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protein sources and improved
starter feed formulation for
broiler chicks**

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Executive Summary

Four experiments were conducted with the objective of developing suitable pre-starter diets for use by the Australian meat chicken industry. Three of the experiments were focused on identifying supplements that would enhance the nutritive value of pre-starter diets, in order to maximise response of chicks placed on such diets. For this purpose an animal protein supplement, spray-dried porcine plasma (SDPP), and a plant protein product, HP AviStart (HPA), were investigated. The fourth experiment examined the potential of a complex dietary formulation that would enable the chicks to derive complementary benefits from SDPP and Soycomil® (SCM), a soybean concentrate. The diets for Experiment 4 were also formulated with 10% more digestible amino acids than currently specified for the Ross 308 broiler (Aviagen 2007).

Inclusion of SDPP led to an increase in final (35 day) LW and improvement in FCR, without an increase in feed intake. There were also increases in the weight of most of the commercial meat parts, including breast, thighs and drumsticks. The supplement was effective at all levels between 5 and 20 g/kg diet. Shorter-term feeding of the supplement for 5 rather than 10 days was equally effective although the longer-term feeding tended to enhance feed efficiency. The response on the wheat-based diets was slightly better than on the maize diets but these differences were not significant. The supplement had no effect on visceral organ weights but increased villus height and also enhanced the activities of some pancreatic and jejunal enzymes. Feeding SDPP resulted in a reduction in feed costs per unit final LW. The costs of feed per kg LW at 35 days were \$0.72, \$0.71, \$0.89 and \$0.67 with diets containing 0, 5, 10 and 20 g SDPP/kg, respectively.

HPA reduced feed intake on a maize-based but not wheat-based diet. Live weight was also improved on the wheat-based diets containing HPA but not on the maize-based diets. The weight of most visceral organs was reduced by inclusion of HPA in the starter diets. Carcass yield and nutrient digestibility were not affected but the supplement enhanced the development of the intestinal mucosa.

Results of the experiment on complex feed formulation did not reveal any significant benefits of SDPP or SCM on LW or FCR. It is not certain if any beneficial fractions are eliminated from SBM in the process of creating SCM. Feeding digestible amino acids at 10 % higher than the breeder-recommended levels significantly improved weight gain and FCR. It can be concluded that SDPP would be useful as a supplement for feeding pre-starter and starter chicks although the response seems to be dependent on feed formulation. The birds also benefited from digestible amino acids fed at levels that are higher than the current recommendations (Aviagen 2007).

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Background

In the commercial hatchery, broiler chicks in a set batch of eggs would hatch within several hours of one another (Bigot *et al.*, 2001), followed by several more hours of sorting the chicks and delivery to farms. As a result, some chicks could be without food or water for up to 36 hours post-hatch (Noy and Sklan, 1999). While this results in weight loss, the most negative effect is on the development of the gastrointestinal tract (GIT). The GIT develops most rapidly within the first 4-7 days of life (Uni *et al.*, 1995; Iji *et al.*, 2001). Access to feed within this period would aid the development of the GIT (Gonzales *et al.*, 2003; Bigot *et al.*, 2003). Poor early development of the GIT can adversely affect health, feed efficiency and growth.

The benefits of early feeding of chicks have been demonstrated by several researchers (Uni *et al.*, 1998; Noy and Sklan, 1999; Noy *et al.*, 2001; Bhuiyan *et al.*, 2011). Chicks could be provided with nutrients at the embryonic stage, in the form of *in ovo* feeding. Equipment for the *in ovo* delivery of nutrients to the developing embryo has been developed by Uni and her research collaborators (Uni and Ferket, 2004). Alongside the development of appropriate delivery technology is the concerted search for suitable products that can be delivered *in ovo* or in a manner that can be ingested by the chick at hatch. *In ovo* feeding has not been widely adopted by the poultry industry for a variety of reasons, notable among which is poor hatchability associated with the process. It is most likely that chicks will continue to be set and hatched in the same way for some time to come. It will be most useful to develop a feeding strategy that can be applied after hatching, which will offset the setback that they encounter prior to delivery to farms. An early feeding strategy after placement at the farm would be more practical than *in ovo* feeding.

Most early-life diets are based on the premise of supplying energy to the bird. Such diets do not adequately support intestinal development or health. There may be a need to supply nutrients that will enable the utilization of this readily available energy source by the developing chick since a major requirement for nutrients at this stage is towards the development of intestinal structure and function. Apart from the supply of nutrients, ingredients that are used in the formulation of early diets should be free of allergens, which have been shown to adversely affect gut development and health in piglets (Dreau and Lalles, 1999). The response by the pig industry is to utilise high-quality proteins, which are low in allergens in creep diets. Such diets support a healthy development of the GIT and promote rapid growth of pre-wean and weaner piglets. While there may be differences in response to these allergens by poultry, it may be necessary to test similar ingredients in poultry chick diets, in order to develop a practical early feed formulation for the industry.

It is noticeable that the availability of appropriate nutrients prior to or immediately after hatch may result in a more uniform gut development, a more stable gut microflora and a more mature immune system. The overall objectives of the proposed project are to identify suitable supplements that can be incorporated into diets for early feeding of broiler chicks, and to identify the most suitable basal ingredient combinations with these supplement(s).

Objectives

The approach to the study was two-fold; one to identify suitable product(s) for inclusion in pre-starter and starter diets for broiler chicks, and the other to develop suitable starter diets for broiler chicks. Once the most suitable diet has been identified, the optimum level of inclusion of the "active" product would be determined, and the product will be formulated into diets based on different key cereal grains, to identify the most suitable base. Finally, the mechanisms associated with the functions of this product in the diet would be explored, with a view to improving the response to it.

The major aim of the project was to develop an effective starter diet for the chicken meat industry that will enhance the health and growth of chicks in early life, and improve final product quality. The specific objectives of the project were to:

- Identify a product that can be included in starter diets;
- Determine the optimum level of inclusion of the product in diets;
- Identify the most suitable cereal grain base for this product in diets, and
- Examine some of the mechanisms of action of the supplement(s) that were tested.

Methodology

Experimental designs

Experiment 1 was a 4 x 2 factorial experiment, designed to investigate the effect of starter level of spray-dried porcine plasma (SDPP) on broiler performance and digestive physiology up to 35 days of age. The nutrient composition of SDPP is shown in Appendix 1. Four inclusion levels of SDPP (0, 5, 10 and 20 g/kg in either maize or wheat-based diets) were included in starter diets (Table 1), which were fed from hatch to 10 days, after which the birds were switched to commercial type grower (11-24 days) and finisher (25-35 days) diets (Table 2). SDPP replaced meat meal in this study. The diets were identical in nutrient profiles and formulated to meet most of the breeder specifications (Aviagen 2007). Four hundred and eighty Ross 308 day-old male chicks (initial weight, 41.0±0.92 g) were randomly assigned to the eight treatments, each with six replicates, ten chickens per replicate. Chickens were reared in multi-tiered brooder cages (600 x 420 x 23 cm) placed in a climate-controlled room up to 24 days, then the birds were transferred to metabolic cages and reared to 35 days. Feed and water were provided *ad libitum*. The room temperature was gradually decreased from 35°C at start to 24°C at 21 days of age. Lighting was 18h throughout the study, except in the first week during which 23h lighting per day was provided. Titanium dioxide (TiO₂), as an indigestible marker, was incorporated to the grower diet at a rate of 5 g/kg diet to enable assessment of nutrient digestibility.

Experiment 2 was similar in design to experiment 1, except that SDPP was replaced by a processed soya product, HP Avistart (HPA), produced by Hamlet Protein As, Denmark. The nutrient composition of HPA is shown in Appendix 2. HPA was included in maize- or wheat-based starter diets at 0, 25, 50 or 100 g/kg (Table 3). The diets were fed from hatch to 10 days, after which the birds (male Ross 308) were transferred to similar grower and finisher diets, based on maize or wheat (Table 4). Titanium dioxide was also included in the grower diet, to enable assessment of nutrient digestibility. All other rearing conditions were as described for experiment 1.

In experiment 3, the effect of duration of feeding SDPP was tested. The supplement was included in starter diets (Table 5) at 0, 10 or 20 g/kg and fed to Ross 308 male chicks for 5 or 10 days. Subsequently, chicks were changed to grower diets (6 or 11 days to 24 days) then finisher diets (25-35 days) similar to the wheat-based diets fed in experiment 1. Rearing conditions were similar to those used in experiment 1.

Experiment 4 was designed to assess the benefits of inclusion of two main protein sources; soybean meal (SBM) or Soycomil® (SCM), a soybean concentrate produced by ADM. The nutrient composition of SCM is shown in Appendix 2. In addition, SDPP (0 or 20 g/kg) or 10% extra digestible amino acids in starter diets were fed to Ross 308 male broiler chickens for the first 10 days of age (Tables 6 and 7). The experiment was thus a 2x2x2 factorial arrangement of treatments in a completely randomised design. A total of 576 day-old chicks were assigned to the 8 treatments, each replicated 6 times with 12 birds per replicate in 48 floor pens. Diets were formulated to be isoenergetic and isonitrogenous. For digestible amino acid concentrations, the levels recommended for Ross 308 (Aviagen, 2007) or levels 10% higher were tested. From day 10 to 35 all the birds were given the same grower and finisher diets (Tables 8 and 9). Temperature

was set at 33-34°C on the first day of the experiment and then gradually decreased by 1°C every second day until a stable temperature of 24°C was reached at 21 days. A lighting program of 18h light and 6h darkness was maintained throughout the trial except the first week when birds had 23h of light. Birds had access to feed and water *ad libitum*.

Table 1: Ingredient and nutrient composition (g/kg) of starter diets used in experiment 1.

Ingredients	Wheat base				Maize base			
	SDPP (g/kg)				SDPP (g/kg)			
	0.0	5.0	10.0	20.0	0.0	5.0	10.0	20.0
Wheat	619.2	623.2	624.3	626.3	-	-	-	-
Maize	-	-	-	-	584.8	597.0	585.3	586.5
Soybean meal	260	258	257	255	302	299	300.2	298
Meat meal	40	35	30	20	40	35	30	20
Oil	36.6	34.3	33.5	33.0	30	20	30	30
SDPP	-	5	10	20	-	5	10	20
Choline chloride	1.4	1.4	1.4	1.4	1.5	0.9	1.5	1.5
Di Ca phos¹	13.0	13.8	15.0	17.0	14.0	14.1	16.0	18.0
Sodium bic²	3.7	3.0	3.0	2.0	3.5	3.2	2.9	1.8
Limestone	10.0	10.5	11.0	11.8	9.1	10.5	10.3	11.4
Salt	1.3	1.2	0.7	0.3	2.0	1.5	1.3	1.0
L-Lysine	5.9	5.8	5.5	5.0	5.3	5.0	4.8	4.5
DL Methionine	3.5	3.45	3.4	3.2	3.0	3.0	2.9	2.8
Threonine	3.4	3.4	3.2	3.0	2.8	2.8	2.8	2.5
Trace minerals³	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Vitamins⁴	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Avizyme⁵	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Phyzyme XP⁶	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Nutrient composition⁷								
ME (MJ/kg)	12.67	12.73	12.71	12.67	12.70	12.72	12.71	12.72
Crude protein	219.0	220.0	220.0	222.0	213.0	214.0	215.0	216.0
Lysine	14.2	14.3	14.2	14.1	13.8	13.7	13.7	13.8
Methionine	6.2	6.1	6.1	5.9	5.9	5.9	5.8	5.7
Arginine	13.0	13.0	13.0	13.1	13	13	13.1	13.1
Meth + cyst⁸	9.2	9.3	9.3	9.3	8.4	8.5	8.5	8.6
Threonine	9.6	9.7	9.6	9.7	9.0	9.1	9.3	9.2
Calcium	10.6	10.6	10.7	10.7	10.4	10.6	10.5	10.6
Available Phos⁹	5.3	5.4	5.5	5.6	5.3	5.2	5.5	5.6
Sodium	2.2	2.0	1.9	1.7	2.3	2.1	2.0	1.8
Choline	1874	1864	1853	1832	1688	1667	1665	1642

¹Di Calcium phosphate; ²sodium bicarbonate; ^{3,4}Supplied as premixes, detailed composition in Appendix 4; ⁵xylanase+amylase+protease, ⁶phytase; ⁷Amino acids are expressed as digestible; ⁸methionine+cystine; ⁹Available phosphorus.

In all experiments, crumbles (following cold pelleting) were fed during the starter phase. Subsequently cold pelleted diets were fed in the grower and finisher phases. The nutrient specifications of the three main supplements, SDPP, HPA and SCM are shown in Appendices 1-3.

The current market prices are \$8000, \$1780 and \$1650 per tonne for SDPP, HPA and SCM, respectively.

Table 2: Ingredient and nutrient composition (g/kg) of grower and finisher diets used in experiment 1.

Ingredients g/kg	Grower		Finisher	
	Wheat base	Maize base	Wheat base	Maize base
Wheat	629.2	-	647.1	-
Maize	-	619.6	-	640.2
Soybean meal	230	265	213.6	260
Meat meal	54.1	56.9	55.4	51.4
Oil	60.0	32.0	60.0	29.1
SDPP	-	-	-	-
Choline chloride	1.7	0.8	1.5	0.6
Dicalcium phosphate	5.6	6.7	3.8	6.1
Sodium bicarbonate	3.3	3.5	5.7	-
Limestone	7.8	7.0	7.2	7.0
Salt	1.0	1.1	1.1	1.0
L-Lysine	2.5	2.5	1.0	1.0
DL Methionine	3.2	3.4	2.6	2.6
Threonine	1.4	1.4	0.8	0.8
Titanium dioxide	5	5	-	-
Trace minerals ¹	0.75	0.75	0.75	0.75
Vitamins ²	0.5	0.5	0.5	0.5
Avizyme ³	0.5	0.5	0.5	0.5
Phyzyme XP ⁴	0.1	0.1	0.1	0.1
Nutrient composition⁵				
ME poultry (MJ/kg)	13.05	13.05	13.11	13.11
Crude protein	208.4	204.2	201.3	198.7
Lysine	11.1	11.1	9.7	9.7
Methionine	5.9	6.2	5.2	5.4
Arginine	12.5	12.5	12.1	12.2
Methionine + cysteine	8.8	8.7	8.1	7.9
Threonine	7.4	7.5	6.7	6.8
Calcium	9.2	9.3	8.6	8.7
Available phosphorus	4.45	4.6	4.1	4.3
Sodium	2.0	2.0	2.7	0.9
Choline	1.5	1.5	1.4	1.4

^{1,2}Supplied as premixes, detailed composition in Appendix 4; ³xylanase+amylase+protease, ⁴phytase;

⁵Amino acids are expressed as digestible.

Sample collection

On days 10, 24 and 35, the birds and feed were weighed to measure the LW, feed intake and feed conversion ratio (FCR; feed intake/LW gain). On days 10 and 24, two birds from each cage were randomly selected and euthanased by cervical dislocation. The abdominal cavity was opened, to obtain visceral organs, which were weighed. On day 24 tissue samples (approximately 4 cm) were collected from the proximal jejunum, flushed with buffered saline and fixed in 10 % neutral buffered formalin for histomorphological analysis. The entire pancreas and part of the jejunum were also taken for assessment of digestive enzyme activities. Ileal digesta were collected into plastic containers and frozen immediately, for subsequent measurement of nutrient digestibility. After freeze-drying (Martin Christ Gerfriertocknungsanlagen, GmbH, Germany), the ileal digesta

samples were ground in a small coffee grinder and stored at 4 °C in airtight containers for chemical analyses of TiO₂, gross energy, protein and dry matter.

Table 3: Ingredient and nutrient composition (g/kg) of starter diets fed in experiment 2

Starter	Wheat base					Maize base		
Ingredient	1	2	3	4	5	6	7	8
Ingredient g/kg								
Canola meal	40	40	40	40	46	50	50	50
Wheat	600.3	581.54	574.00	597.16	-	-	-	-
Maize	-	-	-	-	584.32	564.45	557.30	575.74
Soybean meal	229.3	236.00	227.00	168.88	263.00	265.50	256.00	192.00
Meat meal	40	15	-	-	40	15	-	-
Oil	52.7	57.41	59.50	55.00	27.79	33.67	36.01	31.82
PSP	-	25	50	100	-	25	50	100
Choline chloride	0.57	0.67	0.78	1.01	0.99	1.10	1.19	1.45
DiCa phos ¹	10.83	15.05	17.28	15.84	12.34	16.47	18.67	17.18
Na bicarbonate ²	4.16	4.41	4.58	3.15	4.57	4.84	4.96	5.09
Limestone	10.19	13.39	15.50	16.59	9.47	12.65	14.78	15.76
Salt	0.63	0.90	1.04	2.00	0.63	0.87	1.04	0.92
L-Lysine	4.51	4.22	4.06	4.15	4.39	4.20	3.96	4.05
DL-Methionine	2.35	2.28	2.23	2.24	2.48	2.39	2.34	2.29
Threonine	2.29	2.13	2.02	1.98	2.03	1.87	1.76	1.68
Trace minerals ³	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Vitamins ⁴	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Avizyme ⁵	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Phyzyme XP ⁶	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Nutrient composition⁷								
ME (MJ/kg)	12.56	12.56	12.56	12.56	12.56	12.56	12.56	12.56
Crude protein	214.3	216.4	217.7	218.1	210.1	212.8	214.1	214.2
Lysine	12.9	13.0	13.2	13.4	12.9	13.1	13.1	13.3
Methionine	5.1	5.1	5.1	5.1	5.4	5.4	5.4	5.4
Arginine	12.7	12.0	11.2	9.2	12.6	12.0	11.1	13.3
Meth+cyst ⁸	8.1	8.2	8.3	8.4	8.1	8.1	8.1	8.3
Threonine	8.4	8.5	8.5	8.7	8.2	8.3	8.3	8.5
Calcium	10.4	10.6	10.8	10.8	10.4	10.6	10.8	10.8
Available P ⁹	5.0	5.0	5.0	5.0	5.1	5.1	5.1	5.1
Sodium	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1
Choline	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6

¹Dicalcium phosphate; ²sodium bicarbonate; ^{3,4}Supplied as premixes, detailed composition in Appendix 4; ⁵xylanase+amylase+protease, ⁶phytase; ⁷Amino acids are expressed as digestible; ⁸methionine+cystine; ⁹Available phosphorus.

Measurements and analyses

Growth performance

Feed intake (FI) and live weight (LW) were recorded on days 10, 24 and 35 for determination of average FI and LW. Mortality rate was recorded and FCR was corrected for mortality. Carcass weight and the weight of breast (boneless), thigh and drumsticks were recorded on day 35.

Visceral organ weight

LW and the weight of small intestine (with content), proventriculus plus gizzard, liver, heart, spleen, pancreas and bursa of Fabricius were recorded on days 10 and 24. The relative organ weight was calculated as mass per unit of LW (g/100 of LW).

Table 4: Ingredient and nutrient composition (g/kg) of grower and finisher diets fed in experiment 2

Ingredients g/kg	Grower		Finisher	
	Wheat base	Maize base	Wheat base	Maize base
Wheat	629.2	-	647.1	-
Maize	-	619.6	-	640.2
Soybean meal	230	265	213.6	260
Meat meal	54.1	56.9	55.4	51.4
Oil	60	32	60	29.1
Choline chloride	1.7	0.8	1.5	0.6
Dicalcium phosphate	5.6	6.7	3.8	6.1
Sodium bicarbonate	3.3	3.5	5.7	-
Limestone	7.8	7.0	7.2	7.0
Salt	1.0	1.1	1.1	1.0
L-Lysine	2.5	2.5	1.0	1.0
DL-Methionine	3.2	3.4	2.6	2.6
Threonine	1.4	1.4	0.8	0.8
Titanium dioxide	5	5	-	-
Trace minerals ¹	0.75	0.75	0.75	0.75
Vitamins ²	0.5	0.5	0.5	0.5
Avizyme ³	0.5	0.5	0.5	0.5
Phyzyme XP ⁴	0.1	0.1	0.1	0.1
Nutrient composition⁵				
ME poultry (MJ/kg)	13.05	13.05	13.11	13.11
Crude protein	208.4	204.2	201.3	198.7
Lysine	11.1	11.1	9.7	9.7
Methionine	5.9	6.2	5.2	5.4
Arginine	12.5	12.5	12.1	12.2
Methionine + cystine	8.8	8.7	8.1	7.9
Threonine	7.4	7.5	6.7	6.8
Calcium	9.2	9.3	8.6	8.7
Available phosphorus	4.45	4.6	4.1	4.3
Sodium	2.0	2.0	2.7	0.9
Choline	1.5	1.5	1.4	1.4

^{1,2}Supplied as premixes, detailed composition in Appendix 4; ³xylanase+amylase+protease, ⁴phytase;

⁵Amino acids are expressed as digestible.

Table 5: Ingredient and nutrient composition (g/kg) of starter diets fed in experiment 3.

Ingredient	Starter		
	Control	10 g/kg SDP	20 g/kg SDP
Wheat	606.7	614.6	621.4
Soybean meal	272.5	265.1	258.5
Meat meal	40	30	20
Oil	38	36	34.5
SDPP	-	10	20
Choline chloride	0.7	0.7	0.8
Dicalcium phosphate	11.2	12.6	14.1
Sodium bicarbonate	3.0	2.4	2.2
Limestone	10.7	11.9	13.0
Salt	1.0	1.0	0.9
Titanium dioxide	5	5	5
L-Lysine	3.6	3.4	3.0
DL Methionine	3.7	3.5	3.3
Threonine	2.0	1.8	1.6
Trace minerals ¹	0.75	0.75	0.75
Vitamins ²	0.5	0.5	0.5
Avizyme ³	0.5	0.5	0.5
Phyzyme XP ⁴	0.1	0.1	0.1
Nutrient composition³			
ME poultry (MJ/kg)	12.66	12.65	12.66
Crude protein	220	220	220
Lysine	12.7	12.8	12.7
Methionine	6.47	6.25	6.02
Arginine	13.33	13.24	13.2
Methionine + cystine	9.5	9.4	9.4
Threonine	8.32	8.33	8.34
Calcium	10.54	10.51	10.50
Available Phosphorus	5.01	5.00	5.02
Sodium	1.86	1.86	1.92
Choline	1610	1601	1611

^{1,2}Supplied as premixes, detailed composition in Appendix 4; ³xylanase+amylase+protease, ⁴phytase;

⁵Amino acids are expressed as digestible.

Tissue protein and digestive enzyme analyses

To evaluate the activities of digestive enzymes and protein concentration, the jejunal tissue was processed according to the method described by Shirazi-Beechey *et al.* (1991). The frozen tissue sample was cut up into an ice-cold buffer (100 mM mannitol, 2 mM HEPES/Tris, 7.1) and the mucosa was then stripped into the buffer using a vortex mixer at high speed for 1 min. The mixture was filtered through a Buchner funnel and homogenized at medium speed (No 2. 1300 r/min) for 30 s on an Ultra Turrax T 25 Basic Homogenizer (IKA® Works, Wilmington, NC, USA). A sub-sample of the homogenate was transferred into Eppendorf tubes (Eppendorf South Pacific, North Ryde, Australia) and stored in a freezer (-20 °C) for digestive enzyme analyses. The pancreatic tissue was processed in a similar way to the jejunum except that Milli-Q water (Millipore Australia, North Ryde, Australia) was used in place of buffer and the entire tissue was homogenized. The homogenized mixture was transferred to a new tube and centrifuged at high speed (30 000 xg) for 20 minutes at 5 °C. Aliquots of the supernatant were collected and used for various enzyme assays and total protein measurement according to Nitsan *et al.* (1974).

Table 6: Ingredient composition of starter diets fed in experiment 4

<i>Ingredient (%)</i>	Recommended dig. amino acids				10 % extra dig. amino acids			
	SBM	SCM	SBM-SDPP	SCM-SDPP	SBM	SCM	SBM-SDPP	SCM-SDPP
Wheat	40.4	54.1	43	53.7	34	50.1	37	55.6
Sorghum	20.0	20.0	20.0	20.0	20.0	20.0	20.0	15.0
SBM	27.1	-	23.6	-	32.9	-	28.9	-
SCM	-	16.7	-	15.1	-	19.8	-	17.0
Canola meal	2.0	2.0	2.0	3.3	2.0	2.9	2.0	3.5
Meat meal	3.5	3.5	3.2	2.0	3.5	3.5	3.5	3.5
SDPP	-	-	2.0	2.0	-	-	2	2
Canola oil	4.0	0.7	3.3	0.7	4.5	0.7	3.8	0.8
Limestone	0.9	0.9	1.0	1.2	0.9	0.9	1.0	1.0
Dicalcium phosphate	0.29	0.31	0.23	0.47	0.27	0.28	0.16	0.18
Xylanase	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Phytase	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Salt	0.25	0.23	0.23	0.23	0.25	0.23	0.22	0.21
Sodium bicarbonate	0.36	0.38	0.24	0.23	0.36	0.39	0.24	0.25
Vitamin premix	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Trace mineral premix	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075
Choline chloride	0.108	0.113	0.119	0.124	0.095	0.103	0.107	0.112
L-lysine	0.34	0.34	0.3	0.28	0.34	0.35	0.3	0.31
DL- methionine	0.36	0.33	0.33	0.29	0.42	0.39	0.39	0.35
L- threonine	0.19	0.16	0.15	0.12	0.21	0.18	0.17	0.14
Salinomycin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Zinc bacitracin	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033

^{1,2}Supplied as premixes, detailed composition in Appendix 4.

Table 7: Nutrient composition (g/kg) of starter diets fed in experiment 4

	Recommended digestible amino acids				10 % extra digestible amino acids			
	SBM	SCM	SBM-SDPP	SCM-SDPP	SBM	SCM	SBM-SDPP	SCM-SDPP
ME (kcal/kg)	3050	3050	3050	3050	3050	3050	3050	3050
Crude protein	220.0	228.0	228.0	228.0	248.0	248.0	248.0	247.0
Digestible Arg	13.2	13.2	13.1	13.1	14.9	14.7	14.6	14.4
Digestible Lys	12.7	12.7	12.7	12.7	14.0	14.0	14.0	14.0
Digestible Met	6.5	6.3	6.1	5.8	7.3	7.0	6.9	6.6
Digestible M+C	9.4	9.4	9.4	9.4	10.3	10.3	10.3	10.3
Digestible Trp	2.4	2.0	2.5	2.1	2.7	2.2	2.8	2.3
Digestible Ile	8.8	8.8	8.8	8.8	9.8	9.6	9.7	9.7
Digestible Thr	8.3	8.3	8.3	8.3	9.1	9.1	9.1	9.1
Calcium Available	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Phosphorus	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Sodium	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Chloride	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0

The activities of jejunal and pancreatic enzymes were measured by incubation with fixed substrate concentrations as standardized for poultry by Iji *et al.* (2001). On the jejunal homogenates, the assays were conducted for mucosal protein and the activities of alkaline phosphatase (EC 3.1.3.1), maltase (EC 3.2.1.20) and sucrase (EC 3.2.1.10). For pancreas, assays were conducted for tissue protein and chemotrypsin amidase (EC 3.4.21.1). The enzymatic activities were measured according to the methods previously described for other species ((Holdsworth, 1970; Serviere-Zaragoza *et al.*, 1997). Tissue protein content in both jejunum and pancreas was measured using the Coomassie dye-binding procedure described by Bradford (1976).

Table 8: Ingredient composition of grower and finisher diets fed in experiment 4

Ingredient (%)	Grower	Finisher
Wheat	42.2	43.9
Sorghum	25.0	25.0
SBM	20.2	18.2
Canola meal	3.0	3.0
Meat meal	3.0	3.0
Canola oil	4.0	4.6
Limestone	0.91	0.68
Dicalcium phosphate	0.12	0.30
Xylanase	0.005	0.005
Phytase	0.01	0.01

Salt	0.12	0.15
Sodium bicarbonate	0.30	0.25
Vitamin premix ¹	0.05	0.05
Trace mineral premix ²	0.075	0.075
Choline chloride	0.128	0.133
L- lysine	0.32	0.21
DL- methionine	0.30	0.24
L- threonine	0.16	0.10
Salinomycin	0.05	0.05
Zinc bacitracin	0.033	0.033

^{1,2}Supplied as premixes, detailed composition in Appendix 4.

Table 9: Nutrient composition (g/kg) of grower and finisher diets fed in experiment 4

	Grower	Finisher
ME (kcal/kg)	3150.0	3200.0
Crude protein	204.0	195.0
Digestible Arg	11.4	10.8
Digestible Lys	11.0	9.7
Digestible Met	5.67	4.92
Digestible M+C	8.4	7.6
Digestible Trp	2.1	2.0
Digestible Ile	7.9	7.5
Digestible Thr	7.3	6.5
Calcium	8.0	7.5
Available phosphorus	3.5	3.8
Sodium	1.8	1.8
Chloride	2.2	2.2

Ileal digestibility of nutrients

The digestibility of crude protein (CP), gross energy (GE) and dry matter (DM) of feed and freeze-dried ileal digesta samples were analysed along with the indigestible TiO₂ marker. The TiO₂ content of the ileal digesta and diet samples was measured according to the method described by Short *et al.* (1996). The nitrogen content of ileal digesta samples and diet samples was determined according to the DUMAS combustion technique following the method described by Sweeney (1989) using a LECO[®] FP-2000 automatic nitrogen analyser (Leco Corporation, St. Joseph, MI, USA). Nitrogen was freed by combustion at high temperature in pure oxygen and was measured by thermal conductivity detection and converted to equivalent CP by a numerical factor of 6.25. The furnace temperature was maintained at 105 °C for hydrolysis of sample in ultra-purity oxygen. To interpret the detector response as percentage nitrogen (w/w) calibration was done using pure primary standard of ethylenediaminetetra-acetic acid (EDTA).

The gross energy of diet and ileal digesta samples was determined using an IKA[®]- WERKE bomb calorimeter (C7000, GMBH & CO., Staufen, Germany) at the University of New England. The GE value of the samples was obtained as MJ/kg directly from the digital system of the calorimeter.

The digestibility coefficient of nutrients was calculated using the following equation:

$$\text{Digestibility} = 1 - \frac{\text{Digesta nutrient (g/kg DM)}/\text{Digesta TiO}_2 \text{ (g/kg DM)}}{\text{Diet nutrient (g/kg DM)}/\text{Diet TiO}_2 \text{ (g/kg DM)}}$$

Statistical analysis of data

All data collected were analysed by linear regression or general linear model of Minitab version 16.2.4 (Minitab Inc., 2013), as appropriate for the experimental design. Differences between mean values were considered significant at $P \leq 0.05$.

Animal ethics

The experiments were approved by the Animal Ethics Committee of the University of New England. 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 (NHMRC, 2013).

Results

Experiment 1

Gross response

Feed intake to 10 or 35 days, on both diet types was not affected by starter level of SDPP (Table 10). Rising levels of SDPP resulted in increased LW at 10 and 35 days on both the maize-based diet birds ($P < 0.01$ and $P < 0.05$) and the wheat-based diet ($P < 0.001$ and $P < 0.05$). On the wheat-based diet, SDPP reduced ($P < 0.001$) FCR for the two periods assessed while on the maize-based diet, there was a reduction in FCR between hatch and 10 days ($P < 0.01$) and between hatch and 35 days ($P < 0.05$).

Table 10: Feed intake (g/bird), live weight (g) and FCR (g feed/g weight gain) of birds between hatch and 35d of age after placement on starter diets containing different levels of SDPP.

Cereal	Response	Age (days)	SDPP levels g/kg				SEM
			0	5	10	20	
Maize	Feed intake	1-10	337.4	327.7	327.8	334.4	3.18
		1-35	4144.0	3957.8	3923.6	4029.0	41.49
	Live weight	10	320.6 ^b	332.0 ^{ab}	341.2 ^a	345.6 ^a	2.72 ^{**}
		35	2608.7 ^{ab}	2544.4 ^b	2629.4 ^{ab}	2676.0 ^a	15.28 [*]
	FCR	1-10	1.20 ^a	1.13 ^b	1.10 ^b	1.09 ^b	0.009 ^{**}
		1-35	1.61 ^a	1.58 ^{ab}	1.52 ^b	1.53 ^b	0.012 [*]
Wheat	Feed intake	1-10	329.5	343.5	326.0	332.4	3.13
		1-35	4079.3	4039.1	3940.8	3923.3	45.27
	Live weight	10	316.1 ^b	347.8 ^a	344.4 ^a	352.5 ^a	2.24 ^{***}
		35	2538.1 ^b	2578.4 ^{ab}	2567.9 ^b	2735.4 ^a	27.61 [*]
	FCR	1-10	1.20 ^a	1.12 ^b	1.07 ^c	1.07 ^c	0.008 ^{***}
		1-35	1.64 ^a	1.60 ^a	1.56 ^a	1.46 ^b	0.014 ^{***}

¹ Each value represents the mean of 6 replicates for each treatment group; a,b,c – Mean values on the same row not sharing a superscript are significantly different (^{*} $P < 0.05$; ^{**} $P < 0.01$; ^{***} $P < 0.001$).

Visceral organ weight

At 10 days of age, SDPP significantly increased ($P < 0.01$) the relative weight of proventriculus plus gizzard and spleen in wheat-based diet (Table 11). However SDPP had no significant effect on the relative weight of the small intestine, heart, liver, bursa of Fabricius and pancreas. The relative weight of visceral organs was not affected by SDPP supplementation on maize-based diets. At 24

days, the relative weight of visceral organs was not affected by the starter inclusion levels of SDPP on both wheat-based and maize-based diets (Table 12).

Table 11: Relative weight of small intestine, proventriculus-gizzard, heart, liver, spleen, bursa and pancreas (g/100g of LW) of broiler chicken at day 10 at various SDPP inclusion levels on either maize or wheat-based diet.

Cereal		SDPP levels g/kg				SEM	P value
		0	5	10	20		
Maize	Small Int ²	8.11 ^a	7.84 ^a	7.87 ^a	8.45 ^a	0.145	0.29
	Pro+Gizz ³	3.52 ^a	3.55 ^a	3.42 ^a	3.70 ^a	0.062	0.34
	Heart	0.817 ^a	0.865 ^a	0.869 ^a	0.829 ^a	0.025	0.98
	Liver	3.94 ^a	4.09 ^a	3.75 ^a	4.03 ^a	0.084	0.92
	Spleen	0.075 ^a	0.064 ^a	0.092 ^a	0.069 ^a	0.003	0.92
	Bursa	0.15 ^a	0.16 ^a	0.19 ^a	0.13 ^a	0.007	0.57
	Pancreas	0.40 ^a	0.47 ^a	0.44 ^a	0.38 ^a	0.011	0.19
Wheat	Small Int ²	8.39 ^a	7.86 ^a	7.76 ^a	7.86 ^a	0.224	0.46
	Pro+Gizz ³	3.49 ^b	3.56 ^b	3.42 ^b	4.17 ^a	0.084 ^{**}	0.01
	Heart	0.88 ^a	0.93 ^a	0.88 ^a	0.87 ^a	0.028	0.72
	Liver	3.84 ^a	4.06 ^a	3.72 ^a	4.13 ^a	0.064	0.26
	Spleen	0.075	0.078	0.087	0.093	0.003	0.02
	Bursa	0.16 ^a	0.17 ^a	0.14 ^a	0.17 ^a	0.007	0.88
	Pancreas	0.47 ^a	0.43 ^a	0.42 ^a	0.47 ^a	0.012	0.94

¹ Each value represents the mean of 6 replicates for each treatment group; ^{a,b,c} – Mean values on the same row not sharing a superscript are significantly different (*P<0.05; **P<0.01; ***P<0.001); ²small intestine with digesta; ³Proventriculus and Gizzard; SEM= Standard error of mean

Table 12: Relative weight of small intestine, proventriculus-gizzard, heart, liver, spleen, bursa and pancreas (g/100g of LW) of broiler chicken at day 24 at various SDPP inclusion levels on either maize or wheat-based diet¹.

Cereal	Organ g/100g BW	SDPP levels g/kg				SEM	P value
		0	5	10	20		
Maize	Small int ²	6.02 ^a	6.14 ^a	6.13 ^a	5.60 ^a	0.156	0.28
	Pro+Gizz ³	2.02 ^a	1.97 ^a	2.09 ^a	2.01 ^a	0.051	0.95
	Heart	0.74 ^a	0.83 ^a	0.77 ^a	0.76 ^a	0.016	0.79
	Liver	2.62 ^a	2.61 ^a	2.80 ^a	2.72 ^a	0.052	0.37
	Spleen	0.079 ^a	0.078 ^a	0.077 ^a	0.082 ^a	0.003	0.78
	Bursa	0.17 ^a	0.18 ^a	0.17 ^a	0.16 ^a	0.005	0.24
	Pancreas	0.24 ^a	0.26 ^a	0.24 ^a	0.24 ^a	0.007	0.19
Wheat	Small int ²	6.03 ^a	5.50 ^a	5.72 ^a	5.53 ^a	0.193	0.47
	Pro+Gizz ³	1.88 ^a	1.71 ^a	2.04 ^a	1.90 ^a	0.057	0.54
	Heart	0.84 ^a	0.73 ^a	0.76 ^a	0.86 ^a	0.020	0.44
	Liver	2.75 ^a	2.71 ^a	2.70 ^a	2.52 ^a	0.062	0.18
	Spleen	0.08 ^a	0.10 ^a	0.09 ^a	0.07 ^a	0.004	0.42
	Bursa	0.14 ^a	0.17 ^a	0.17 ^a	0.19 ^a	0.010	0.14
	Pancreas	0.20 ^a	0.20 ^a	0.20 ^a	0.22 ^a	0.006	0.23

¹ Each value represents the mean of 6 replicates for each treatment group; ² small intestine with digesta; ³ Proventriculus and Gizzard; SEM= Standard error of mean

Carcass parts yield

In general, starter levels of SDPP had no significant effects on carcass yield and the relative weight of carcass parts on either wheat-based or maize-based diets at 35 days of age. However carcass yield percentage was marginally increased by rising level of SDPP in the starter diets of broiler chickens comparing to the control (Table 13).

Table 13: Carcass and meat parts yield (g) of chickens at 35 days of age at various SDPP inclusion levels on either maize or wheat-based diets.

Cereal	Carcass part	SDPP levels (g/kg)				SEM	p
		0	5	10	20		
Maize	Carcass yield (%)	75.6	76.0	75.8	76.4	0.30	0.62
	Breast	571.9	564.8	584.8	578.4	8.43	0.63
	Thigh	293.5	305.4	317.7	309.6	5.03	0.27
	Drumstick	250.3	244.4	250.1	249.1	2.77	0.92
	Wings	198.7	207.2	200.1	204.3	2.21	0.62
	Neck	147.5	150.2	134.0	145.4	4.43	0.70
Wheat	Carcass yield (%)	73.5	75.5	74.3	76.0	0.44	0.13
	Breast	580.0	548.7	548.1	580.0	9.76	0.79
	Thigh	312.0	303.0	308.4	318.4	6.48	0.58
	Drumstick	258.3	240.3	254.6	263.0	3.42	0.29
	Wings	209.2	199.9	207.6	213.1	2.63	0.33
	Neck	136.7	146.5	149.6	163.1	4.77	0.06

¹ Each value represents the mean of 6 replicates for each treatment group; SEM= Standard error of mean

Nutrient digestibility

On both wheat-based and maize-based diets, there was no significant effect of dietary inclusion of SDPP in starter diet on ileal digestibility of ileal protein, gross energy and dry matter at 24 days of age. However chickens that were fed at low SDPP level (5 g/kg) on maize-based diet tended to have better ($P<0.09$) protein digestibility. On wheat-based diet, nutrient digestibility was slightly increased in birds that received SDPP in their starter diets compared to the control. Nutrient digestibility was generally higher in chicks that were fed on starter diets containing 10 g SDPP/kg diet (Table 14).

Tissue protein content and activities of digestive enzymes

There was no significant effect of SDPP on tissue protein content of the jejunal mucosa of 24 day old birds on both wheat-based and maize-based diets (Table 15). On the wheat-based diets, the activity of maltase was significantly increased ($P<0.01$) in chickens fed on the diet containing the highest level of SDPP. However, this effect was absent in birds on the maize-based diets. Similarly, the activity of sucrase increased significantly ($P<0.006$) in chickens on the wheat-based diet with the highest level of SDPP, but this effect was absent in birds on the maize-based diets. There was a significant increase ($P<0.02$) in alkaline phosphatase activity of birds on the maize-based diet which contained the highest SDPP level. However, there was no significant variation in alkaline phosphatase activity in chicks on the wheat-based diets.

Pancreatic tissue protein and chymotrypsin amidase at 24 days of age were not affected by dietary inclusion of SDPP in the maize-based diets (Table 15). However, on the wheat-based diets pancreatic tissue protein was reduced ($P<0.02$) by rising level of SDPP, and the activity of chymotrypsin amidase increased ($P<0.01$) in birds that received the highest level of SDPP. Dietary inclusion of SDPP had no significant effect on the pancreatic lipase activity of birds on either diet type.

Table 14: Ileal digestibility of protein, gross energy and dry matter at 24 days of age¹

Cereal		SDPP levels g/kg				SEM
		0	5	10	20	
Maize	Protein	0.78	0.78	0.73	0.72	0.01
	Gross energy	0.76	0.75	0.71	0.72	0.01
	DM	0.79	0.87	0.88	0.84	0.01
Wheat	Protein	0.75	0.75	0.83	0.79	0.02
	Gross energy	0.68	0.68	0.74	0.69	0.03
	DM	0.86	0.85	0.87	0.85	0.01

¹ Each value represents the mean of 6 replicates for each treatment group; SEM= Standard error of mean

Table 15: Tissue protein and digestive enzyme activities (ηmol/mg protein) in the jejunum and pancreas of broiler chickens at 24 days of age

Cereal	Tissue	Enzyme	SDPP levels g/kg				SEM
			0	5	10	20	
Maize	Jejunum	Protein ⁴	26.9 ^a	26.9 ^a	27.0 ^a	26.2 ^a	0.57
		Maltase ⁵	1.81 ^a	1.68 ^a	1.53 ^a	1.88 ^a	0.05
		Sucrase ⁵	0.16 ^a	0.13 ^a	0.14 ^a	0.14 ^a	0.004
		AP ^{2,5}	0.09 ^{ab}	0.08 ^b	0.08 ^b	0.11 ^a	0.003*
	Pancreas	Protein	21.8 ^a	21.8 ^a	25.2 ^a	23.5 ^a	0.95
		CA ^{3,5}	6.5 ^a	6.6 ^a	5.5 ^a	5.9 ^a	0.28
		Lipase	1.1 ^a	1.2 ^a	1.0 ^a	0.9 ^a	0.055
Wheat	Jejunum	Protein	27.7 ^a	27.0 ^a	26.6 ^a	28.2 ^a	0.77
		Maltase	1.6 ^b	1.4 ^b	1.5 ^b	1.9 ^a	0.039**
		Sucrase	0.13 ^b	0.11 ^b	0.13 ^b	0.16 ^a	0.003**
		AP	0.07 ^a	0.06 ^a	0.06 ^a	0.07 ^a	0.003
	Pancreas	Protein	26.7 ^a	25.4 ^{ab}	26.1 ^a	21.4 ^b	0.773*
		CA	6.4 ^b	6.1 ^b	6.3 ^b	7.7 ^a	0.217**
		Lipase	1.1 ^a	1.1 ^a	1.1 ^a	1.2 ^a	0.021

¹ Each value represents the mean of 6 replicates for each treatment group; ^{a,b,c} – Mean values on the same row not sharing a superscript are significantly different (*P<0.05; **P<0.01; ***P<0.001); ²AP, Alkaline Phosphatase; ³CA, Chymotrypsin amidase; ⁴Protein (mg/g tissue). SEM= Standard error of mean.

Morphometry of jejunal mucosa

Feeding SDPP to the birds in the first ten days resulted in an increase in the height of jejunal villi at 24 days of age, regardless of grain base (Table 16). However, this effect was only significant (P<0.01) for the birds on the wheat-based diets. Crypt depth in the jejunum increased due to the inclusion of SDPP in the starter diet, however, it was only significant (P<0.03) for the birds that received SDPP in the maize-based diet. There were significant reductions in villus/crypt ratio in birds on both the maize-based (P<0.008) and wheat-based (P<0.025) diets.

Table 16: Jejunal morphometry of chickens at 24 days in response to SDPP level and cereal grain base

Cereal		SDPP levels g/kg				SEM
		0	5	10	20	
Maize	Villus height(μm)	1915.4 ^a	1840.5 ^a	1917.1 ^a	1976.1 ^a	15.43
	Crypt depth (μm)	147.5 ^c	197.5 ^a	189.0 ^{ab}	176.5 ^b	2.52*
	Villus/crypt ratio	13.7 ^a	9.6 ^c	10.8 ^b	11.1 ^b	0.19***
Wheat	Villus height(μm)	1881.7 ^b	1929.4 ^a	2017.4 ^a	1980.5 ^a	15.69**

	b				
Crypt depth (µm)	134.5 ^a	197.5 ^a	168.9 ^a	162.3 ^a	2.18
Villus/crypt ratio	13.9 ^a	10.4 ^c	11.5 ^b	12.0 ^b	0.15 [*]

¹ Each value represents the mean of 6 replicates for each treatment group; ^{a,b,c} – Mean values on the same row not sharing a superscript are significantly different (*P<0.05; **P<0.01; ***P<0.001); SEM= Standard error of mean

Economic analysis of feeding SDPP

The economics of feeding SDPP to broiler chicks is shown in Table 17. These data have been derived from the average of responses and costs on the maize- and wheat-based diets. In general, feed intake declined with an increase in the level of SDPP in the diet while there was an increase in LW of the birds. FCR also improved with rising level of SDPP in the starter diet.

During the starter period, feed cost increased with rising dietary level of SDPP. However, in the following grower and finisher periods (11-35 d), feed costs actually declined. The total costs of feed were \$1.84, \$1.81, \$1.77 and \$1.83 per bird, which equated to feed costs per kg LW of about \$0.72, \$0.71, \$0.89 and \$0.67 per bird.

Table 17: Economic analysis of feeding SDPP to broiler chicks on maize- and wheat-based diets.

	Period (days)	SDPP levels g/kg			
		0	5	10	20
Feed intake (kg/bird)	0-10	0.386	0.383	0.372	0.377
Feed intake (kg/bird)	0-35	4.2	4.1	4.0	4.0
LW (g)	10	318.0	340.0	342.5	349.5
LW (g)	35	2573.5	2561.0	2598.5	2705.5
FCR	0-10	1.20	1.13	1.09	1.08
FCR	0-35	1.63	1.59	1.54	1.49
Feed cost (\$/bird)	0-10	0.18	0.19	0.20	0.22
Feed cost (\$/bird)	11-35	1.66	1.62	1.59	1.60
Total feed cost (\$/bird)	0-35	1.84	1.81	1.79	1.83
Cost/kg LW (\$/bird)	0-35	0.72	0.71	0.69	0.67

Experiment 2

Gross responses

Feed intake up to 10 or 35 days on the wheat-based diet was not affected by starter level of processed soy product (HPA), however, on the maize-based diet it was significantly decreased (P<0.01) for the birds that received the highest level of HPA (Table 18). On the wheat-based diet, LW at 10 (P<0.01) and 35 (P<0.05) days was increased by rising levels of HPA while on the maize-based diets, LW was improved but not significantly affected by rising levels of HPA throughout the test period. On the wheat-based diet, HPA reduced (P<0.01) FCR for the two periods assessed while on the maize-based diet, there was no reduction in FCR between hatch and 10 days, but FCR tended to improve (P<0.08) between hatch and 35 days.

Table 18: Feed intake (g/bird), live weight (g) and FCR (g feed/g weight gain) of birds between hatch and 35d of age¹.

Cereal	Response	Age (days)	HPA levels (g/kg)				SEM
			0	25	50	100	
Maize	FI	1-10	304.0 ^a	299.7 ^a	302.0 ^a	293.1 ^a	3.33
		1-35	3931.9 ^a	3814.0 ^{ab}	3819.4 ^{ab}	3763.1 ^b	20.76 ^{**}
	BW	1-10	295.8 ^a	300.4 ^a	318.2 ^a	293.4 ^a	3.15
		1-35	2519.6 ^a	2518.3 ^a	2672.8 ^a	2546.6 ^a	27.29
	FCR	1-10	1.17 ^a	1.13 ^a	1.07 ^a	1.14 ^a	0.007
		1-35	1.58 ^a	1.54 ^a	1.45 ^a	1.50 ^a	0.016
Wheat	FI	1-10	311.2 ^a	302.6 ^a	300.0 ^a	303.6 ^a	2.89
		1-35	3742.5 ^a	3703.8 ^a	3789.6 ^a	3753.8 ^a	33.70
	BW	1-10	303.1 ^a	303.7 ^a	304.1 ^a	323.1 ^b	2.42 ^{**}
		1-35	2429.8 ^a	2437.0 ^a	2539.0 ^a	2626.2 ^a	33.88 [*]
	FCR	1-10	1.16 ^a	1.13 ^{ab}	1.12 ^b	1.06 ^c	0.006 ^{***}
		1-35	1.56 ^a	1.54 ^{ab}	1.51 ^{ab}	1.45 ^b	0.016 ^{**}

¹ Each value represents the mean of 6 replicates for each treatment group; a,b,c – Mean values on the same row not sharing a superscript are significantly different (*P<0.05; **P<0.01; ***P<0.001).

Visceral organ weight

At 10 days of age, there was no significant effect of dietary inclusion of HPA on visceral organ weight of birds on both the maize-based and wheat-based diets (Table 19). However, on the chickens fed maize-based diets, there was a marginal increase in the weight of the small intestine (P<0.08) and liver (P <0.07) of the birds fed on starter diet with the highest level of HPA.

Table 19: Relative weight of visceral organs (g/100g of LW) of broiler chicken at 10 days of age¹.

Cereal	Organ	HPA levels (g/kg)				SEM
		0	25	50	100	
Maize	Small Int ²	8.3 ^a	8.2 ^a	8.3 ^a	8.4 ^a	0.124
	Pro+Gizz ³	3.7 ^a	3.7 ^a	3.5 ^a	3.7 ^a	0.07
	Heart	0.87 ^a	0.88 ^a	0.93 ^a	0.92 ^a	0.029
	Liver	4.0 ^a	4.1 ^a	4.2 ^a	4.4 ^a	0.078
	Spleen	0.08 ^a	0.08 ^a	0.10 ^a	0.07 ^a	0.007
	Bursa	0.16 ^a	0.16 ^a	0.17 ^a	0.19 ^a	0.011
	Pancreas	0.46 ^a	0.43 ^a	0.44 ^a	0.46 ^a	0.011
Wheat	Small Int	7.9 ^a	7.4 ^a	6.9 ^a	8.3 ^a	0.287
	Pro+Gizz	3.2 ^a	3.5 ^a	3.6 ^a	3.4 ^a	0.057
	Heart	0.88 ^a	1.00 ^a	0.91 ^a	0.94 ^a	0.030
	Liver	4.1 ^a	3.8 ^a	4.0 ^a	4.1 ^a	0.069
	Spleen	0.06 ^a	0.08 ^a	0.06 ^a	0.08 ^a	0.004
	Bursa	0.16 ^a	0.16 ^a	0.13 ^a	0.17 ^a	0.008
	Pancreas	0.40 ^a	0.43 ^a	0.40 ^a	0.43 ^a	0.016

¹ Each value represents the mean of 6 replicates for each treatment group; a,b,c – Mean values on the same row not sharing a superscript are significantly different (*P<0.05; **P<0.01; ***P<0.001); ² small intestine with digesta; ³ Proventriculus and Gizzard; SEM= Standard error of mean.

Table 20: Relative weight of visceral organs (g/100g of LW) of broiler chicken at 24 days of age¹.

Cereal	Organ	HPA levels (g/kg)				SEM
		0	25	50	100	
Maize	Small Int ²	4.9 ^a	4.5 ^{ab}	4.5 ^{ab}	4.0 ^b	0.091 ^{**}
	Pro+Gizz ³	2.0	2.1	2.1	2.0	0.044
	Heart	0.76	0.79	0.74	0.73	0.021
	Liver	2.7 ^a	2.7 ^a	2.7	2.7	0.065
	Spleen	0.08	0.08	0.08	0.08	0.003
	Bursa	0.14	0.14	0.16	0.16	0.008
	Pancreas	0.23	0.24	0.25	0.23	0.007
Wheat	Small Int ²	4.4	4.6	4.1	4.0	0.137
	Pro+Gizz ³	1.7	1.6	1.7	1.5	0.047
	Heart	0.8	0.9	0.8	0.7	0.019
	Liver	2.5	2.6	2.6	2.6	0.076
	Spleen	0.08	0.08	0.08	0.07	0.004
	Bursa	0.16	0.18	0.20	0.15	0.009
	Pancreas	0.20	0.21	0.19	0.17	0.006

¹ Each value represents the mean of 6 replicates for each treatment group; ^{a,b,c} – Mean values on the same row not sharing a superscript are significantly different (*P<0.05; **P<0.01; ***P<0.001); ² small intestine with digesta; ³ Proventriculus and Gizzard; SEM= Standard error of mean

At 24 days of age, the relative weight of the small intestine of chickens on the maize-based diets was significantly reduced (P<0.003) by rising level of HPA in the diet (Table 20). On wheat-based diets, the relative weight of the pancreas tended to decrease (P< 0.056) in birds that received the highest level of HPA in comparison to the control and other experimental groups. The weight of other visceral organs was not affected by dietary inclusion of HPA.

Table 21: Carcass yield and meat parts yield (g) of broiler chicken at 35 days of age¹.

Cereal	Carcass part	HPA levels g/kg				SEM	p
		0	25	50	100		
Maize	Carcass yield (%)	75.0	76.0	76.0	76.0	0.002	0.79
	Breast	674.5	714.2	739.1	694.4	9.70	0.62
	Thigh	304.0	334.2	336.8	321.3	4.50	0.42
	Drumstick	242.6	245.1	254.9	238.3	3.23	0.66
Wheat	Carcass yield (%)	75.0	75.0	76.0	77.0	0.002	0.058
	Breast	655.2	638.2	703.7	694.4	9.67	0.07
	Thigh	294.6	299.4	330.4	323.1	6.01	0.06
	Drumstick	235.0	231.4	248.5	240.7	3.53	0.37

¹ Each value represents the mean of 6 replicates for each treatment group; SEM= Standard error of mean

Carcass parts yield

Starter level of HPA had no significant effect on carcass parts yield of chickens on both maize-based and wheat-based diets at 35 days of age (Table 21). However, there were slight increments in parts yield at 35 days of age as a result of inclusion of the supplement in the starter diet. On the wheat-based diets, the supplement tended to increase breast meat yield (P<0.07) and weight of thighs (P<0.06).

Table 22: Digestibility coefficient of ileal protein, gross energy and dry matter of broiler chickens at 24 days of age given different HPA starter levels¹

Cereal		HPA levels (g/kg)				SEM
		0	25	50	100	
Maize	Protein	0.72	0.82	0.78	0.80	0.002
	Gross energy	0.70	0.79	0.74	0.77	0.024
	DM	0.84	0.87	0.86	0.87	0.011
Wheat	Protein	0.74	0.77	0.79	0.78	0.009
	Gross energy	0.69	0.72	0.74	0.73	0.011
	DM	0.84	0.85	0.83	0.84	0.007

¹ Each value represents the mean of 6 replicates for each treatment group; SEM= Standard error of mean

Nutrient digestibility

There was no significant effect of HPA on the ileal digestibility coefficient of protein, gross energy and dry matter in birds of both maize-based diet and wheat-based diets on day 24 of age (Table 22). However, the digestibility of protein, gross energy and dry matter was marginally improved by rising levels of HPA in the starter diets.

Table 23: Tissue protein content and digestive enzyme activities (nmol/mg protein) in the jejunum and pancreas of broiler chickens at 24 days of age¹

Cereal	Tissue	Enzyme	HPA levels (g/kg)				SEM
			0	25	50	100	
Maize	Jejunum	Protein ⁴	18.4 ^a	21.3 ^a	18.4 ^a	19.3 ^a	0.70
		Maltase ⁵	2.0 ^b	2.3 ^{ab}	2.7 ^a	2.4 ^a	0.08*
		Sucrase ⁵	0.14 ^a	0.18 ^a	0.17 ^a	0.17 ^a	0.007
		AP ^{2,5}	0.102 ^a	0.097 ^a	0.117 ^a	0.108 ^a	0.005
	Pancreas	Protein ⁴	54.7 ^a	52.5 ^a	41.5 ^a	56.8 ^a	1.90
		CA ^{3,5}	2.6 ^a	2.8 ^a	3.4 ^a	2.6 ^a	0.08
		Lipase ⁵	0.54 ^a	0.55 ^a	0.67 ^a	0.49 ^a	0.014
Wheat	Jejunum	Protein ⁴	18.4 ^a	19.1 ^a	19.4 ^a	18.9 ^a	0.48
		Maltase ⁵	2.2 ^a	2.4 ^a	2.1 ^a	2.5 ^a	0.07
		Sucrase ⁵	0.18 ^a	0.17 ^a	0.16 ^a	0.17 ^a	0.007
		AP ^{2,5}	0.102 ^a	0.099 ^a	0.094 ^a	0.109 ^a	0.005
	Pancreas	Protein ⁴	50.6 ^a	53.2 ^a	53.1 ^a	47.9 ^a	1.99
		CA ^{3,5}	3.2 ^{ab}	3.0 ^a	3.2 ^{ab}	4.0 ^a	0.14*
		Lipase ⁵	0.64 ^a	0.63 ^a	0.60 ^a	0.75 ^a	0.027

¹ Each value represents the mean of 6 replicates for each treatment group; ^{a,b,c} – Mean values on the same row not sharing a superscript are significantly different (*P<0.05; **P<0.01; ***P<0.001); ²AP, Alkaline Phosphatase; ³CA, Chymotrypsin amidase; ⁴Protein (mg/g tissue);

⁵Enzymes (nmol/mg protein) SEM= Standard error of mean

Tissue protein content and digestive enzyme activities

Tissue protein content and activities of sucrase and alkaline phosphatase in the jejunum at 21 days of age were not significantly affected by HPA inclusion to the starter diets, regardless of cereal base (Table 23). However, there was a significant increase (P<0.04) in maltase activity in chickens on the maize-based diets as a result of HPA inclusion to their starter diet. This effect was absent in birds on the wheat-based diets.

There was no significant effect of HPA on tissue protein content and lipase activity in the pancreas of birds on diet types at 24 days of age. However, chymotrypsin amidase activity increased significantly (P<0.03) in the birds that received the highest level of HPA in the wheat-based diet. This effect was not present in birds on the maize-based diets.

Morphometry of jejunal mucosa

Including HPA in the starter diets had significant effects on the jejunal histology at 24 days of age (Table 24). On both types of diets, villus height increased ($P<0.01$) with rising level of HPA in the starter diets. Crypt depth was reduced in birds on both the maize-based ($P<0.05$) and wheat-based ($P<0.01$) diets as a result of inclusion of HPA. Inclusion of the supplement resulted in significant changes in the villus/crypt ratio on the maize- ($P<0.05$) and wheat-based ($P<0.01$) diets at 24 days of age but there was no clearly defined trend. There was no significant effect of HPA on the villus surface area, however, villus surface area was numerically increased in the birds that received starter diets containing HPA.

Table 24: Jejunal villus height (μm), crypt depth (μm), villus height /crypt depth ratio and villus surface area (mm^2) of chickens fed the different diets at 24 days of age.

Cereal	Response	HPA levels (g/kg)				SEM
		0	25	50	100	
Maize	Villus height	1796.0 ^c	1801.1 ^{b^c}	1939.2 ^a	1850.6 ^b	9.18 ^{**}
	Crypt depth	148.4 ^a	131.8 ^b	124.7 ^b	131.0 ^b	2.15 [*]
	Villus/crypt	12.6 ^b	14.6 ^a	15.9 ^a	11.5 ^a	0.24 [*]
	Villus SA ¹	0.72	0.63	0.83	0.73	0.01
Wheat	Villus height	1619.1 ^c	1691.5 ^{b^c}	1816.8 ^a	1759.0 ^{ab}	17.90 ^{**}
	Crypt depth	144.3 ^a	139.9 ^{ab}	131.0 ^b	129.7 ^b	1.82 ^{**}
	Villus/crypt	12.0 ^b	11.8 ^b	14.0 ^a	11.3 ^a	0.21 ^{**}
	Villus SA ¹	0.59	0.68	0.72	0.64	0.01

a,b,c – Mean values on the same row not sharing a superscript are significantly different (* $P<0.05$; ** $P<0.01$; *** $P<0.001$); Villus SA¹ = villus surface area (mm^2); SEM= Standard error of mean

Experiment 3

Feed intake to 10 days was reduced ($P<0.01$) with rising levels of SDPP in the diet, regardless of duration of feeding the supplement (Table 25). However, LW at 10 days was increased ($P<0.01$) with rising levels of SDPP, as was a reduction ($P<0.001$) in FCR. There were no significant effects of the supplement on mortality up to 10 days.

Table 25: Feed intake (g/bird), LW (g), FCR and liveability of birds (1-10 days) on different levels of SDPP, fed over 5 or 10 days in the starter phase.

Feeding duration	SDPP level (g/kg)	Feed intake (g)	LW (g)	FCR	Liveability
5 days	0	326.0 ^a	327.2 ^b	1.14 ^a	1.00
	10	317.8 ^{ab}	332.3 ^b	1.09 ^{ab}	0.97
	20	307.6 ^{ab}	331.2 ^b	1.06 ^b	0.98
10 days	0	326.0 ^a	327.2 ^b	1.14 ^a	1.00
	10	301.2 ^b	329.2 ^b	1.04 ^{bc}	0.98
	20	305.7 ^{ab}	344.2 ^a	1.01 ^c	1.00
SEM		2.35	1.20	0.01	0.005
Sources of variation					
Feeding duration		0.200	0.180	0.026	0.300
Level		0.004	0.004	0.000	0.161
Feeding duration × Level		0.303	0.025	0.268	0.759

a,b,c – Mean values on the same column, not sharing are significantly different, as indicated for each factor. SEM is standard error of means.

There was a slight increase in feed consumption to 24 days as a result of inclusion of SDPP in the starter diet, but this effect was not significant (Table 26). LW at 24 days was increased ($P<0.01$)

due to feeding of SDPP over 5 or 10 days in the starter diet. FCR also declined ($P < 0.001$) with an increase in SDPP in the diet, irrespective of feeding duration.

The response to the supplement to 35 days is shown in Table 27. There were no significant effects of the supplement on feed consumption although LW tended to increase ($P < 0.06$) with an increase in SDPP in the starter diet. FCR was, however, improved ($P < 0.01$) by rising levels of SDPP and was better ($P < 0.001$) when the supplement was fed for 10 rather than 5 days in the starter period. Flock uniformity and mortality were unaffected by treatment.

Table 26: Feed intake (g/bird), LW, FCR and liveability of birds (1-24 days) on different levels of SDPP, fed over 5 or 10 days in the starter phase.

Feeding duration	SDPP level (g/kg)	Feed intake (g)	LW (g)	FCR	Liveability
5 days	0	1732.5	1367.6 ^b	1.31 ^a	0.97
	10	1763.3	1432.0 ^{ab}	1.27 ^{ab}	0.95
	20	1782.6	1462.9 ^a	1.25 ^b	0.98
10 days	0	1732.5	1367.6 ^b	1.31 ^a	0.97
	10	1733.8	1449.3 ^a	1.23 ^b	0.98
	20	1737.8	1446.1 ^a	1.24 ^b	0.98
SEM		10.58	9.48	0.01	0.007
Sources of variation					
Feeding duration		0.251	0.994	0.156	0.485
Level		0.568	0.002	0.000	0.611
Feeding duration × Level		0.683	0.765	0.487	0.611

a,b,c – Mean values on the same column, not sharing are significantly different, as indicated for each factor. SEM is standard error of means.

Table 27: Feed intake (g/bird), LW (g), FCR and liveability of birds (1-35 days) on different levels of SDPP, fed over 5 or 10 days in the starter phase.

Feeding duration	SDPP level (g/kg)	Feed intake	LW	FCR	Liveability	Flock uniformity
5 days	0	3755.0 ^a	2513.9 ^a	1.52 ^a	0.93	91.04
	10	3892.5 ^a	2635.4 ^a	1.50 ^a	0.93	90.88
	20	3778.0 ^a	2569.3 ^a	1.49 ^{ab}	0.93	89.05
10 days	0	3755.0 ^a	2513.9 ^a	1.52 ^a	0.93	91.04
	10	3871.4 ^a	2669.8 ^a	1.47 ^{bc}	0.95	90.82
	20	3830.6 ^a	2676.4 ^a	1.45 ^c	0.95	96.35
SEM		36.34	24.21	0.004	0.009	0.93
Sources of variation						
Feeding duration		0.887	0.34	0.004	0.574	0.20
Level		0.376	0.06	0.000	0.923	0.68
Feeding duration × Level		0.916	0.65	0.077	0.923	0.20

a,b,c – Mean values on the same column, not sharing are significantly different, as indicated for each factor. SEM is standard error of means.

Experiment 4

Table 28 summarises the gross response of birds to diets containing soybean meal (SBM) or Soycomil R (SCM); SDPP, and digestible amino acids. Feed intake was generally higher on diets containing SBM than on diets containing SCM, and this was significant ($P < 0.001$) during the grower stage. Feed intake was also reduced by inclusion of SDPP at the starter ($P < 0.05$) and grower ($P < 0.05$) phases and over the test cycle (1-35 days, $P < 0.01$). The inclusion of extra amino acids in the diets resulted in increased feed consumption during the starter ($P < 0.05$), grower ($P < 0.001$) and 1-35 days ($P < 0.05$) phases. There were no interactions between the three main test factors, with regards to feed consumption.

LW gain was higher on the SBM-containing diets than on the SCM diets, these differences being significant at the starter ($P < 0.001$) and grower ($P < 0.01$) phases. LW gain was also reduced ($P < 0.001$) by SDPP during the starter phase but this was not significant at the grower and finisher phases. Formulating extra amino acids to the diet had a beneficial effect on LW gain, resulting in improvement during the starter ($P < 0.001$) and grower ($P < 0.01$) phases and 1-35 days ($P < 0.01$). The interaction between SDPP and amino acid supplementation was significant in the starter and grower phases ($P < 0.01$) and also over the entire feeding period ($P < 0.05$).

FCR was better on the SBM diets than on the SCM diets but this was significant ($P < 0.1$) only in the starter phase (Table 28). The SDPP groups also had poorer ($P < 0.05$ during the starter phase) FCR than the control groups. Higher digestible amino acids improved the FCR during the starter ($P < 0.001$) and grower ($P < 0.05$) phases but not when considered over the entire production cycle.

Table 28. Growth performance of broilers on starter diets containing different protein sources, SDPP and amino acid concentrations.

Treatment	SDPP ¹ (%)	Feed intake (g/bird)			LW gain (g/bird)			FCR		
		0-10	11-24	0-35	0-10	11-24	0-35	0-10	11-24	0-35
<i>Amino acid and protein source</i>										
Normal (SBM) ²	0	297.3	1473.3	3566.1	287.1	1079.7	2452.9	1.04	1.37	1.46
Normal (SCM) ³	0	287.1	1456.1	3584.4	268.3	1064.9	2481.3	1.07	1.37	1.45
Normal (SBM)	2	280.4	1417.0	3474.6	262.8	1032.2	2406.8	1.07	1.37	1.44
Normal (SCM)	2	268.3	1393.2	3413.4	239.8	1022.7	2354.2	1.12	1.36	1.45
10% extra (SBM)	0	296.0	1523.8	3644.1	302.0	1098.7	2513.1	0.98	1.39	1.45
10% extra (SCM)	0	295.9	1485.2	3624.3	290.5	1050.7	2471.1	1.02	1.41	1.47
10% extra (SBM)	2	293.3	1530.7	3600.8	295.3	1121.3	2536.1	0.99	1.37	1.42
10% extra (SCM)	2	289.8	1451.3	3540.2	280.4	1052.9	2462.5	1.03	1.38	1.44
	SEM	2.25	7.50	18.83	1.67	5.87	11.47	0.011	0.005	0.006
<i>Main effects</i>										
Protein source	SBM	291.7	1486.2 ^a	3571.4	286.8 ^a	1082.9 ^a	2477.2	1.02 ^b	1.37	1.44
	SCM	285.2	1446.4 ^b	3540.6	269.7 ^b	1047.8 ^b	2442.3	1.06 ^a	1.38	1.45
SDPP (%)	0	294.1 ^a	1484.6 ^a	3604.7 ^a	286.9 ^a	1073.5	2479.6	1.03 ^a	1.38	1.45
	2	282.9 ^b	1448.0 ^b	3507.2 ^b	269.5 ^b	1057.2	2439.9	1.05 ^b	1.37	1.44
Amino acids	Normal	283.2 ^b	1434.9 ^b	3509.6 ^b	264.5 ^b	1049.8 ^b	2423.8 ^b	1.07 ^a	1.37 ^b	1.45
	+10 %	293.7 ^a	1497.7 ^a	3602.3 ^a	292.0 ^a	1080.8 ^a	2495.7 ^a	1.01 ^b	1.39 ^a	1.44
<i>Main effects and interactions (P values)</i>										
Protein source		0.16	0.010	0.39	<.0001	0.002	0.11	0.002	0.38	0.48
SDPP		0.017	0.017	0.010	<.0001	0.14	0.069	0.037	0.12	0.14
Amino acids		0.024	0.0001	0.014	<.0001	0.006	0.002	<.0001	0.034	0.67
Protein source × SDPP		0.77	0.42	0.41	0.56	0.73	0.19	0.76	0.47	0.71
Protein source × amino acids		0.30	0.19	0.79	0.25	0.038	0.28	0.91	0.19	0.40
SDPP × amino acids		0.14	0.12	0.35	0.009	0.011	0.033	0.31	0.093	0.23
SDPP × amino acids × protein source		0.93	0.56	0.79	0.948	0.55	0.56	0.78	0.99	0.73

a,b,c – Mean values on the same column, not sharing are significantly different, as indicated for each factor. SEM is standard error of means. ¹Sprayed dried porcine plasma. ²Soybean meal. ³Soy concentrate (Soycomil®, ADM).

LW gain was higher on the SBM-containing diets than on the SCM diets, these differences being significant at the starter ($P<0.001$) and grower ($P<0.01$) phases. LW gain was also reduced ($P<0.001$) by SDPP during the starter phase but this was not significant at the grower and finisher phases. Formulating extra amino acids to the diet had a beneficial effect on LW gain, resulting in improvement during the starter ($P<0.001$) and grower ($P<0.01$) phases and 1-35 days ($P<0.01$). The interaction between SDPP and amino acid supplementation was significant in the starter and grower phases ($P<0.01$) and also over the entire feeding period ($P<0.05$).

Table 29. Visceral organ weight of 10-day old broiler chicks on starter diets containing different protein sources, SDPP and amino acid concentrations.

<i>Amino acid and protein source</i>	SDPP¹ (%)	Provent/ Gizzard	Liver	Small intestine	Spleen
Normal (SBM)²	0	3.4	3.8	4.4	0.07
Normal (SCM)³	0	3.6	4.4	4.1	0.08
Normal (SBM)	2	3.6	4.0	4.6	0.09
Normal (SCM)	2	3.7	5.0	4.0	0.12
10% extra (SBM)	0	3.6	4.0	4.7	0.09
10% extra (SCM)	0	3.4	3.9	4.2	0.07
10% extra (SBM)	2	3.7	4.1	4.8	0.09
10% extra (SCM)	2	3.3	4.7	4.4	0.08
	SEM				
Main effects					
Protein source	SBM	3.6	4.1 ^b	4.6 ^a	0.08
	SCM	3.5	4.6 ^a	4.2 ^b	0.09
SDPP (%)	0	3.5	4.0 ^b	4.3	0.08
	2	3.6	4.7 ^a	4.5	0.09
Amino acids	Normal	3.6	4.5	4.3 ^b	0.09
	+10 %	3.5	4.2	4.5 ^a	0.08
Main effects and interactions					
Protein source		0.463	0.014	0.001	0.821
SDPP		0.707	0.004	0.348	0.343
Amino acids		0.571	0.116	0.043	0.417
Protein x SDPP		0.693	0.213	0.789	0.477
Protein x amino acids		0.153	0.212	0.970	0.123
SDPP x amino acids		0.618	0.382	0.507	0.602
Protein x SDPP x amino acids		0.955			0.653
			0.787	0.593	

a,b,c – Mean values on the same column, not sharing are significantly different, as indicated for each factor. SEM is standard error of means. ¹Sprayed dried porcine plasma. ²Soybean meal. ³Soy concentrate (Soycomil®, ADM).

FCR was better on the SBM diets than on the SCM diets but this was significant ($P<0.1$) only in the starter phase (Table 28). The SDPP groups also had poorer ($P<0.05$ at the starter phase) FCR than the control groups. Higher digestible amino acids improved the FCR at the starter ($P<0.001$) and grower ($P<0.05$) phases but not when considered over the entire production cycle.

The relative weight of some visceral organs at 10 and 24 days of age are shown in Tables 29 and 30, respectively. At 10 days of age, the weight of the liver was higher ($P<0.05$) in chicks on the SCM diets than chicks on the SBM diets. Supplementation with SDPP also resulted in increased ($P<0.01$) weight of the liver. The weight of the small intestine was increased by feeding SBM ($P<0.001$) and higher levels of amino acids ($P<0.05$). There were no significant effects of treatments on visceral organ weights at 24 days of age.

Table 30. Visceral organ weight of 24-day old broiler chicks on starter diets containing different protein sources, SDPP and amino acid concentrations.

<i>Amino acid and protein source</i>	SDPP¹ (%)	Provent /Gizzar d	Liver	Small intestine	Splee n
Normal (SBM)²	0	1.8	2.7	3.6	0.07
Normal (SCM)³	0	2.0	3.0	3.2	0.09
Normal (SBM)	2	1.9	2.8	3.0	0.07
Normal (SCM)	2	2.0	3.1	3.1	0.08
10% extra (SBM)	0	1.9	2.9	3.1	0.08
10% extra (SCM)	0	2.0	2.9	3.2	0.08
10% extra (SBM)	2	1.9	2.9	3.1	0.09
10% extra (SCM)	2	2.1	2.9	3.2	0.09
	SEM				
Main effects					
Protein source	SBM	1.9	2.8	3.2	0.08
	SCM	2.0	2.9	3.2	0.09
SDPP (%)	0	1.9	2.9	3.3	0.08
	2	2.0	2.9	3.1	0.08
Amino acids	Normal	1.9	2.9	3.2	0.08
	+10 %	2.0	2.9	3.2	0.09
Main effects and interactions					
Protein source		0.166	0.203	0.856	0.204
SDPP		0.484	0.599	0.095	0.582
Amino acids		0.501	0.898	0.677	0.393
Protein x SDPP		0.953	0.646	0.299	0.464
Protein x amino acids		0.863	0.072	0.227	0.393
SDPP x Amino acids		0.947	0.413	0.143	0.069
Protein x SDPP x amino acids		0.577	0.908	0.435	0.681

a,b,c – Mean values on the same column, not sharing are significantly different, as indicated for each factor. SEM is standard error of means. ¹Sprayed dried porcine plasma. ²Soybean meal. ³Soy concentrate (Soycomil®, ADM).

Discussion of Results

Gross response

The results of experiment 2 demonstrate that the addition of HPA to the starter diet improved the LW and FCR of broilers up to 10 or 35 days on both grain-based diets. The supplement was more effective in the wheat-based diet than in the maize-based diets. Overall, including HPA in the starter diet increased the productivity of broiler chickens. This could be due to the reduction of poorly digestible and anti-nutritional factors, and further concentrating the highly digestible protein in the product (Batal & Parsons, 2003). Such processing may increase the availability and utilization of essential nutrients such as amino acids and energy, which, in turn, would have a positive effect on the growth performance of the birds, especially in early life. The results are in agreement with the findings of trials on the same product conducted in Belgium and USA (van der Eijk, 2013). Jiang *et al.* (2006) also found that substitution of HPA300 for SBM resulted in better growth performance of broiler chickens. Šiugždaitė *et al.* (2008) observed the same positive effect with the substitution of HP soy concentrate for fish meal on weaned pigs.

Spray-dried porcine plasma had a similar effect on LW and FCR on wheat- or maize-based diets. It is worth noting that LW and FCR continued to improve during the post-starter period when all birds

were fed the same common grower and finisher diets. This would ensure that the product could be used over a short period of time and not greatly increase the cost of production. This was further demonstrated in the third experiment, in which it was shown that SDPP could be used for 5 rather than 10 days, and still sustain the same level of productivity. It would appear that the supplement has a beneficial effect on the early development of the GIT, resulting in life-time improvement in productivity. It is generally known that GIT development is most rapid in the first 5-10 days of life (Uni *et al.*, 1998; Iji *et al.*, 2001; Smulikowska *et al.*, 2002) and SDPP seems to drive this development. The mechanisms by which the product functions may be multifaceted. Jaing *et al.* (2000a) attributed the effect to a reduction in cell density and number in the lamina propria of the small intestine, as a sign of reduced local inflammation. Other researchers have attributed the response to the level of immunoglobulins in SDPP (Thomson *et al.*, 1994; Pierce *et al.*, 2005). Other factors present in the plasma, such as biologically active peptides, growth factors, and other components that specifically exist in SDPP may also be involved.

Feed intake was not affected by SDPP at any stage of the experimental period. This was in line with the findings of Jamroz *et al.* (2011) who observed no differences in feed intake between broilers fed on diets that contained SDPP and control birds. The improvement in growth performance is not always associated with the amount or improvement in feed intake. Including more digestible amino acids definitely led to an improvement in growth and FCR. Such diets would offer more readily available amino acids to young chicks, whose digestive function is still poor. Supplementation with SDPP did not achieve a positive result in this experiment. The reasons for the failure of the supplement to elicit the same response as in the previous experiments are unclear but it may partly be due to differences in formulation and rearing system. The fourth experiment was conducted on litter.

Visceral organ weight

The relative weight of the visceral organs was not affected by the inclusion of HPA or SDPP in the starter diets of young chicks on either maize- or wheat based-diet. The results were similar to those of Mojarrad *et al.* (2012) who tested a soybean product that was processed by a technique similar to that used by Hamlet Protein. The essence of feeding high quality products in the starter period is to improve the development of digestive function. It would appear that any changes in the contribution of the GIT would be functional rather than physical.

Meat yield

The inclusion of the SDPP had no significant effect on meat yield in terms of weight of breast, thigh, drumstick, wing and neck of broilers on either of the grain-based diets. The results were in contrast to those of Bregendahl *et al.* (2005) who stated that skinless, boneless breast meat yield increased when SDPP was fed to broiler chickens. Inclusion of HPA in the starter diets of broiler chickens on either maize-based or wheat-based diets marginally improved the meat yield and carcass parts weight, especially breast and thigh weights. The results are in agreement with the findings of van der Eijk (2013) who reported that the inclusion of HPA at a level of 5 % in broiler starter diet for 7 days improved carcass yield and breast weight in comparison with the control. A medium level of HPA in maize-based diet and a high level of HPA in wheat-based diet produced the best outcomes. This improvement may be due to the better performance of HPA-supplemented groups, which resulted in better protein deposition in muscle tissue than other groups. It may also be related to the heavier LW of the HPA-supplemented groups. There is a positive relationship between live LW and carcass yield and its parts weight.

Nutrient digestibility

Spray-dried plasma did not have much effect on nutrient digestibility. These results are in line with the findings of Jamroz *et al.* (2012) who noticed no significant effects of SDPP feeding on the ileal digestibility of protein and dry matter in broiler chickens. However, although non-significant, there

was an improvement in the digestibility of protein, gross energy and dry matter due to the inclusion of HPA in the starter diets, irrespective of the cereal base. The current findings are in line with those reported by van der Eijk (2013) that ileal nutrient digestibility was improved in birds on diet containing HP compared to birds without the supplement. The supplement, as an ingredient, is more highly digestible than SBM due to its low levels of anti-nutritive factors, such as trypsin-inhibitors and oligosaccharides (Batal & Parsons, 2002, 2003). Trypsin-inhibitors reduce the activity of trypsin and chymotrypsin, thus reducing the digestibility of dietary protein (Gallaher & Schneeman, 1986). Previous research has indicated that soybean oligosaccharides may reduce the utilization of energy (van Kempen *et al.*, 2006) and may have a negative influence on the digestibility of dry matter in the ileum in piglets (Wiggins, 1984).

Tissue protein and digestive enzyme activities

The application of SDPP to the starter diets affected the activity of certain digestive enzymes in both the jejunum and pancreas. There are no reports in literature on the effects of SDPP or similar products on basic digestive function. However, the presence of a highly digestible product such as SDPP would stimulate digestive enzyme activities. This response would be complemented by those of rapid growth and increased development of the intestinal mucosa. The activity of pancreatic (Sklan & Noy, 2000) and intestinal mucosal (Uni *et al.*, 1999) enzymes are well correlated to the LW of birds. The activities of digestive enzymes can be influenced by the form (Gabriel *et al.*, 2003) and type of cereal grains (Almirall *et al.*, 1995) used in diets for poultry. Therefore, variation in the activities of certain digestive enzymes between the two grain-based diets could be due to the differences in the chemical composition of the grains, including the nature of anti-nutritional factors such as soluble non-starch polysaccharides (NSP) in wheat. The differences in basal diets may determine how SDPP functions although there does not appear to be much variation between maize and wheat.

In the current study, HPA inclusion in the starter diet had no significant effect on jejunal tissue protein content and the activities of sucrase and alkaline phosphatase. There was an improvement in maltase activity with rising level of HPA, but it was only significant with the maize-based diet. The results were mostly consistent with the findings of Jankowski *et al.* (2009) who reported that the activity of mucosal maltase was not affected by dietary inclusion of HPA in diets for turkey. The supplement is not an important source of simple carbohydrates of the type that are targeted by terminal carbohydrases.

Inclusion of HPA in the diets had no significant effect on pancreatic tissue protein and the activities of pancreatic enzymes in either of the grain-based diets. There was a marginal decrease in the pancreatic tissue protein of birds that received the highest HPA level on the wheat-based diets and the same was found for chickens that received a medium level of HPA in the maize-based diet. This may have a positive effect on the use of nutrients as they are re-directed to muscle deposition in the rest of the body.

There was a marginal increase in the activity of chymotrypsin amidase in the birds that received the highest and the medium levels of HPA in wheat- and maize-based diets, respectively. The improvement in the activity of chymotrypsin amidase may be due to the presence of readily digestible protein source (HPA) in the intestinal lumen, as was highlighted above.

Jejunal histomorphology

The jejunal villi of SDPP-fed chickens were longer and crypts were deeper on both grain-based diets. These results are supported by those of King *et al.* (2005) who observed an increase in villus height and crypt depth of broiler chickens in response to SDPP inclusion in diets. Chickens fed on diets containing SDPP had long finger-like intestinal villi, unlike those on the control diet (Jamroz *et al.*, 2011; 2012). Owusu-Asiedu *et al.* (2003) also reported that intestinal villus height was increased due to SDPP supplementation to pig diets.

The magnitude of this histological response to SDPP is difficult to explain from a purely nutritional point of view. It may be mediated through improvements in intestinal health and barrier function. Supplementation of SDPP to animal diets can in part reduce wearing of epithelial structure, thus improving intestinal mucosal barrier function (Pérez-Bosque *et al.*, 2006). Spray-dried plasma is a feed ingredient composed of a diverse mixture of functional proteins and other biologically important components, some of little nutritional value. Oral consumption of SDPP maintains gut barrier function and reduces or modulates the overstimulation of the inflammatory response. Maintenance of gut barrier function is critical for normal nutrient absorption and reduces exposure to toxins or pathogens that may be present in the intestinal lumen (Campbell *et al.*, 2010).

Diet constituents could influence the morphology of brush border membranes by altering villus height and crypt depth, thereby modifying the surface area available for digestion and absorption (Sharma & Schumacher, 2001). In the current study, inclusion of HPA in the starter diet significantly improved the morphological traits of the small intestine, as measured at the jejunum. Birds that received HPA in the starter phase had significantly longer villi, less crypt depth, higher villus height:crypt depth ratio and larger villus surface area at 24 days than the control. In young chicks, the presence of feed stimulates the growth of the villi, and the availability of nutrients will significantly increase the villus height (Moran, 1982). The results of this research are in agreement with the findings of Xu *et al.* (2012) who reported that the villus height and villus:crypt ratio were increased in the duodenum and jejunum of egg-laying hens on a diet supplemented with processed (fermented) soybean meal compared with those that received plain SBM. Feng *et al.* (2007) observed an improvement in the intestinal morphology of broiler chicks as a result of replacement of SBM with fermented SBM in the starter diet. The longer villi and shallower crypts of chickens on HPA-supplemented starter diets may be primarily due to the stimulatory effect of the product. This needs to be confirmed through further studies on cellular dynamics in the mucosa. The increased villus height to crypt depth ratio and villus surface area of the chickens that were fed on the starter diets containing HPA would greatly equip the intestines for absorption. Digestion and absorption are believed to improve as the ratio between villus height to crypt depth increases (Pluske *et al.*, 1996). Although raw SBM has not been used in this study, residual anti-nutritive factors, including lectins, have been shown to cause morphological changes in the intestine when they come into contact with the specific receptors on the epithelial cell surface in the small intestine wall, destroying the brush-border structure of the intestine (Qin, 2003). This will adversely affect nutrient digestion and absorption.

Duration of feeding starter supplements

A number of feeding strategies have been investigated regarding pre-starter feeding of broiler chickens. Such strategies have included assessment of different supplements, as was done in the current project. A major barrier to the adoption of some products is the cost. The current cost of SDPP, for example, is around \$8000 per tonne. Feeding the supplement to the birds from hatch to 42 days at 2 % of the diet would require about 100 g or \$0.8 per chick. This is a major deterrent to its application by producers. The results of the third experiment demonstrated that an adequate response can be obtained when the product is fed for only 5 days, which coincides with when chicks still have a minimal feed consumption. The economic analysis of using the product over 10 days also showed that feed cost per unit LW was improved through supplementation with SDPP. This will be an incentive for the poultry industry to utilize the product once there is more certainty that it would support increased economic return. Such certainty can be obtained from better understanding of feed formulations, which enables response to the product. We have demonstrated such formulations in two of the experiments conducted in this project. A third experiment, in which there was a positive response to SDPP under Australian conditions, will be presented at the APSS in 2014. It must be pointed out that all three experiments were conducted in cages. The product would need to be tested on litter, to fully reflect Australian rearing conditions. The lack of response to DSPP in the fourth experiment, conducted on litter supports the need for further testing on litter.

Implications

The results obtained from the experiments in this project hold great promise for the poultry industry:

- SDPP can be used in starter diets of chicks to improve growth and FCR, and also reduce relative cost of production.
- SDPP is effective in wheat- or maize-based diets and can be fed for between 5 and 10 days, to achieve the same results.
- SDPP may not be effective in all feed formulations.
- Extra digestible amino acids promote growth starter and led to an increase in 35-day weight of nearly 3 %.

Recommendations

- SDPP should be tested in more diet formulations, to ensure wider application of the product.
- SDPP should be tested in broilers reared on litter, as this is the rearing system used in Australia, to confirm the results obtained in cages.
- A commercial-scale study should be conducted, to establish if SDPP is effective under practical situations.
- SDPP should be fed at no more than 10 g/kg diet and over 5 days, to reduce the cost of production and maximise profit.

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Plain English Compendium Summary

Project Title:	Use of novel protein sources and improved starter feed formulation for broiler chicks
Poultry CRC Project No.:	2.1.9
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Project Overview	Four experiments were conducted to identify suitable supplements to be used in developing starter diets for broiler chickens. In one experiment, the response to spray-dried porcine plasma (SDPP) in wheat- or maize-based diets was tested. The product was also fed for 5 or 10 days. A similar experiment examined the response to a processed plant product, AviStart, in diets similar to those used in the first experiment. A fourth experiment evaluated a pre-starter diet containing Soycomil R in place of soybean meal and higher (10 %) levels of digestible amino acids. All experiments except the fourth were conducted in cages. The animal by-product, SDPP, improved weight gain and FCR during the starter, grower and finisher periods. It could be fed over 5 days rather than 10 days, in order to reduce the cost of feeding. In general, SDPP improved feed costs per unit weight gain, and could be fed in wheat- or maize-based diets. AviStart improved weight gain and FCR but was marginally effective in wheat- than in maize-based diets. Spray-dried porcine plasma and Soycomil R were not effective in the fourth experiment although there was an improvement in growth and FCR as a result of increased levels of digestible amino acids.
Background	The production cycle of meat chickens has been reduced to such an extent that the starter phase represents more than a quarter of the cycle. Poor feeding within the starter phase would have a tremendous negative impact on the final weight of the birds. Such poor feeding could be due to delayed access to feed/water or use of poor ingredients in formulating the starter diet. It is difficult to address the former although there are attempts at <i>in ovo</i> feeding, to improve post-hatch growth. The objective of the current study was to identify

	ingredients and feed formulae that can improve the growth of broiler chicks, post-hatch.
Research	The research consisted of four feeding trials as described above. In all experiments, the diets containing the supplements were fed for 10 days after which the chicks were transferred to regular grower and finisher diets. The birds were weighed at the start and then at 10, 24 and 35 days of age. Feed consumption was also measured over the same periods so that FCR could be calculated. Sub-samples were taken on days 10, 24 and 35, to measure the development of digestive function.
Project Progress	Completed
Implications	The results of this project suggest that it is possible to improve growth and FCR if starter diets are supplemented with SDPP. The product could be fed for 5 or 10 days. A plant product, AviStart had a similar effect. These products would require further testing particularly on litter, in order to fully recommend them to the industry.
Publications	<p>A PhD student, Sleman Mohammad, conducted the first three experiments of this project and will include the data in his thesis. To date, the student has presented a conference paper from one of the experiments. The details of this presentation are:</p> <p>Mohammed, S., Swick, R.A. and Iji, P.A. (2013). Response of broiler chickens to rising levels of processed soya product. European Symposium on Poultry Nutrition, Potsdam, Germany, 19, p148.</p> <p>Another conference paper has been developed for presentation to the Poultry Science Association conference in the USA in 2014. The title of the paper is:</p> <p>Mohammad, S.S., Swick, R.A. and Iji, P.A. Gross response and digestive physiology of broiler chickens on diets containing spray-dried plasma over different feeding durations.</p>

Appendix 1: Nutrient composition (g/kg) of spray-dried porcine plasma (SDPP) used in the study

Nutrient (g/kg)	
Dry matter	920.0
ME Poultry MJ/kg	15.99
Crude protein	780.0
Crude fat	3.0
Ash	100.0
Amino acid (Total)	
Arginine	47.0
Lysine	68.0
Methionine	7.0
Cystine	28.0
Methionine +Cystine	35.0
Tryptophan	14.0
Glycine	30.0
Histidine	28.0
Leucine	78.0
Isoleucine	29.0
Phenylalanine	4.600
Threonine	48.0
Valine	9.0
Amino acids (Digestible)	
Arginine	42.3
Lysine	61.2
Methionine	6.3
Cystine %	25.2
Methionine + Cystine	31.5
Tryptophan	12.6
Leucine	70.2
Isoleucine	26.1
Threonine	43.2
Valine	47.7
Minerals	
Calcium	1.50
Sodium	22.0
Phosphorus (available)	13.0
Phosphorus (total)	13.0
Chloride	11.0
Magnesium	0.3
Iron (mg/kg)	90.0

Source: APC, 2425 SE Oak Tree Court, Ankeny, IA 50021, USA

Appendix 2: Nutrient composition (g/kg) of HP Avistart (HPA) used in the study.

Nutrient (g/kg)	
Dry matter	930.0
ME (MJ/kg)	10.86
Crude protein	556.0
Crude fat	25.0
Starch	30.0
Total amino acid	
Arginine	38.9
Lysine	33.9
Methionine	7.5
Cystine	7.8
Methionine + Cystine	15.3
Tryptophan	7.5
Glycine	23.7
Histidine	14.2
Leucine	41.4
Isoleucine	25.6
Phenylalanine	27.5
Threonine	21.7
Valine	26.7
Serine	28.6
Alanine	24.6
Aspartic acid	62.2
Glutamic acid	99.4
Proline	29.6
Digestible amino acids	
Arginine	
Lysine	33.9
Methionine	7.5
Cystine	7.8
Methionine +Cystine	15.3
Tryptophan	7.5
Threonine	21.7
Minerals	
Calcium	2.50
Sodium	0.40
Phosphorus	8.00
Chloride	0.625
Magnesium	3.5
Iron (ppm)	200.00
Manganese (ppm)	50.00
Zinc (ppm)	60.00

Source: Hamlet Protein A/S. P.O. Box 130 . Saturnvej 51 . DK-8700 Horsens . Denmark.

Appendix 3: Nutrient composition (g/kg) of Soycomil R (SCM) used in the study.

Nutrient (g/kg)	
Dry matter	940.0
ME (MJ/kg)	13.41
Crude protein	650.0
Ether extract	10.0
Crude fibre	35.0
Ash	35.0
Nitrogen-free extract	
Soluble	20.0
Insoluble	160.0
Trypsin activity (mg/g)	2.0
Total Amino acids (g/kg)	
Lysine	42.3
Methionine	9.1
Met+Cys	18.9
Isoleucine	27.3
Tryptophan	31.9
Arginine	49.4
Phenylalanine	34.5
Valine	33.8
Histidine	18.2
Leucine	52.0
Minerals	
Calcium	3.5
Phosphorus	8.0
Available P	2.4
Potassium	22.0
Sodium	0.11
Magnesium	3.35
Chloride	1.0
Iron	130.0
Zinc	35.0
Copper	12.0

Source: ADM Specialty Ingredients (Europe) B.V. P.O. Box 2 · 1540 AA Koog aan de Zaan · Netherlands.

Appendix 4: Nutrient composition of premixes included in the diets.

Trace mineral supplied per kilogram of diet: Cu (sulphate), 16 mg; Fe (sulphate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulphate and oxide), 120 mg; Zn (sulphate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg.

Vitamin supplied per kilogram of diet: Vitamin A (retinol), 12000 IU; Vitamin D₃ (cholecalciferol), 5000 IU; Vitamin E (tocopheryl acetate), 75 mg, Vitamin K (menadione), 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; Vitamin B₁₂ (cyanocobalamin), 16 µg; biotin, 200 µg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg.