}	37.40^{a}	32.80^{c}	38.30^{a}	33.70^{bc}	36.20^{ab}	34.30^{bc}	0.450
Yolk weight$^{\#}$ (g)	3.53	4.77	4.28	4.75	6.11	5.35	4.45	0.263
Chick length (CL) (cm)	18.90^{ab}	19.10^{a}	17.50^{d}	18.60^{abc}	17.70^{d}	18.00^{cd}	18.10^{bcd}	0.140
Shank-to-toe length (cm)	5.12^{ab}	5.15^{a}	4.78^{cd}	5.06^{ab}	4.54^{d}	4.90^{abc}	4.83^{bc}	0.048
Toe length (cm)	3.13^{a}	3.13^{a}	2.97^{ab}	3.15^{a}	2.77^{b}	3.00^{a}	3.05^{a}	0.038
BW:CL	2.15	2.21	2.14	2.13	2.28	2.35	2.14	0.031
Ross 308**								
Body weight* (g)	46.50	47.20	N/A	41.90	N/A	44.10	45.00	1.050
Yolk-free body mass (g)	40.20	41.50	N/A	37.50	N/A	38.20	39.70	1.040
Yolk weight$^{\#}$ (g)	6.33	5.76	N/A	4.36	N/A	5.83	5.26	0.337
Chick length (CL) (cm)	18.50	19.40	N/A	18.60	N/A	19.30	18.07	0.200
Shank-to-toe length (cm)	5.07^{ab}	5.25^{a}	N/A	4.97^{ab}	N/A	5.20^{a}	4.73^{b}	0.056
Toe length (cm)	3.23	3.27	N/A	3.07	N/A	3.17	3.00	0.037
BW:CL	2.52	2.43	N/A	2.25	N/A	2.28	2.50	0.046

$^{a-d}$Mean values on the same row not sharing a superscript are significantly different ($p<0.05$), *Average body weight at hatch, **Ross 308 SEM were based on analysis for IOF treatments with available data only, $^{\#}$Content+membrane, BW:CL: Body weight to chick length ratio, N/A: Not available, SEM: Standard error of mean

Table 2: Effect of IOF on growth responses of broiler chicks (1-5 days)

				Concentration of HPA (mg mL^{-1})				
Growth performance	Control	SH	MQ water	T-HPA	H-HPA	M-HPA	L-HPA	SEM
Cobb 500								
FI (g)	64.70^{bc}	63.30^{c}	83.30^{a}	73.00^{b}	52.40^{d}	63.00^{c}	56.20^{cd}	2.29
BWG (g)	84.50	120.80	94.40	104.30	81.70	101.50	91.10	1.80
FCR (g g^{-1})	0.76^{ab}	0.57^{c}	0.88^{a}	0.70^{abc}	0.64^{bc}	0.62^{bc}	0.62^{bc}	0.02
Ross 308*								
FI (g)	58.20^{c}	109.10^{a}	N/A	74.80^{b}	N/A	56.00^{c}	56.80^{c}	5.63
BWG (g)	97.30^{b}	102.50^{a}	N/A	105.20^{a}	N/A	102.80^{a}	105.00^{a}	0.81
FCR (g g^{-1})	0.60^{bc}	1.01^{a}	N/A	0.71^{b}	N/A	0.54^{c}	0.54^{c}	0.05

$^{a-d}$Mean values on the same row not sharing a superscript are significantly different ($p<0.05$), Ave: Average, BWG: Body weight gain, FI: Feed intake, FCR: Feed conversion ratio, *Ross 308 SEM were based on analysis for IOF treatments with available data only, N/A: Not available, SEM: Standard error of mean

Effect of IOF supplementation of HPA solution on the development of internal organs at hatch: At hatch, the relative weight of liver was higher ($p<0.05$) in the control group compared to the rest of the groups in Cobb 500 broiler chicks (Table 3). Gizzard and proventriculus weight was similar ($p>0.05$) in the control, SH and T-HPA groups, while the H-HPA group was only less heavier ($p<0.05$) than the control and SH groups. The weight of the bursa of Fabricius was smaller ($p<0.05$) in the H-HPA group compared to the control, SH and T-HPA groups. There was no significant ($p>0.05$) effect of treatments on the relative weights of pancreas and small intestine at hatch. In Ross 308 chicks, no significant ($p>0.05$) difference was observed in the relative weights of all internal organs across treatment groups.

Table 3: Effect of IOF on weight of internal organs (g/100 g b.wt.) of broiler chicks at hatch

				Concentration of HPA (mg mL^{-1})				
Internal organs (g)	Control	SH	MQ water	T-HPA	H-HPA	M-HPA	L-HPA	SEM
Cobb 500								
Heart	0.33	0.31	0.27	0.31	0.27	0.31	0.31	0.007
Liver	1.41^{a}	1.13^{b}	0.96^{b}	1.07^{b}	0.96^{b}	1.03^{b}	1.04^{b}	0.035
Giz+Prov	3.28^{a}	2.95^{ab}	2.49^{bc}	2.77^{abc}	2.37^{c}	2.67^{bc}	2.69^{bc}	0.080
Pancreas	0.078	0.085	0.07	0.092	0.073	0.262	0.102	0.023
Small Int	1.40	1.45	1.23	1.33	1.17	1.17	1.41	0.033
Bursa	0.048^{a}	0.045^{a}	0.030^{bc}	0.052^{a}	0.028^{c}	0.037^{abc}	0.038^{abc}	0.263
Ross 308*								
Heart	0.36	0.35	N/A	0.31	N/A	0.39	0.40	0.015
Liver	1.21	1.25	N/A	1.00	N/A	1.29	1.15	0.036
Giz+Prov	2.91	3.04	N/A	2.57	N/A	2.94	2.81	0.474
Pancreas	0.11	0.10	N/A	0.09	N/A	0.10	0.83	0.007
Small Int	1.53	1.42	N/A	1.27	N/A	1.32	1.58	0.059
Bursa	0.050	0.065	N/A	0.040	N/A	0.058	0.040	0.337

$^{a-c}$Mean values on the same row not sharing a superscript are significantly different ($p<0.05$), Giz+Prov: Gizzard and proventriculus, Int: Intestine, *Ross 308 SEM were based on analysis for IOF treatments with available data only, N/A: Not available, SEM: Standard error of mean

Table 4: Effect of IOF on relative weight of internal organs (g/100 g b.wt.) of 5 days old

				Concentration of HPA (mg mL^{-1})				
Internal organs (g)	Control	SH	MQ water	T-HPA	H-HPA	M-HPA	L-HPA	SEM
Cobb 500								
Heart	0.81	0.90	0.88	1.00	0.84	1.05	0.95	0.025
Liver	5.26	6.40	5.27	6.57	5.13	6.08	5.36	0.175
Giz+Prov	4.85	5.63	5.89	5.94	4.87	5.79	5.29	0.130
Pancreas	0.49	0.49	0.47	0.54	0.49	0.47	0.51	0.014
Small Int	5.10^{b}	6.47^{ab}	7.51^{a}	6.61^{ab}	5.45^{b}	6.70^{ab}	6.64^{ab}	0.188
Bursa	0.10	0.12	0.08	0.14	0.10	0.12	0.10	0.006
Ross 308*								
Heart	0.90^{bc}	0.84^{c}	N/A	1.04^{ab}	N/A	1.03^{ab}	1.19^{a}	0.035
Liver	5.80	5.52	N/A	6.27	N/A	6.38	6.06	0.183
Giz+Prov	5.38^{a}	4.58^{b}	N/A	5.96^{a}	N/A	5.48^{a}	5.45^{a}	0.142
Pancreas	0.54	0.52	N/A	0.57	N/A	0.63	0.50	0.024
Small Int	5.65	5.34	N/A	6.25	N/A	6.16	6.14	0.135
Bursa	0.16	0.16	N/A	0.20	N/A	0.15	0.15	0.010

$^{a-c}$Mean values on the same row not sharing a superscript are significantly different ($p<0.05$), Giz+Prov: Gizzard and proventriculus, Int: Intestine, *Ross 308 SEM were based on analysis for IOF treatments with available data only, N/A: Not available, SEM: Standard error of mean

The results of the effect of IOF supplementation of HPA solution on the internal organs of broiler chicks at early posthatch stage (5 days old) are presented in Table 4. No significant ($p>0.05$) difference was observed in all internal organs measured in Cobb 500 broiler chicks, except in small intestine, where the MQ group had a significantly ($p<0.05$) higher value of this trait than the control group and the H-HPA groups.

In Ross 308 broiler chicks, the relative weight of the heart in the L-HPA group was significantly ($p<0.05$) higher than values recorded in the control and SH groups. The relative weight of the gizzard and proventriculus was lower ($p<0.05$) in the SH group than in the control and all the *in ovo* injected groups.

The data presented in Table 5 for day 10 are only for Cobb 500, as there was not enough data to report for internal organs for Ross 308. The weight of internal organs of broiler chicks between HPA treatment was not significantly ($p>0.05$) different at day 10 for Cobb 500 broiler chicks, except in the relative weight of the heart, which was higher ($p<0.05$) in the M-HPA group than in the other groups, including the control.

Effect of IOF supplementation of HPA on tissue protein contents and digestive enzyme activities: In Cobb 500, the jejunal tissue protein concentration at 5 day was highest ($p<0.05$) in the T-HPA group and followed by the H-HPA group, while the lowest concentration was found in the control group (Table 6). There was no effect of IOF

Table 5: Effect of IOF on internal organs (g/100 g b.wt.) of 10 days old Cobb 500 broiler chicks

	Concentration of HPA (mg mL^{-1})				
Internal organs (g)	Control (mg mL^{-1})	SH (mg mL^{-1})	150 mg mL^{-1}	37.5 mg mL^{-1}	SEM
Heart	1.85^{b}	2.00^{b}	1.84^{b}	2.32^{a}	0.069
Liver	9.40	11.62	12.95	11.17	0.641
Gizzard+proventriculus	8.25	8.50	9.36	9.22	0.347
Pancreas	0.96	1.06	1.23	0.99	0.0438
Small intestine	10.86	14.16	12.98	12.49	0.438
Bursa	0.37	0.40	0.39	0.34	0.0157
Spleen	0.21	0.19	0.22	0.17	0.0099

^{a,b}Mean values on the same row not sharing a superscript are significantly different, ($p<0.05$), SEM: Standard error of mean

Table 6: Effect of IOF on tissue protein concentrations and enzyme activities of broiler chickens on 5 days of age

					Concentration of HPA (mg mL^{-1})			
Intestinal enzymes	Control	SH	MQ water	T-HPA	H-HPA	M-HPA	L-HPA	SEM
Cobb 500								
Jejunum								
Protein$^{\#}$	37.0^{b}	45.0^{ab}	46.0^{ab}	48.0^{ab}	63.0^{a}	43.1^{ab}	36.0^{b}	2.30
Maltase$^{\#\#}$	2.83	2.05	2.55	2.00	2.00	2.11	2.33	0.11
Sucrase$^{\#\#}$	0.24	0.25	0.20	0.47	0.18	0.50	0.22	0.05
AMP*$^{\#\#}$	57.3	50.0	57.0	42.5	51.0	45.3	54.0	1.86
Pancreas								
Protein$^{\#}$	28.4	25.2	27.0	37.0	36.0	25.0	28.2	1.62
CA**	2.00	1.70	1.10	2.06	2.20	2.00	2.80	0.15
Trypsin$^{\#\#}$	0.84	0.64	0.89	0.89	0.70	0.66	0.80	0.05
Ross 308$^{\$}$								
Jejunum								
Protein$^{\#}$	45.4	48.0	N/A	35.0	N/A	43.0	39.0	2.4
Maltase$^{\#\#}$	2.50	2.25	N/A	2.80	N/A	2.42	2.20	0.15
Sucrase$^{\#\#}$	0.05	0.12	N/A	0.08	N/A	0.04	0.08	0.01
AMP*$^{\#\#}$	51.00	55.00	N/A	68.00	N/A	53.00	59.00	3.15
Pancreas								
Protein$^{\#}$	27.2	25.0	N/A	23.0	N/A	22.5	20.0	1.60
CA**	1.74	3.42	N/A	1.24	N/A	2.27	2.61	0.40
Trypsin$^{\#\#}$	0.83	0.70	N/A	0.85	N/A	0.80	0.90	0.80

^{a,b}Mean values on the same row not sharing a superscript are significantly different, $p<0.05$, *AMP: Aminopeptidase-N (μmol mg^{-1} protein), **CA: Chymotrypsin amidase (μmol mg^{-1} protein), $^{\#}$Protein (mg g^{-1} tissue), $^{\#\#}$Enzymes (μmol mg^{-1} protein), $^{\$}$Ross 308 SEM were based on analyses for IOF treatments with available data only, N/A: Not available, SEM: Standard error of mean

Table 7: Effect of IOF on tissue protein concentration and enzyme activities of 10 days old Cobb 500 broiler chicks

			Concentration of HPA (mg mL^{-1})		
Intestinal enzymes	Control	SH	T-HPA	M-HPA	SEM
Jejunum					
Protein$^{\#}$	46.00	35.00	30.10	35.60	2.76
Maltase$^{\#\#}$	2.08	2.00	2.14	2.70	0.20
Sucrase$^{\#\#}$	0.14^{a}	0.08^{b}	0.05^{b}	0.04^{b}	0.012
AMP*$^{\#\#}$	51.00	47.20	59.00	61.50	2.95
Pancreas					
Protein$^{\#}$	7.00	10.00	11.30	11.20	0.70
CA**	3.04	2.22	2.60	2.40	0.34
Trypsin$^{\#\#}$	4.00	4.00	4.00	4.00	0.001

^{a,b}Mean values on the same row not sharing a superscript are significantly different, $p<0.05$, *AMP: Aminopeptidase-N (μmol mg^{-1} protein), **CA: Chymotrypsin amidase (μmol mg^{-1} protein), $^{\#}$Protein (mg g^{-1} tissue), $^{\#\#}$Enzymes (μmol mg^{-1} protein), N/A: Not available, SEM: Standard error of mean

supplementation of HPA solution on the activities of jejunal enzymes. In Cobb 500, the pancreatic tissue protein concentrations and enzyme activities were similar ($p>0.05$) on day 5. The tissue protein contents of jejunum and pancreas in Ross 308 did not differ ($p>0.05$) between HPA treatments. On day 10, the activity of sucrase was significantly higher ($p<0.05$) in the control group (Table 7) compared to other treatment.

DISCUSSION

The results of the present study showed that IOF of the solution of HPA produced a maximum hatchability performance of 70% when fertile eggs were injected with the 37.5 mg mL^{-1} concentration of the test product. This was closely followed by the sham (perforated but not injected with solution) group. These rates of hatchability were both achieved in eggs from Cobb 500 broiler strain. Injecting eggs with MQ water resulted in greatly depressed hatchability, with the most unhatched fertile eggs observed in this study. Generally, there were better hatchability performance records obtained in eggs from Cobb 500 broiler breeder strain than their Ross 308 counterpart. The highest (150 mg mL^{-1}) concentration of the IOF solution of HPA resulted in the most pipped-but-not-hatched eggs in both strains.

The maximum hatchability observed in the present study is close to what was observed in other studies[29]. However, the rates of hatchability obtained are generally lower than those set by industry standards and when compared to some reports from other studies using other protein-rich supplements[30] and close to some observations when other non-protein supplements were provided *in ovo*[31]. It is not certain why this is so but it may be that the IOF solutions are lower in energy needed to support embryos during pipping and hatching. At the same time, it is not clear if this is as a result of increase in early mortality of embryos. Previous results on the chemical composition of the test product indicated low levels of glucose and lactate in the solution, although close to that of the amniotic fluid[32]. The solution was injected at 1 mL, which is a frequently reported volume in IOF studies. However, in this case, this volume may be more or less than required to optimize hatchability using the test product. This may also be attributed to incubation problems as the facility (incubator) was being used for the first time and probably was not fine-tuned enough. The M-HPA achieved the highest hatchability in this present study. Results of the chemical composition of the solutions of the test product from a previous study in our laboratory revealed that this level of concentration had higher glucose and lactate content[32], which would improve the energy status of embryos[15,33].

The results obtained in this study tend to agree with previous findings. In other studies, hatchability was either not affected[34] or was reduced by IOF of nutrients or supplements[17]. Another important factor may be the physical properties of the IOF solution (osmolality, viscosity, pH and pCO_2), of which most were divergent from those of the late-incubation amniotic fluid as previously reported. It is important to mention the fact that more hatched eggs were observed in Cobb 500 broiler breeder strain than in Ross 308 may be related to the age of the breeder hens as it is known that eggs laid by older breeders present higher infertility[35].

The highest concentration of HPA (150 mg mL^{-1}) resulted in the highest chick weight at hatch and yolk-free body mass in Cobb 500, although these were only marginally higher than in the chicks in the control group. The *in ovo* injection of MQ water and L-HPA solution depressed chick weight at hatch. This may be explained by the unavailability of nutrient in the MQ water or the low nutrient content of the L-HPA solution. The chicks in these two groups may have depended solely on the yolk content reserves for development and maintenance, while other groups leveraged on the extra nutrients supplied by richer HPA solutions injected. This may further explain the yolk weight being marginally less heavy in the groups that had no IOF (control and MQ) and or had L-HPA solution injected (L-HPA group), probably as a result of more or rather, better yolk utilization in these groups, especially in Cobb 500 strain. That the IOF of HPA did not have any effect on residual yolk sac weight is in line with a report by Zhai *et al.*[29]. Generally, chick quality seems to decrease with *in ovo* injection of MQ water and decreasing concentration of HPA, beginning from 75 mg mL^{-1} concentration. The different treatment did not have any effect on BW:CL ratio in both strains of broiler chicks. However, in practice, hatching weight and chick length are largely measured to evaluate chick quality because there is a critical relationship between 1 day old chick quality and posthatch broiler performance[36,37]. The findings of this study did not concur with the postulation by Mukhtar *et al.*[38], that a bigger, day-old chick has a large residual yolk and a small yolk-free body mass. Also, the differences for chick hatch weight between treatments can be largely explained by variations in residual yolk mass[39], which is known to be extremely variable at hatch. Although, not an effect of IOF, in most cases, longer chick length portends better growth potential as observed with the sham group and also reported by Petek *et al.*[40].

Between hatch and 5 days, IOF supplementation of HPA did not affect BWG in Cobb 500 broiler chicks as there was no significant difference between the IOF treatments and the control group. There is no known reason why FI was higher in the MQ group at the time of this study but it does not seem that the FI increased with increased concentration of HPA solution as seen in the feed consumption and conversion ratio dynamics observed in the Cobb 500 chicks in this study, although the FCR was similar in with the control group. However, it is possible that the highest concentration of HPA could increase early BWG within the first 5 days of age.

In Ross 308, the SH and all IOF treatment groups had better BWG than the control, while SH and the T-HPA groups consumed more feed than the rest. The HPA solution may have contributed faster development of the GIT at the early stage, which would have improved feed intake and nutrient utilization.

With some nutrients in the HPA solution to support the available glycogen reserves in the developing embryo, there may have been less dependence on muscle protein for gluconeogenesis, thereby increasing early growth and development. This idea is supported by previous studies that showed that injecting IOF solution rich in carbohydrates and amino acids replenished the glycogen reserves depleted during the prenatal period and also increased BW[9]. As for the improved BW gain in the SH group, there is no clear explanation, except to mention that the embryos may have benefitted from increased oxygen availability for metabolic process but there is no evidence in this present study to support that.

At day 10, there was not enough data to report growth responses for Ross 308 broiler chicks. In Cobb 500 broiler chicks, an improvement was observed in the BWG of the SH group. With the application of IOF at 150 mg mL^{-1} of HPA solution, FI was improved and better than in the control broiler chicks but this concentration of the IOF solution did not improve FCR at day 10. The improvement in BWG observed in SH group may be related to the access to more oxygen by the embryos through the perforated hole, which may have had positive effect on embryonic metabolic activity. But with respect to the IOF groups, results obtained tended to agree with other researchers[30,41] who reported no significant effect of IOF of nutrients on BWG up to 14 day post-hatch in broiler chicks. The inability of the IOF supplement used in the present study to effect substantial improvement in gross responses of broiler chicks may be as a result of insufficient dose or inadequate energy content. The test product is rich in amino acids as reported in a previous study[32] but may be limited in energy as evidenced from the low concentrations of glucose and lactate. However, it may be inappropriate to conclude on the potential of the test product as the results could be different if chicks were reared to market weight.

At hatch, significant differences were observed in the internal organ weight of neo-natal chicks. In Cobb 500, IOF did not improve liver weight as liver from the control group was heavier. Gizzard plus proventriculus weight was comparable in the control, SH and T-HPA groups but lighter in the other IOF treatments. This seems to suggest that a high concentration of the test product may enhance the digestive functions of the gizzard plus proventriculus. The MQ and H-HPA treatments had negative effects on the weight of the bursa of fabricius. This may result in limited development of the organ and hence delay immune-competence in neo-natal chicks. Contrary to the observations in Cobb 500, there was no effect of IOF supplementation on internal organ development of neo-natal chicks from Ross 308. There is no known explanation for this outcome but it may be as a result of a strain specific factor that may not have been observed during this study.

The most important effect of IOF of HPA solution on internal organs were elicited between the *in ovo* administration of 37.5 and 18.75 mg mL^{-1} of HPA solutions in broiler chicks. A large heart weight indicates good blood, oxygen and nutrient supply to other organs, which is important for better development and growth. Similarly, large pancreas and liver are indicators of enzymatic and metabolic activities in broiler chicks, which are important in nutritional and physiological activities.

In this study, the top and high concentrations of HPA marginally improved tissue protein concentration in chicks supplemented with the test product via IOF while both as well as the medium concentration also increased digestive enzyme activities, although these effects were not significant. There are no reports in literature on the effects of supplementing HPA through IOF. The generally non-significant effects observed may have resulted from unavailability of or inadequate substrates to act on, since the HPA solutions used did not contain much of the target substrate for the enzymes. However, slight improvements were effected by supplementation of higher concentrations of HPA solution *in ovo*. This could mean that the test product could potentially facilitate enzyme functions by enhancing their secretion, which could in turn improve digestive activities in broiler chicks.

This study is an addition to the body of knowledge in the development and identification of a novel product for use in IOF from soy protein to mitigate delayed access to feed and has shown that the product tested has potential to improve early growth and performance in broiler chicks. The findings in this study will also be beneficial to the poultry industry seeking products with beneficial effects in terms of hatching weight in broiler chicks. This study opens up a new discussion for researchers with interest in early nutrition, especially *in ovo* feeding to further experiment with the product tested.

CONCLUSION AND FUTURE RECOMMENDATIONS

This study was set up to explore the effect of a novel *in ovo* feed product on hatchability indices, early post-hatch performance and digestive physiology of broiler chicks. This study showed that the product has potential to improve hatching weight of broiler chicks and early post-hatch performance till 10 days. A major constraint to its use is inability to form homogenous solutions. As HPA has not been tested elsewhere as an IOF supplement, it is difficult to make a definitive statement on its suitability. As such, it may be premature to recommend its use as an IOF supplement. Further development and trial studies with HPA is therefore, necessary to ascertain its suitability for *in ovo* feeding.

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