PHYSIOLOGICAL RESPONSES OF BROILER CHICKENS TO DIETARY HIGH-QUALITY PROTEINS

By

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and it has not been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis and all sources used, have been acknowledged.

Sleman Beski

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LIST OF ABBREVIATIONS

A/G	Albumin/Globulin
AEC	Animal ethics committee
ANF(s)	Anti-nutritional factor/s
ANOVA	Analysis of variance
AP	Alkaline phosphatase
CA	Chymotrypsin amidase
CFU	Colony forming unit
CP	Crude protein
d	Day (s)
DM	Dry matter
ED	Embryonic day
FCR	Feed conversion ratio
FI	Feed intake
GALT	Gut associated lymphoid tissue
GE	Gross energy
GIT	Gastrointestinal tract
h	Hours
Hb	Hemoglobin
HDL	High density lipoprotein
HP	Hamlet protein
IFA	Infeed antibiotics
LB	Luria bertani

LDL	Low density lipoprotein
LW	Live weight
ME	Metabolizable energy
NRC	National Research Council
PCV	Packet Cell Volume
PSP	Processed soy protein
SBM	Soybean meal
SCFA	Short chain fatty acid
SDP	Spray dried plasma
SDPP	Spray dried porcine plasma
SPC	Soybean protein concentrate
SPI	Soybean protein isolate
TiO_2	Titanium dioxide
UnC	Unchallenged
VSA	Villus surface area

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SUMMARY

Four experiments were conducted to assess the influence of high-quality protein products on the performance, digestive physiology and intestinal integrity, immune development, and survivability of young broiler chickens. Two sources of high-quality proteins, a processed soy product and spray-dried porcine plasma, were included only in the starter diets of broiler chicks and their effects were investigated. Feeding trials were conducted on male Ross 308 broiler chicks from d-old to 35 ds of age. All feed was provided as pellets and experiments were conducted in environmentally controlled housing. Each experimental chapter has been presented as a stand-alone research paper. This summary provides an over view of the key findings of the research.

Providing newly hatched chicks with starter diets containing high-quality protein staff enhanced growth performance at d 10 of age. The effects of supplemental dietary high-quality proteins in the starter diets appears to have long-term benefits on the growth performance of broiler chickens and their effects were sustained during subsequent grower and finisher stages of broiler production cycle.

Inclusion of processed soy product and spray-dried porcine plasma was effective at ensuring optimum feed intake and high growth performance throughout the experimental period. These benefits were most likely due to improved intestine mucosal integrity, nutrient digestibility and pancreatic and intestinal digestive enzyme activities.

The inclusion of a bioactive-nutraceutical such as spray-dried porcine plasma appears to have benefits on the immunity of the broiler chickens, limiting the high risk of immunological and physiological challenges particularly when chicks were faced

disease challenge. The non-nutritional benefits were complimentary to subsequent survivability of broiler life cycle.

The implications and overall findings from this project are discussed in chapter 7 along with recommendations for the poultry industry and further research. The results of these studies provide evidence that inclusion of high-quality protein products to starter diets of broiler chicks is a promising tool to enhance the performance, intestinal and immune development, survivability of broiler chickens. The results also highlight the nutritional and non-nutritional roles of early feeding of spray-dried porcine plasma in chick development. Beyond providing nutrients for growth, spray-dried plasmas can maintain feed intake, down-regulate the immune system, promote rapid development of gastrointestinal system.

There is a need for further investigation into use of high quality protein products in layer chickens diets. A wider range of exploring various proteins feed staff should be considered, taking advantages and exploiting the most available processing techniques.

CHAPTER 1 GENERAL INTRODUCTION

Agricultural industries, including animal production, have dramatically changed over the past few decades (Lassen *et al.*, 2006). The change has been driven by the transition from traditional agriculture as a style of life to a business (Fredeen & Harmon, 1983). The poultry industry, as an important component of the agricultural sector, has been developed in several areas such as nutrition, genetics and management to maximize the efficiency of growth and meat yield. Agriculture is continuously faced with the problem of diminishing resources and increased population growth. To remain competitive and profitable in the global marketplace, poultry producers have realized that efficiency is the key focus. Genetic selection has improved carcass qualities (yield of meat and composition), and bird productivity (growth rates); while technology has improved labour efficiency, nutrition (least-cost formulation and feed supplements), health (development of new vaccines and medications) and reproduction. However, the drive for efficiency has also meant considerable compromises for the newly hatched birds.

The attainment of a good 7-d weight, together with good health status is increasingly important because this period represents an ever-increasing percentage of the broiler production cycle and is strongly correlated with the market weight (Kleyn & Chrystal, 2008). These goals can be achieved if the early development in both physiological and anatomical perspectives of broilers, particularly in the gut, is viewed as a single entity. The young chicks have undeveloped digestive system (Noy & Sklan, 1998a). A dramatic development of gastrointestinal tract initiates in the last few days of incubation, and continues for several days posthatch until it reaches to the phase of developmental

maturation (Uni *et al.*, 2003a). Early development of broilers is dependent on their nutritional status. To achieve the desired performance objectives, improvement in the nutritional status and aspects of young broiler feeding is necessary to assure the adequate utilization of essential nutrients by the bird (Kleyn & Chrystal, 2008). Nutrition is a tool which can be exploited to assist the immune and digestive maturity of the young birds and aid their transition from yolk dependence to exogenous feed utilization.

Providing high-quality protein for human consumption is the key role of animal production. To cope with market demand of protein (meat), broilers are reaching market age sooner each year (Kleyn & Chrystal, 2008). However, animal nutrition strategies have been changed after the ban on the use of antibiotics to control disease and promote growth performance of animals. Therefore advances in nutrition will be fundamental to securing this rapid growth achievement and maintaining sustainable broiler production, particularly for young birds. Accordingly, the common definition of nutrition simply supplying nutrients for maintenance and growth has become obsolete. Specialist areas such as immuno-nutrition, are rapidly gaining attention (Field *et al.*, 2000; Okamoto *et al.*, 2009). When formulating broiler diets, choosing ingredients to maximize nutrient availability, rather than simply meeting energy or amino acid levels, is necessary (Ravindran, 2005).

There is an increased interest in the development of management and feeding schemes to stimulate intestinal development and welfare in newly hatched chicks, in order to improve growth efficiency while minimizing antibiotics usage. Understanding the mechanism of action of antibiotics on animal physiology, together with the proper use of disease models and *in vitro* methods, helps in finding ideal alternatives to in-feed antibiotics (De Lange *et al.*, 2010). Given the substantial improvements made in the understanding of intestinal

nutrient assimilation, a complimentary objective in nutrition might be to formulate diets for young birds with consideration for the optimization of growth, and function and health of the gut as a priority. Minimization of the concentration of anti-nutritional factors and supplementation with immunologically active compounds are the main focus of gut health-promoting broiler diets. These diet characteristics are influenced by feed ingredient composition and feed processing.

It is necessary to find safe, easily digestible and nutritious raw materials for the production of starter compound feeds which also positively affect the intestinal microflora and is close to the raw material further applied in poultry feeding. Most attention is given to protein products, due to the importance of protein as a major constituent of the biologically active compounds in the body. It also assists in the synthesis of body tissue that is used in renovation and growth of the body (Oluyemi & Roberts, 1979). Furthermore, protein is also involved in the production of enzymes, hormones and blood, all of which have important roles in the physiology of any living organism (Zamora & Fields, 1979). Some new natural components have been introduced to animal nutrition to support the growth performance and health of animals in both unsanitary and conventional conditions. Some of these products belong to the broad class nutraceuticals, which could be defined as raw materials (or part of a raw material) that can provide both medical and nutritional benefits to the animals, including the prevention and treatment of disease (Kalra, 2003).

Numerous potentially valuable protein sources for animal nutrition will be exploited if they properly processed. Several processing methods have been developed to expand the availability of a wide variety of feedstuffs and introduce them into the animal feed

industry. The most common method used for plant and animal protein processing is thermal treatment (Papadopoulos, 1989).

The broad aim of the work reported within this thesis was to test high-quality protein products of both animal and plant origins such as spray-dried plasma and processed soy product as alternative processed protein sources in the nutrition of neonatal broiler chicks. The general hypothesis is that feeding these products to broiler chicks at an earlier age can assist early gut development and digestive physiology and improve broiler growth performance and immunity. The main objectives of the present study were to improve our understanding of the impact of these dietary protein products on gastrointestinal and immune development, survivability and performance. The studies examined structural, digestive, blood and immune parameters together with performance data to assess the immune and intestinal integrity of broilers in response to these novel protein products.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

The poultry industry has made advances in many areas including nutrition, genetics, and management to maximize the efficiency of growth performance and meat yield. The production cycle of broilers continues to shorten and modern broilers are reaching market age sooner each year (Kleyn & Chrystal, 2008). This trend of broiler chicken selection for early maturation and marketing age means that the nutrition of chickens during the posthatch period needs consideration (Takahashi *et al.*, 2011). Toward the end of incubation and as a very new hatchling, egg yolk is the main nutrient source that provides the chick with almost all of its required nutrients (Aviagen, 2009). It has been shown that in the first 48 hours after hatching, the yolk aids the maintenance and development of the small intestine. During this period the chick must make the transition from utilizing lipids supplied by the yolk as its energy source, to using exogenous feed which is rich in protein and carbohydrates (Sklan, 2001). By the time feed enters the gut, the remaining yolk will be mobilized within the chick's body. Getting chicks off to a good start is an important factor in maximizing their productivity. If the chicks are fed directly after hatch, they will efficiently utilize these nutrients for growth. Therefore, timely transportation of the chicks to the rearing farm and feeding them immediately on arrival are necessary for better broiler performance (Aviagen, 2009). It is worth mentioning that the quality of feed ingredients is the key factor in establishing the growth potentiality of broilers. Therefore providing chicks with high quality products, particularly in the starter diets, will make a difference and promote strong early growth.

2.2 Role of early nutrition in chick development

The first days after hatching are critical in the broiler's life cycle (Pezeshkian, 2002; Gonzales *et al.*, 2003). Feeding the broiler chick during the first week of life represents a challenge to nutritionists and flock managers because this period represents an ever-increasing growing percentage of the broiler production cycle. At this time, physiologically and anatomically the chick has yet to develop. A good start leads to a uniform flock of chicks with a good first week weight, which is strongly correlated to the final market or slaughter weight (Kleyn & Chrystal, 2008). The newly hatched birds are required to adapt from dependence on the yolk to exogenous feed posthatch (Sklan & Noy, 2000). Although they are anatomically mature, the digestive, immune and thermo-regulatory systems require more development (Maiorka *et al.*, 2006). Therefore, early access to feed will accelerate the growth and development of these systems. In addition to exogenous feed utilization, the residual yolk will also be exploited by newly hatched chicks for their early posthatch development (Noy & Sklan, 2001).

The feeding onset time as well as the form of the supplemented nutrients are critical for posthatch development (Noy *et al.*, 2001). Immediately after hatching, subsequent performance characteristics of chicks can be greatly influenced by nutrient intake (Tabeidian *et al.*, 2011). For the development of the digestive and immune systems, early nutrition is essential. It enhances the development of the gastrointestinal tract (GIT) and increases the growth performance of birds (Speake *et al.*, 1998). Besides, posthatch nutrition helps to develop chicks' immunity and increases their resistance to pathogens (Bigot *et al.*, 2001; Uni & Ferket, 2004). Exogenous nutrition is accompanied by rapid development of the digestive tract and its associated structures for the proper digestion of

the ingested food. The development of the GIT and organ growth will improve the efficiency of assimilation of ingested nutrients' for muscle development (Moore *et al.*, 2005). In fact, the performance in the first week of life has been shown to have a correlation on the final performance of the flock (Kenny, 2005). Early access to feed enhances body weight gain, rapid satellite cell proliferation, and enables the development of passive immunity (Henderson *et al.*, 2008) and small intestine (Noy & Sklan, 1998a). Depression in mucosal development occurs as a result of delaying posthatch nutrition (Noy & Sklan, 2001). Morphological changes resulting from late nutrition results in clumping of microvilli on the first d and abnormal crypt structure between d 7 and 9 posthatch (Uni *et al.*, 1998) which in turn have a negative impact on the absorption process and subsequent nutrient utilization and metabolism.

2.3 Digestive tract development of young chicks

In poultry, the dramatic development of the GIT initiates in the last few ds of incubation, and continues for several ds posthatch until it reaches the developmental maturation (Uni *et al.*, 2003a).The GIT continues to grow and goes through fundamental changes in its morphology and physiology in order to improve the digestion and absorption processes of food (Maiorka *et al.*, 2006). The morphological changes include an increase in the intestinal size causing incremental improvement in the absorptive capacity of the intestinal surface; however, the physiological changes are associated with the production efficiency of digestive enzymes produced by the by pancreas and intestine that improves feed digestion (Nitsan *et al.*, 1991).

Directly after hatch, to adapt to the carbohydrate- and protein-based diets, the chick's GIT encounters numerous exogenous nutrient sources which stimulate the GIT into a state of rapid development (Lumpkins, 2007). This is an important feature of growth, particularly the development of the digestive functional organs (*e.g.*, pancreas and intestine) in the early post-hatch period of the chick's life (Nitsan *et al.*, 1991). It has been observed that in the days after hatching there is a rapid increase in the weight of the proventriculus, gizzard, and small intestine relative to body weight compared to the weight of other tissues and organs in both chickens and turkeys (Noy & Sklan, 1997).

Increasing the weight as well as the secretory activity of the pancreas and digestive organs is necessary to achieve optimal early chick growth (Nitsan *et al.*, 1991). As mentioned previously, in broiler production, the first posthatch period is critical. Broiler weight achieved in their first seven days has a linear relationship with their market weight (Saki, 2005). Therefore, posthatch feeding could have an effect on the broiler chicks' performance (Yang *et al.*, 2009). It has been shown that pre-starter diet positively affects the growth performance of broiler chicks (Saki, 2005; Swennen *et al.*, 2007).

Many reports have shown that early access to feed increases initial chick growth, which can continue throughout the broilers' production cycle (Uni et al., 1999; Noy & Sklan, 2001). Manipulating the pre-starter diet can modify the development and growth of broiler chicks (Nir *et al.*, 1993).

2.3.1 Structural development

Prior to hatching, the yolk is the main energy source for the embryo. The remaining yolk is absorbed into the abdominal cavity and continues thereafter to supply energy for a few

ds posthatch. Exogenous feed intake initiates rapid development of the intestinal mucosa and the associated structures for assimilation of the nutrients present in the gut (Santin *et al.*, 2001). In broiler chicks, rapid intestinal development occurs between ds three and eight posthatch (Dror *et al.*, 1977). Development of the small intestine involves mucosal growth and increase in digestive function. During this phase the enzymes needed for digestion are developed and this action can be accelerated by posthatch intake of exogenous feed.

The function of the GIT is closely related to its microscopic structure. During the posthatch period, the small intestine grows in weight much faster than the chick's body weight (Sell *et al.*, 1991; Sklan, 2001) because of rapid enterocyte proliferation and differentiation (Geyra *et al.*, 2001b). Directly posthatch, the small intestine of young birds shows rapid morphological and functional development. The morphological changes take place by maturation and differentiation of enterocytes, which lead to subsequent growth of villi and increased crypt depth (Uni *et al.*, 1995). This in turn facilitates increased nutrient uptake by the growing bird, through increasing the surface area and the mucosal nutrient transporters of the GIT (Moran Jr, 1985b). It is well-known that the structure and morphology of villi play a vital role in the digestive and absorptive efficiency of the digestive system (Zulkifli *et al.*, 2009). Large and long intestinal villi make the nutrient absorption in the GIT more efficient (Yamauchi *et al.*, 1993). Therefore, early growth and development of these tiny structures is very important. It has been found that there are initial rapid increases in the villus size and area in the first two ds of a bird's life, after which the growth rate decreases and reaches a plateau five to ten ds after hatch (Uni *et al.*, 1999). Intestinal crypts begin to form at hatch and are clearly defined at several ds post-

hatch, increasing in both cell numbers and size (Uni *et al.*, 2000; Geyra *et al.*, 2001b). Iji *et al.* (2001a) found substantial structural development in the gut mucosa at hatch, with gross changes occurring in the mucosal structure over the first 21 d, which was attributed to exposure to dietary nutrition. They reported an increase in the villus size and surface area and the depth of crypt in chicks from hatch to 21 d of age. Experiments done on the morphology of the intestine indicated that from four to 21 d of age, large villi and deeper crypts may be observed with a slight alteration in density of enterocytes with age (Uni *et al.*, 1998). Intestinal villus morphology was found to be influenced by many factors such as diet type and genetic structure (Ravindran, 2003).

Previous studies have observed that early access to feed after hatch accelerates the development of the small intestinal mucosal layer (Noy & Sklan, 1998a); and a delay in accessing exogenous feed retards the development of the small intestinal mucosal layer (Geyra *et al.*, 2001b; Uni *et al.*, 2003b). Furthermore, delaying access to feed for 24-48 h resulted in decreased villus length (Yamauchi *et al.*, 1996), decreased crypt size and villi:crypt ratio and reduced enterocyte migration rate (Geyra *et al.*, 2001a). In addition, changes in mucin dynamics caused by delayed access to feed for 48 hours reduces the small intestinal absorptive capacity and protective functions (Uni *et al.*, 2003b).

The gastrointestinal modifications that occur in the young bird as it matures allow the bird to achieve full digestive capacity, and recent research has found that the digestibility of feed ingredients increases as broilers grow with age (Parsons, 2004).

2.3.2 Digestive enzyme activity

The digestive system of the young birds is anatomically complete but still immature and requires physiological and morphological development. The physiological development is interrelated with the intensification in the digestive enzyme activity and releasing capacity of the pancreas and intestinal membrane, as mentioned earlier. Pancreatic and small intestinal enzyme secretion, are low at hatch (Sell, 1996), and the birds are functionally immature at this stage; however, with the intake of exogenous feed the enzyme activity will start to increase. After hatch, there is a modification in the small intestinal enzymatic activity in order to acclimatize to the incoming substrate (Noy & Sklan, 1997). The enzymes not only perform luminal digestion but also the final stages of the hydrolysis of nutrients from the brush border membrane.

Numerous studies have indicated the existence of enzymes and measured the pancreatic enzyme activity prior to and posthatching. Trypsin and amylase have been identified on d 18 of embryonic life, and lipase has also been present prior to hatch. Pancreatic secretion of all these enzymes intensified after hatching, with a variation in the rate of increase among different enzymes (Noy & Sklan, 1997).

There are some variations in the release of some pancreatic and intestinal enzymes from d 4 to 21 posthatch. After seven days, the secretion of the pancreatic enzymes and bile are constant per gram of feed intake (Traber *et al.*, 1991). Examples of such enzymes are sacharase-isomaltase, peptidase, and phosphatidases (Maiorka *et al.*, 2006). The activities of lipase, amylase, and protease increase during the first week of life. The activity of pancreatic amylase has been shown to increase threefold from d 1 to d 10 after hatch;

however, trypsin and lipase activity has been reported to increase five- to sixfold. The activity of maltase and sucrase, which are important enzymes in carbohydrate digestion, increased steadily (Uni *et al.*, 1998).

Nir *et al.* (1993) reported that the activity of pancreatic and GIT digestive enzymes increased with age. Sklan and Noy (2000) observed that in chicks that had early posthatch feed access had higher trypsin, amylase and lipase activities in the intestinal mucosa, but a constant intake of feed resulted in a constant secretion of these enzymes .

The carbohydrate digestion mechanism already exists in newly hatched chicks. Poultry in general have the ability to digest carbohydrate immediately after hatch (Maiorka *et al.*, 2006). The enzymes that are responsible for complete carbohydrate digestion are present on the surface of the enterocyte brush border. The levels of these enzymes that are secreted are relative to the substrate concentration present in the intestinal tract (Moran Jr, 1985a). Alpha-amylase, which is specifically responsible for starch digestion, may be observed on d 18 of incubation and the specific activity of this enzyme reaches its peak at d 4 posthatch (Marchaim and Kulka, 1967). Noy and Sklan (2002) found that amylase activity reached its maximum level on d 8 after hatch.

The protein digestive system undergoes tremendous changes during the period posthatch and these changes depend on the concentration and composition of nutrients in the diet (Noy & Sklan, 1997). According to Noy and Sklan (1997) the protein digestion taking place in the small intestine increases from 78% at d 4 to 92% at d 21 posthatch. The enzyme, pepsin, is important for the initial digestion of protein in the feed. Pancreatic proteases, peptidases, and chymotrypsin contribute to further breakdown of protein

present in feed (Uni *et al.*, 1999). Elevated levels of the enzymes, trypsin and chymotrypsin, may be observed at d 11 (Sklan & Noy, 2000).

During incubation, lipids are the main energy source to the embryo. The metabolism of lipids depends on enzymes, like pancreatic lipase, the presence of bile salts and fatty acid-binding protein (Maiorka *et al.*, 2006). According to Maiorka *et al.* (2006) fatty acid digestion increased from 82% at d four to 89% at 21 d of age. The enzyme, lipase, may be observed on d 4 posthatch and the maximum level of lipase may be observed on d 8 in the period posthatch (Noy & Sklan, 1999). Due to the restricted amount of lipase that is produced, the capacity of the bird to digest lipids present in the diet in the period posthatch is limited. Therefore, the amount of lipids that are included in pre-starter and starter diets should be restricted. But as the bird ages, the capacity of lipids and fats digestion will increase and lipid content can increase accordingly (Rutz *et al.*, 2007).

Overall, the changes that occur in the GIT of the newly hatched chick as it matures, allow the bird to achieve full digestive capacity, and this has been confirmed by Sulistiyanto *et al.* (1999) and Batal and Parsons (2002), who found that the increment in the digestibility of nutrients by broilers is an age-related process. Functionally, the digestion of exogenous nutrients improves due to the reduction in the digesta passage rate and hydrophobic yolk in the GIT as well as an intensification in the secretion of bile acids, pancreatic and brush border enzymes (Noy & Sklan, 1995; Uni *et al.*, 1996).

2.4 Development of immune functions

Distinguishing between self and non-self and the proper response to identification of each, is the simple function of every immune system (Korver, 2006). In general, unlike

mammals, birds possess quite similar defense actions in terms of general organization and mechanisms of immunity, and this system is directly affected by the animals' genetics, physiology, nutrition, and environment (Qureshi *et al.*, 1998; Sharma, 2003). . The avian immune system has a unique morphological and functional peculiarity. Humoral and cell-mediated immune reactions are reliant on two anatomically separate organs, the thymus and bursa of Fabricius (Neumann, 1998). Lymphoid organs are the basic units of the avian immune system. Among them, the bursa of Fabricius (in which B type lymphocytes develop and differentiate) and the thymus (which is responsible for the differentiation and development of T-type lymphocytes) are considered to be primary lymphoid organs (Qureshi *et al.*, 1998). The rest are called the secondary lymphoid organs and are distributed all over the body and occupied by large numbers of lymphocytes and antigen-presenting cells. . .

Immune system development in broilers starts during the embryonic period and continues after hatching (Klasing, 1998; Alex *et al.*, 2006; Yegani & Korver, 2008). The development of the primary lymphoid organs, such as the thymus and bursa, initiate in early embryonic life and they involute later (Sklan, 2005). Then the migration of lymphocytes from the primary to the secondary lymphoid organs starts (Sklan, 2005). (Dibner & Richards, 2004). . The first sign of bursal development arises on the 4^{th} ED, and receives the stem cells derived from the yolk sac at 7.5^{th} ED. However, the humoral response maturation will only occur after a week post-hatching (Mast & Goddeeris, 1999). The other secondary immune organs are only partially developed at hatching, and they are capable of efficiently responding as active immune components only 10 d after hatching (Mast & Goddeeris, 1999). .

Despite the anatomically complete immune system, as well as the developed immune competence at the end of embryonic life, the responses of the newly hatched chick are poor, and the acquired maternal antibodies provide the major part of its defence system (Rose, 1979). The primary immune organs exist and are occupied by lymphatic cells. Nevertheless, the secondary immune organs are still not fully active at hatch (Schat & Myers, 1991). In the broiler chick, the expression of the secondary responses, as designated by the existence of germinal centres or circulating IgG and IgA, initiate between the 1st and 4th week after hatching (Leslie, 1975).

In poultry, the development of the immune system is a continuous active process beginning during embryonic life and only completed weeks or months posthatch (Nnadi & Ezema, 2010). The first week posthatch is a period of rapid expansion of leukocyte populations, together with a rapid increase in the size of the lymphoid organs (Panda *et al.*, 2010), lymphoid organ seeding, and the educational actions that create the unique lymphocyte clones that will mediate immunity later in life (Klasing, 1998). The increases in the number of these cells and size of organs are necessary for the development of the acquired immunity. The period after hatching is crucial for the development of the gut immune system which is defined as gut-associated lymphoid tissue (GALT). In chickens, GALT consists of the bursa of Fabricius, caecal tonsils, Peyer's patches, and lymphoid aggregates in the urodeum and proctodeum (Befus *et al.*, 1980). The development of lymphoid tissue associated with the intestine is essential for chick survival, particularly during the first two weeks of life when maternal antibodies are important for humoral response (Yun *et al.*, 2000). The bursa of Fabricius has a main role in the production of antibodies. By the time of hatch, the bursal channel opens and, instantly, antigen

transportation from the environment into the lumen and lymphoid follicles of the bursa begins (Yegani & Korver, 2008). Therefore, the transition period from embryo to posthatch stage is critical for the normal development of the bird. With the beginning of food intake and microbial colonization, the immune system must differentiate between harmless food components and potentially harmful pathogens. At this stage, the immune system is immature and the chick must rely on innate effector mechanisms and maternal antibodies. Thus, achieving a rapid transition from an innate response to the acquired immune system, which has more efficient and targeted responses, may reduce the production losses in commercial poultry caused by the activation of the immune system (Korver, 2006). Some practices, normally adopted on the first days after hatching, may affect bird development, particularly, the digestive, immune, and thermo-regulatory systems. These systems pass through considerable morphological and physiological changes after hatching, such as hyperplasia, hypoplasia, and cell differentiation (Alex *et al.*, 2006). Therefore, maternal nutrition as well as chicks' early nutrition may play important roles in the development and function of the bird's immune system (Nnadi & Ezema, 2010). Development of the immune system, particularity GALT, has a linear relationship with the time of feeding. It has been shown by Shira *et al.* (2005) that there is a delay in the activity of GALT during the first 14 d of a bird's life due to a delay of in feeding for the first three d posthatch. The lower systemic and antibody responses in the intestine following rectal immunization of antigens have also been observed. Furthermore, the T and B lymphocytes' occupation and the expression of T-lymphocyte-specific genes have been found to be delayed in the hindgut as a result of delay in access to feed. It has

also been observed that an increase in the sizes of the B- and T-cell populations in the bursa is delayed due to posthatch delay in access to feed (Yegani & Korver, 2008)

2.5 Protein nutrition of broiler chickens

The main objective of poultry nutrition is the optimization of the birds' productivity and performance through feeding them diets that meet their bodies' requirements. When formulating broiler diets, the main emphasis is placed on the crude protein (CP). Protein is an important element of the diet needed for survival of animals because protein and its amino acid contents are used as construction units of the structural proteins, metabolic proteins, enzymes, and precursors of several body constituents. However, protein is also one of the most expensive ingredients in poultry diets. Therefore, nutritionally and economically, proper protein usage is essential in all feeding systems, and wasteful usage increases the cost of production. Broilers have high dietary CP requirements, so identification of the optimum protein concentration in broiler diets, for either maximizing broiler performance or profit, requires more knowledge about birds' requirements for protein and amino acids and their effects on the birds' growth performance and development. It also requires knowledge about the protein sources available that can be used in poultry diets.

2.5.1 Protein and amino acid requirements

Nutrient requirement, simply defined, is the minimum dietary amount needed for maximum performance, and during formulation nutritionists often provide a higher protein level than required as a buffer to avoid any possible nutrient shortages and subsequent performance decline (Sterling *et al.*, 2005).

The critical constituent of poultry diets is protein, and together with the other main nutrients such as carbohydrates, fat, water, vitamins, and minerals, is indispensable for life (Cheeke, 2005). Proteins are polymers that are composed of α-amino acids which are linked together by peptide bonds (Perry *et al.,* 2003). Proteins are broken down and hydrolyzed in the digestive system into amino acids. Then, after absorption, the amino acids will be assembled and metabolized to form body proteins that are used in the building of different body tissues (Aviagen., 2009). They also serve vital metabolic roles as blood plasma proteins, enzymes, hormones, and antibodies, each of which has a specific role in the body (Pond *et al.*, 1995).

The usefulness of a protein feedstuff for poultry depends upon its ability to supply a sufficient amount of the essential amino acids that the bird requires, as well as the protein digestibility and the level of toxic substances associated with it (Scanes *et al*., 2003). Therefore, the quality of protein is not determined by its level in the diet, but by its amino acid profile, particularly the essential amino acids. Provision of these indispensable amino acids to the bird is most important and broiler diets should be formulated based on the digestibility of the amino acids (Aviagen., 2009). Protein deficiency, due to the absence of one or more essential amino acids or insufficient protein intake, results in a decrease in growth rate, feed consumption, and utilization and retention of nitrogen, (Church & Varela-Alvarez, 1991). However, an excess of protein intake causes amino acid breakdown via deamination, and excretion as uric acid, which is energetically and economically wasteful (Sklan & Plavnik, 2002). In advanced cases, it can lead to ammonia toxicity (Perry *et al*., 2003). Therefore, providing the bird with accurate levels of its protein requirement is necessary to increase its production and profitability.

The concept of dietary protein requirement has been debated since the discovery that proteins are made from amino acids and that some of them are fundamentals for maximum growth and performance (Pesti, 2009). The National Research Council (1994) demonstrated that the amount of protein needed by poultry is equal to the amount of the amino acid requirements, and that amino acids are involved in various body functions. Although all of the protein's amino acids are demanded by the body, only the essential amino acids need be delivered in the diet.

Essential amino acids are those that the body does not have the ability to synthesize at all or in sufficient amounts to meet physiological needs (Cheeke, 2005). Non-essential amino acids can be synthesized from other amino acids or nitrogen in the bird's body; therefore, they are not necessarily required in the diet. However, it should be considered that if the bird's diet lacks non-essential amino acids or an additional nitrogen source, the body will start to synthesize these from essential amino acids and this may adversely affect the bird's performance. Thus, during diet formulation, and in order to avoid essential and non-essential amino acid deficiencies, it is common to supply the required protein level together with the essential amino acid requirements (NRC, 1994).

The dietary protein and amino acid requirements for broilers change, depending on their growing phase. According to NRC (1994), the growing period of broilers is divided into three stages, starter (0-3 weeks), grower (3-6 weeks), and finisher (6-8 weeks), and the dietary protein requirements in each period are 23, 20 and 18%, respectively. However, due to the very rapid growth of modern broilers as a result of great development in the field of genetics and breeding in poultry, the abovementioned requirements and the phases have been re-defined by breeders. Several factors affect the protein and amino acid

requirements of poultry. These factors include the age of bird, production state, gender, size, species, and strain (Liebert, 2006), as well as the difference in the quality and digestibility of the protein. Amino acid requirements may also be effected by temperature, as consumption is frequently reduced in extreme heat and increased in cold periods (Furlan *et al.*, 2004). Therefore, modifications of amino acid concentration in the diet may be needed in abnormally hot and cold conditions. Feed intake, and consequently amino acid consumption, are also affected by the state of the bird's health, the structure of the provided diet (Maiorka, *et al*., 2005), and by different environmental stressors.

2.5.2 Sources of protein for poultry

In poultry feed formulation, after the energy-yielding raw materials, protein supplements constitute the biggest component, and attention has been focused on the protein and energy levels of the feed. Meeting the bird's requirements for dietary protein contributes considerably to the feed costs (Skinner *et al.*, 1992). Vegetable (plant) and animal protein are the two most important protein sources in poultry rations.

The majority of an animal's dietary protein requirement is supplied by plant protein sources. Worldwide, traditionally, the most used energy and protein sources are firstly maize and secondly soybean. Cereals, like wheat and sorghum, and some plant protein meals are used all over the world as well. Soybean meal is the preferred protein source used in poultry feed manufacturing. Its crude protein content is about 40 to 48%, and this depends on the quantity of hulls removed and the oil extraction process. Compared to the protein meal of other oilseed grains, soybean protein is favoured due to its well-balanced amino acid profile, especially the essential ones, enabling it to balance most cereal-based

diets (Ravindran, 2013). Because of their deficiency in some amino acids, plant proteins usually require a supplementary source of amino acids or other protein sources such as animal protein. Plant proteins are usually cheaper than animal proteins; however, there is a limitation to their use because of their content of anti-nutritional factors. Most of these anti-nutritional factors can be destroyed by thermal processing that causes an increase in the value and protein level of plant proteins (Adeyemo & Longe, 2007).

In general, vegetable (plant) protein sources are nutritionally unbalanced and poor in certain essential amino acids, and this decreases their biological value as they may not furnish the required limiting amino acids needed by birds for egg and meat production. Poultry nutritionists have paid more attention to the use of animal protein sources (Akhter *et al.*, 2008). Animal proteins are well balanced in terms of essential amino acids that are necessary for body growth and development. Therefore, they are usually used to complement the amino acid balance in the diets rather than as the main protein source.

In general, the quality of animal protein sources is dependent on the composition of the raw material used. Animal protein supplements are derived from: poultry and poultry processing, meat packing and rendering operations, fish and fish processing, and milk and dairy processing (Denton *et al.*, 2005). Bone meal, meat meal, poultry meal, hydrolyzed feather meal and to a lesser extent blood meal have all been used as important feedstuffs for poultry feeding (Pearl, 2002). Animal proteins are a beneficial component of poultry diets because they offer a high level of protein/amino acids, a high level of available phosphorus, an amount of other minerals, and moderate levels of energy.

Thus, it is necessary to include one or more of these animal protein sources in chicken diets. Hatchery by-products, feather and blood meals, and spent hens, have also been used for non-ruminant animals (Moritz & Latshaw, 2001). To improve performance, there has also been some interest in substituting part of the soybean meal in poultry diets with animal products. Supplementation of animal protein sources may considerably improve performance parameters over standard diets. However, this may be because of the high concentration of essential amino acids, or it may be due to the lower percentage of indigestible carbohydrates present in soybean meal (Firman & Robbins, 2004).

In various countries, during poultry feed manufacturing, care is taken that animal protein ingredients do not fall below the minimum levels required in the feeds, particularly for young birds, which require a high level of amino acids. The essential amino acid requirements are gradually decreased as the birds age, and it is possible to supply diets that contain lower animal protein content and relatively higher levels of plant protein to meet the demands of older birds (Ravindran, 2013).

2.5.3 Growth response to protein

Providing high quality protein for human consumption is the key role of animal production, and this can be achieved by providing accurate proportions of high quality protein in animal diets. Protein is a food element which assists the synthesis of body tissue that is used in repair and growth of the body (Oluyemi & Roberts, 1979). Proteins are also involved in the production of enzymes, hormones, and blood, all of which have important roles in the physiology of any living animal (Zamora & Fields, 1979). Similar to other living creatures, energy and protein are very important nutrients for broilers. Energy is

necessary for body function, and protein is an essential constituent of all tissues in the animal body. The most expensive nutrient in broiler diets is protein, which has a major influence on the growth performance of the bird (Kamran *et al.*, 2004).

It is not only growth rate and feed utilization efficiency that depend on dietary protein and energy levels, the proportion of carcass and, particularly, abdominal fat is also dependent on dietary protein. The desired responses to adequate protein levels are: maximum live body weight and lean carcass mass, efficient feed utilization; and low carcass fat. The carcass yield changes (weight of edible product mass per live body weight) caused by altering the level of dietary protein may be about 4%, which is highly beneficial from an economic standpoint (Aviagen., 2000).

The level and quality of dietary protein strongly affect broiler growth rate and meat yield (Eits *et al.*, 2005b). The producers need to consider this as well as the cost of the protein to ensure the profitability of the enterprise (Eits *et al.*, 2005a). Many studies have been conducted to evaluate the effect of dietary protein level on broiler performance. Marcu *et al.* (2009) showed that broilers (Ross 308) fed on high dietary protein levels in starter, grower and finisher diets (24, 22, 21%, respectively), had significantly higher body weight, weight gain, better feed conversion and higher slaughter yield value than those fed on low dietary protein (22, 21, 19%, respectively) during the same periods. Pesti (2009) found an improvement in growth and feed utilization efficiency of broilers in response to increasing dietary protein levels. Jahanian (2009) showed a significant increase in daily feed intake and weight gain with an increase in protein level in the broiler diet. Bunchasak *et al.* (2005a) stated that the production performance of layer chickens was considerably affected by dietary protein level, and the hens fed on a low dietary protein (14%) diet had

poorer production performance than those fed on high dietary protein levels (16% and 18%). Namroud *et al.* (2008) indicated that decreasing the dietary protein level below 19% depressed broiler performance and appetite and increased fat deposition in the bird's body.

Researchers have reported that decreasing dietary CP resulted in a decrease in average daily gain (Bregendahl *et al.*, 2002) and decreased feed efficiency and growth rate of broilers (Bregendahl *et al*., 2004). Much research has been undertaken to assess the effect of supplementing low CP diets with amino acids. The findings of such research are not consistent. In order to replace CP, but maintain growth, several researchers have suggested fortification of low-protein diets with amino acids. Reducing the protein level from 28 to 20% in a diet supplemented with the required amount of amino acids did not reduce turkey performance (Baldini *et al.*, 1954). Similarly, it has been observed that by adding lysine and methionine to broiler diet, the level of crude protein can be reduced to a level that does not reduce performance (Uzu, 1983). The same results can be achieved by adding a mixture of essential amino acids and a source of nitrogen or non-essential amino acids to the broiler diets (Corzo *et al.*, 2005). Waldroup *et al.* (2005b) demonstrated that diets low in CP can be supplemented with synthetic amino acids to maintain performance. Widyaratne and Drew (2011) suggested that low-protein diets can support growth performance equal to high-protein diets when highly digestible ingredients are used. Whereas it has been observed that supplementation of low-protein diets with amino acids reduced the growth performance of broiler chickens (Aftab *et al.*, 2006; Kamran *et al.*, 2008), others have stated that the performance was not affected when the birds were fed on low-protein diets (Parr & Summers, 1991; Yamazaki *et al.*, 1998; Aletor *et al.*, 2000;

Kamran *et al.*, 2010). On the other hand, the predominance of information proposes that a decrease in the protein level of more than 3% leads to a significant reduction in the rate and efficiency of growth as well as the carcass composition, regardless of the presence of all the other necessary nutrient requirements (Sterling *et al.*, 2005; Waldroup *et al.*, 2005a).

2.5.4 Immune response to protein

Nutrition has a considerable effect on animal health and immunity. Impairment of the immune responsiveness occurs as a result of nutritional deficiencies, and thereby increases morbidity and mortality (Chew, 1995). Insufficient nutrient consumption can modify the quantitative and qualitative aspects of the immune response to pathogens. For maintenance of normal immune function, several micro- and macronutrients are required. These include amino acids, fatty acids, several vitamins and minerals (Keser *et al.*, 2008). Studies have been carried out on the relation between the level of protein and amino acid intake and immunocompetence. The amount of amino acid required to increase growth and feed efficiency will also improve immunocompetence (Latshaw, 1991). Dietary protein or amino acid deficiencies have long been demonstrated to reduce immune function and increase the sensitivity of animals to infectious challenges or stressful conditions. Recently, and to better understand the relationships between amino acids and immune function, more fundamental research has been done. Indeed amino acids might regulate the activation of T-lymphocytes, B-lymphocytes, natural killer cells and macrophages; and improve cellular redox status, lymphocyte proliferation, as well as antibody and cytokine production.Dietary supplementation with amino acids beyond their requirement for growth

might thus be under certain environmental conditions and have the potential to reduce the use of medication in animal production throughout the world (Geraert & Mercier, 2010).

It has been observed that the immune responses of birds can be modified in response to dietary protein deficiency or excess (Payne *et al.*, 1990), or changes in amino acid levels (Tsiagbe *et al.*, 1987a). Antibody production and the development of antibody-producing cells in response to the T-cell-dependent antigens have been found to be inhibited due to protein deficiency (Carlomagno *et al.*, 1980). Glick *et al.* (1983) found that deficiency in the protein level (about 33% of requirement) in the diet could reduce the number of lymphocytes that populate the thymus gland of chickens. However, the responses vary with strain (Cheema *et al.*, 2003), environment, stress, production, and health status. Thus, nutrient supplementation at the appropriate times and amounts are required for defensive immune responses (Humphrey *et al.*, 2002). Jahanian (2009) found that raising the protein level from 19 to 22.35% in the diet increased the percentage of lymphocytes and subsequently decreased the heterophil-to-lymphocyte ratio, whereas feeding low protein diet caused a reduction in the relative weights of the thymus and bursa of Fabricius. However, Houshmand *et al.* (2012) stated that protein content in the diet significantly affected the growth performance of broilers but had no effects on the immunity or stress signs (except for cholesterol level). Nnadi and Ezema (2010) explained that protein nutrition appears to modulate the growth and some immune parameters in developing chicks but has little or no effect on the relative weights of most lymphoid organs or the liver.

From the immunological aspect, Bunchasak *et al.* (2005b) found that Newcastle disease titer of hens fed 18% CP diet was significantly higher than those of hens fed 16 and 14%

CP diets. All protein fractions in the serum and serum total protein tended to increase in response to a rising level of protein, except for alpha globulin and the albumin:globulin ratio, which tended to decrease. Dietary protein levels had no effects on the size of the spleen. Chicks fed on diets poor in essential amino acids had reduced delayed-type hypersensitivity and secondary IgG responses compared to chicks fed adequate diets, although this could be due to amino acid imbalances rather than their deficiency (Cook, 1991). In general, specific amino acids could negatively affect humoral response more than cellular immunity. Deficiency in the total sulphur amino acids may restrict the available cysteine required to produce glutathione, and as a result decrease antioxidant defences against reactive oxygen species (ROS) produced during an immune response. Bhargava *et al.* (1970) found that deficiency of methionine caused an increase in antibody production. However, Tsiagbe *et al.* (1987b) suggested that the amount of methionine required for growth was less than that required for maximum antibody development and activity.

2.6 Role of specialist protein products in poultry nutrition

2.6.1 Synthetic amino acids

The prohibition of the use of animal protein sources in poultry nutrition in many countries, and also the relatively high costs of these products demand new alternative products. The possible alternative in this situation is the use of plant protein. However it is well known that there is a deficiency in one or more essential amino acids in plant-based protein. Achievement of an optimum balance of nutrients to meet the animal's requirement from a particular range of raw materials is a distinctive problem in feed formulation. As the ratio

between the individual amino acids in protein concentrates varies significantly, there may be occasions when it is impossible, within the variety of raw materials available, to meet the animal's requirement for all amino acids. In these situations, supplementation with free synthetic amino acids would be very successful. In addition to this, dietary supplementation of synthetic amino acid to poultry diets increases feed conversion efficiency, lowers feed cost per unit of weight gain or production, reduces nitrogen excretion, and has other positive effects.

Instead of animal protein feeds in poultry nutrition, plant protein feeds are used with the supplementation of synthetic amino acids (Cmiljanić *et al.*, 2005). Increasing the efficiency of protein and amino acid utilization is crucial for the reduction of feed costs and maximization of meat production with an absolute minimum intake of amino acids. Synthetic amino acids have been found to facilitate the formulation of diets with an ideal amino acid profile (Han & Lee, 2000).

It is well known that one of the important roles of synthetic amino acids in animal nutrition is their ability to enhance the digestibility of amino acids (nitrogen), as well as their promotion of lean meat production. Amino acids are also related to the production of antibodies in animals (Han & Lee, 2000). Therefore, the development of immune function in poultry will be enhanced if they get an adequate amount of amino acids in their diets.

Formulation with commercially available purified essential amino acids (EAA) to achieve broiler requirements not only improves the overall amino acid balance, but allows for a reduction in crude protein (CP), while also improving the general performance of broiler birds (Zarate *et al.*, 2003). Investigations have demonstrated that poultry production can

be considerably improved by the addition of synthetic amino acids with probiotics and enzymes (Cmiljanić *et al*., 2003).

Supplementation with limited amounts of synthetic amino acids (0.1 to 0.3%) to diets of swine and poultry could spare 2 to 3% of dietary protein and considerably reduce nutrient excretion, particularly nitrogen (Han & Lee, 2000). Researchers working on turkeys and broilers have indicated that the crude protein content can be significantly reduced in poultry diet with essential amino acid supplementation, and the birds can achieve similar performance to that achieved on diets with high crude protein content (Moore *et al*., 2001; Brooks, 2003). However, Nonis and Gous (2006) found that dietary supplements of free amino acids were not utilized by broiler breeders.

2.6.2 Processed plant proteins

Plants provide the major portion of protein requirements by animals. However, due to their deficiency in one or more amino acids, plant proteins are usually fortified with synthetic amino acids or another protein source such as processed oil seed meal or animal protein concentrates. Plant proteins contain some anti-nutritional components that naturally exist within their structures, which can adversely affect the quality of the protein and limit its value in animal nutrition. Antinutritional factors (ANFs) are substances produced in natural feedstuffs as byproducts of the different metabolic processes of species (for example, inhibition or activation of nutrients, reduction in the digestive or metabolic utilization of feed) that detract from the nutritive value of the feed (Akande *et al.*, 2010).

The most commonly found antinutrients in plant protein sources are toxic amino acids, saponins, cyanogenic glycosides, tannins, phytic acid, gossypol, oxalates, goitrogens, lectins, protease inhibitors, chlorogenic acid, and amylase inhibitors (Akande *et al.*, 2010). These can be divided into heat-labile and heat-stable factors. Among heat-labile factors are trypsin inhibitors, haemagglutinins, phytate, goitrogens, and anti-vitamin factors. The heat-stable factors include saponins, oestrogens, flatulence factors, and lysinoalanine (Leiner, 1977).

Numerous potentially valuable protein sources for animals will remain unexploited, if ways are not developed to overcome the antinutrient components in these sources. Several processing methods have been developed to expand the availability of a wide variety of feedstuffs and introduce them to the animal feed industry. The most common method used for plant and animal protein processing is thermal treatment (Papadopoulos, 1989). The antinutritional factors in the plant proteins can be decreased by heat treatment and this process increases the quality and protein level of plant proteins such as cottonseed cake (Adeyemo & Longe, 2007). Improper treatment of plant food like beans and pulses may cause human and animal exposure to high levels of these poisonous antinutrient substances (Soetan & Oyewole, 2009).

The most widely used plant protein source in animal nutrition is soybean. However, other cereal sources such as wheat and sorghum as well as some plant protein meals such as canola, sunflower and peas are extensively used as well. In poultry, soybeans are used as soybean meal (SBM) which is made from the grinding of defatted flakes. New varieties of soybeans that have high protein and a lower oligosaccharide content compared to conventional soybeans have lately been developed (Baker *et al.*, 2011). In general,

soybean meal is considered the best plant protein source due to its nutrient composition. Soybeans are excellent sources of protein and energy for poultry and swine. The high protein content, with its well-balanced and highly digestible amino acids, makes SBM a valuable protein for human and non-ruminant animal feeding (Kocher *et al.*, 2002). However, as is a common feature of plant proteins, SBM has a high concentration of ANFs, which decrease its nutritive value (Marsman *et al.*, 1997; Mehri *et al.*, 2010) and limit its inclusion in broiler chicken diets, especially at the starter phase. Based on the fact that old animals are more resistant to anti-nutritional constituents that negatively affect digestion than younger animals, only the good quality ingredients with low levels of antinutrients should be used in starter diets to achieve good health and higher growth rate (Dersjant-Li, 2002).

It has been recognized for many years that thermal processing of soybeans is needed to increase its nutritive value (Palacios *et al.*, 2004) mainly by destroying the antinutritive factors. After introducing the thermal treatment into the isolation process of soy protein products, numerous heat-resistant substances are destroyed (Kim *et al.*, 1978). Consumption of untreated soy protein causes modifications in the intestinal morphology and physiology of broilers and a noticeable immune response (Peisker, 2001).

Various processed soybean products have been used in animal and poultry feeding. These include soybean protein concentrates (SPC), soybean protein isolates (SPI), and products in which the soybean was pretreated with enzymes and/or microorganisms. Processed soy products are distinctly different to soybean meal, thus they have much lower ANF activities, and contain a significantly lower amount of oligosaccharides and antigenic substances. Therefore, their nutritive value is much better than that of soybean meal and

can be incorporated at high levels in animal diets (Peisker, 2001). Replacement of SBM with these processed products in animal diets improves growth performance because SBM may contain enough ANFs to exert their antinutritional effects (Saki *et al.*, 2012). Jankowski *et al.* (2009) concluded that partial or complete replacement of SBM with SPC in the diets of young turkeys enhanced their 8-week body weight. In the same experiment they found that inclusion of SPI in lieu of SBM significantly improved feed utilization. It has been found that 5% replacement of soybean with AviStart (Hamlet Protein) in broiler starter diet resulted in an improvement in body weight and feed efficiency when the diet was fed for seven ds. Similar improvements in body weight, mortality and feed conversion ratio of birds were found when the diet was fed for 10 ds (van der Eijk, 2013). Batal and Parsons (2003) suggested that there may be some possible benefits of feeding SPC or SPI during the first three weeks after hatching and that improperly processed soybeans should not be fed to young chicks. A positive effect of Hamlet protein (HP) soy concentrate on feed intake has been reported(Philpotts & Norton, 2003). It has been demonstrated in experiments with pigs that both SPC and SPI have higher digestible protein and amino acids than SBM (Sohn *et al.*, 1994). Sohn *et al.* (1994) stated that both SPI and SPC have the same qualities and their effects were better than SBM when they were used as milk protein substitutes offered to the pigs aged from 21 to 35 ds. In another study, when extruded SPC was partially substituted for SBM in a high-SBM diet (40%), there was a significant improvement in pig performance; however, this effect was absent when non-extruded SPC was used (Lenehan *et al.*, 2007). Šiugždaité *et al.* (2008) claimed that the body weight of pigs was significantly increased due to the replacement of fish meal with an HP product in pig diet.

Intestinal health and integrity could be depressed by some ANFs which may increase intestinal weight and size and ultimately influence chicken performance (Pusztai, 1993). Plant ANFs are known to react with both the luminal content and epithelial cells because of their resistance to proteolytic breakdown in the gut (Saki *et al.*, 2012). They depress animal growth by interfering with the digestion and absorption of nutrients in the GIT (Soetan & Oyewole, 2009). Soybean ANFs can impair digestion processes (Kissil *et al.*, 2000) through inhibition of digestive enzymes (Moyano Lopez *et al.*, 1999), particularly proteolytic digestive enzymes such as trypsin (Dourado *et al.*, 2011). They can also produce complicated indigestible substances such as phytic acid-protein complexes (Richardson *et al.*, 1985), which reduce protein digestibility and increase endogenous losses (van der Eijk, 2013). Such ANFs (protease, trypsin and chymotrypsin inhibitors, lectin, soy globulins) especially existing in SBM may affect small intestinal characteristics (Jiang *et al.*, 2000a; Batal & Parsons, 2003; Fasina *et al.*, 2004; Saki *et al.*, 2012). Thereby adversely affect the morphology and function of the digestive tract in animals (Dunsford *et al.*, 1989; Batal & Parsons, 2003), and have the potential to affect enzyme activity and consequently broiler performance (Saki *et al.*, 2012). Lectins in SBM exert their antinutritional effects in the animal body through modulating the gut immune system and decreasing the secretion of gut hormones by reducing the production of endocrine cells (Pusztai, 1993). Furthermore, they interfere with the gut microbial ecology and damage the GIT (King *et al.*, 1983; Pusztai, 1991). In addition, soybean agglutinin causes substantial morphological and functional modifications in both the small intestine and pancreas (Grant *et al.*, 1987) by combining with the specific receptors on the epithelial cell surface in the small intestine wall and destroying the brush border mucosa (Qin,

2003). Feeding animals with processed soy or plant protein products that are purified or devoid of antinutritional compounds is important in order to optimize nutrient utilization and improve digestive physiology and subsequent productive performance of animals. Studies have shown that when SPC is used instead of SBM in diets for pigs, the negative impact of feeding SBM on the intestinal tract lining and on the serum antibody titer disappears (Peisker, 2001).

2.6.2.1 Extraction methods

The nutritive value of SBM is determined by the quantity and availability of amino acids along with its preparation processing conditions (Liener, 1981). In the SBM production industry, differences in the processing conditions, such as temperature and time, can be found among processors to improve processing efficiency and the quality of the product. Improper soybean processing can lead to the reduction of the nutrient availability to the animal (Grieshop *et al.*, 2003).

The high extraction efficiency (99%) and capability to handle large amount of soybeans make the solvent extraction the most efficient method for separation of oil from the soybeans. Mechanical oil extraction can also be used in soybean processing. This process has less extraction efficiency (70%) but has the ability to produce SBM free of any chemical substances (Bargale *et al.*, 1999).

2.6.2.2 Manufacture of processed soy products by solvent extraction

The method for manufacturing processed soy products from raw soybean is shown in Figure 2.1. To obtain good quality and large quantities of soy protein products, high

quality raw soybeans should be collected and cleaned at the initiation of production process. Prior to the solvent extraction process, the beans undergo dehulling, cracking, flaking, and steam conditioning. The extracted crude oil is removed, further processed, fractionated, and separated into soy oil and lecithin.

Defatted flakes which remain after oil extraction undergo further processing. Soybean meal is the most common product made from defatted flakes. The protein level of soybean meal can be determined based on the quantity of soy hulls returned to the flakes. No hulls are returned to the meals that have high protein content (48-50% crude protein). Low-protein meals have 42-43% crude protein. The flakes are steam-toasted to eliminate any remaining extraction solvent (a mix of n-hexane and hexane isomers, 50:50, is used as extraction solvent) as well as to deactivate the heat-labile ANFs. Soybean meal is mainly used in animal nutrition. However, grinding, sizing and texturizing of the edible defatted flakes produce vegetable protein products used as meat substitutes and vegetarian foods. The end product of the extraction and precipitation process of the protein from defatted flakes is soy isolate, which contains about 90% protein.

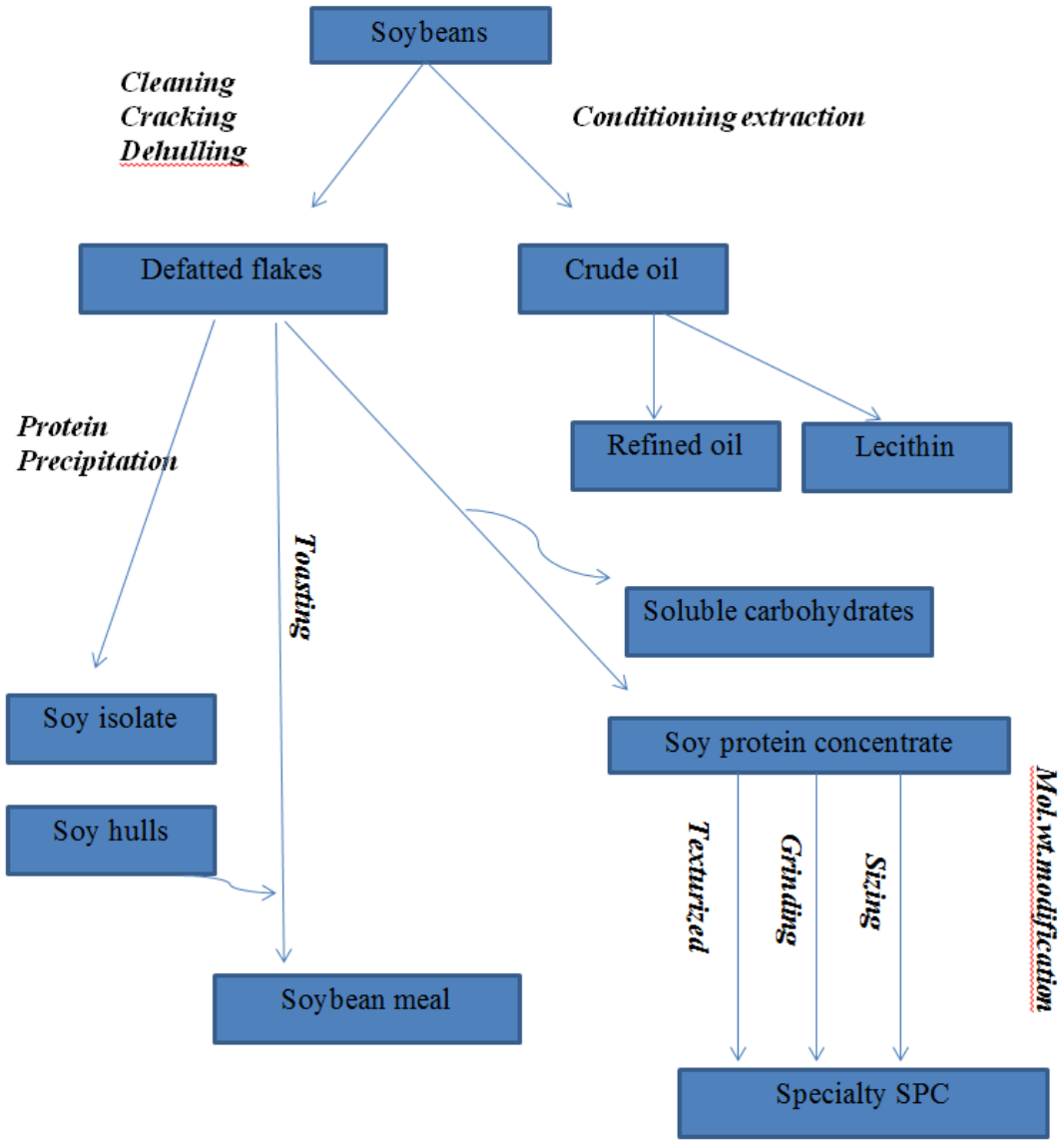


Figure 2.1 Manufacture of soy products (Peisker, 2001)

In the production of specialty soy proteins browning reactions should be avoided so that defatted flakes cannot enter the common desolventizer (toaster). To avoid changing the functional characteristics of the soybean proteins, removal of the solvent (hexane) occurs by applying low-heat vacuum drying. This harvests the white flakes which then undergo further processing. The white flakes are then purified to eliminate soluble carbohydrates,

and the resultant product is called soy protein concentrate (SPC). Two manufacturing methods for the elimination of soluble carbohydrates can be applied: removal by extraction or by enzymatic degradation. The end products of the two methods differ slightly in their nutrient composition. However, the most dominant method used for SPC production is the extraction method. Aqueous alcohol selectively leaches out the soluble carbohydrates. Other ANFs such as estrogens and antigenic factors are also removed with this processing step. Soy protein concentrate can be further purified and antinutritional factors can be further eliminated by modification of the aqueous alcohol mixture, the temperature, and the processing time. Texturizing, grinding and sizing, or modification of the molecular weight can be applied to form protein specialist products for use in the food chain (Peisker, 2001).

2.6.3 Animal and blood by-products

An animal by-product can be simply defined as a part of a slaughtered animal which is not directly contributing to human nutrition (Hazarika, 1994). Protein supplements of animal origin are provided by rendering operations, meat packing, poultry and poultry processing, milk and dairy processing, and fish and fish processing (Denton *et al.*, 2005). Meat and bone offal, blood, bones, intestines, rumen content, and the carcasses of animals rejected by a meat inspector are considered the major categories of animal by-products used in animal nutrition. These by-products are characterized by their high content of good quality protein and energy, reasonable essential amino acid profile and the absence of crude fibre and other antinutritional factors in their composition (Konwar & Barman, 2005). Thus, they are used as valuable sources of protein in animal feeding. Of these animal by-

products, animal nutritionists have shown a preference for the incorporation of blood and blood-derived products in feed.

Blood meal is a by-product of slaughterhouses and is used as a protein source in animal diets. Blood meal is considered one of the richest sources of lysine and a very good source of arginine, methionine, cystine, and leucine; however, it contains less glycine and very much less isoleucine than fishmeal or bone meal (NRC, 1994).

Blood meal is used as a protein supplement, a lysine supplement, and vitamin stabilizer and as a source of trace minerals. Fresh blood has a high protein content of about 17% with a reasonable amino acid balance (Liu, 2002) and approximately 87% crude protein on a dry matter basis. Blood meal contains 9% total lysine with a minimum biological activity of 80% (Konwar & Barman, 2005). For many years dried blood products have been used in the feed industry, and these products have usually been regarded as quality protein sources in starter diets for pigs (Stein, 1996). Blood is prepared by collection after slaughtering and then heating it for protein coagulation. After this, excess water is discarded, and it is dried and powdered (Hazarika, 1994). The quality of the product obtained is greatly influenced by its purity and the method of drying. The temperature at which it is dried is important as overcooked meal is undesirable for animals and its use has a negative effect on the growth efficiency of poultry (Konwar & Barman, 2005). Porcine blood obtained by the modified spray-drying method can be treated as a potentially beneficial source of proteins, amino acids, microelements and some biologically active substances for non-ruminant animals (Jamroz *et al.*, 2011).

Blood meal can be included in poultry and swine diets up to a level of 25% (Hazarika, 1994). Previous reports have indicated that inclusion of 1-4 % blood meal in diet can improve poultry performance (Petkov *et al.*, 1980; Nuarautelli *et al.*, 1987; Anang *et al.*, 2001), while others show no adverse effect of higher levels of dietary blood meal on chicken growth (Hassan *et al.*, 1974; Onwudike, 1981; Donkoh *et al.*, 1999; Donkoh *et al.*, 2002). However, Khawaja *et al.* (2007) stated that supplementation of broiler chicken diet with more than 3% blood meal had a negative effect on feed intake and body weight gain of broilers. A large proportion of blood meal is used for the production of plasma proteins and as a source of protein in pig and poultry diets. The introduction, approximately 10 years ago, of a more gentle drying process in the form of spray drying has dramatically improved the responses obtained by feeding blood products to pigs. Application of various processing methods to the blood has resulted in the production of different blood-derived products, such as spray-dried plasma protein and spray-dried blood cells (Stein, 1996).

2.6.3.1 Spray-dried plasma

Spray-dried plasma protein is a typical source of protein source which is produced by the separation of whole blood into plasma and cell fractions (Stein, 1996). Spray-dried blood products are commonly derived from bovine or porcine origins and are highly digestible protein sources with good amino acid profiles (Castelló *et al.*, 2004; Torrallardona, 2010). Since the late 1980s, spray-dried animal plasma products have been used in North America and Asia (Gatnau & Zimmerman, 1990) and their re-introduction into European animal diets is being considered for non-ruminant feeds (Castelló *et al.*, 2004). Australia has recently started producing spray-dried porcine plasma.

2.6.3.2 Production of spray-dried plasma

Fresh blood is collected at abattoirs and treated with an anticoagulant. The plasma and blood cell fractions are then separated by centrifugation. The plasma is then heated and spray-dried, with the final product being a fine granulated powder (Cole & Sprent, 2001). The product may be further processed to standardize the immunoglobulin concentration. This step facilitates its handling at manufacturing plant, or makes it to be in the form of water-soluble product (Pettigrew, 2006). Although most of the available studies regarding plasma products in animal nutrition have been concentrated on the protein content of plasma, it is worth noting that plasma has some significant physiological effects inside the animal body. It is therefore collected and processed to preserve the functional properties of the proteins. These functional proteins such as spray-dried plasma (SDP), serum, or globulin concentrate are a combination of bioactive compounds such as biologically active peptides, albumin, immunoglobulins, fibrinogen, lipids, growth factors, enzymes, and other components that exert specific biological activities in the intestine in addition to their nutritional value (Campbell, 2011a).

The development and manufacture of SDP products for animal feed was first undertaken in the United States by APC, which remains the largest producer and leads the market in the US and worldwide with its exclusive products including AP-920, Appetein and Solutein. (Pettigrew, 2006).

2.6.3.3 Role of spray-dried plasma in animal nutrition

Processed blood by-products, especially plasma, can be purified and used as high nutritional feedstuffs and a valuable source of proteins and other essential nutrients for

animals. A sufficient drying process in the form of spray drying has drastically improved the nutritional quality of blood by-products and improved the responses obtained by feeding it to animals. Spray-dried plasma products have been used in pig, fish, dog, cattle, and poultry nutrition (Castelló *et al.*, 2004). Spray-dried porcine plasma and similar products have been used by the pig industry to support piglets prior to and after weaning (Kats *et al.*, 1994; Coffey & Cromwell, 2001; Van Dijk *et al.*, 2001). Spray-dried plasmas of both porcine and bovine origins are usually used as sources of highly digestible and palatable proteins in pig production, and their feeding to weaning pigs in the starter phase enhances their performance over this stressful weaning time (Coffey & Cromwell, 2001; Van Dijk *et al.*, 2001) through improved feed intake and feed efficiency (De Lange *et al.*, 2010). Given the advantages of SDP for pig production, its potential benefits in the feeding of other production species have been established. Similarly to pigs, improvements in feed intake, growth rate, and feed efficiency have been reported in calves (Quigley & Wolfe, 2003), broilers (Campbell, 2003), and turkeys (Campbell *et al.*, 2004a) in response to the consumption of dietary spray-dried plasma. In diets of young birds, SDP incorporation has been found to improve the body weight and general growth performance (Jamroz *et al.*, 2012).

The vast majority of researchers have reported that the response to dietary SDP was more pronounced in production conditions with high pathogen exposure than in clean rearing environments. This could be due to the immunological properties of SDP. Spray-dried plasma proteins are processed to preserve the functional properties of proteins, including biologically active peptides such as albumin and IgG (Quigley & Wolfe, 2003). Dietary spray-dried porcine plasma could possibly improve the performance of broilers raised

under challenge conditions, predominantly in the starter phase (Henn *et al.*, 2013). Same observations have been reported in turkey poults (Campbell *et al.*, 2004a) and pigs (Stahly *et al.*, 1994; Coffey & Cromwell, 1995). Spray-dried plasma contains a diversity of functional proteins such as albumin, immunoglobulins, growth factors, and biologically active peptides (Borg *et al.*, 2002). These proteins are more efficient during animal exposure to environmental or immunological challenges. Coffey and Cromwell (1995) stated that pigs housed in a challenging environment grew more efficiently in response to SDP consumption than pigs reared in a sanitary environment. Similar responses to spray-dried serum have also been observed in broilers housed in different environments (Campbell *et al.*, 2003). An improvement in the health and growth efficiency has been observed in animals fed on diets containing SDP and challenged with *Escherichia coli* (Quigley & Drew, 2000; Bosi *et al.*, 2001) and *Cryptosporidium* (Hunt *et al.*, 2002).

The precise mechanisms behind the improved growth and health of broilers fed plasma remain unclear. However, some have linked this improvement to the nutritive properties of plasma proteins. Blood products such as spray-dried whole blood, plasma or red cells have been documented as protein sources with high nutritional value due to their excellent amino acid profile and digestibility, and have been used as ingredients in farm animal diets for many years (Castelló *et al.*, 2004). Therefore, from a nutritive perspective, the improvement in the growth performance of birds fed SDPP may be due to the product being a high quality protein with a good amino acid profile that can support gut development and rapid muscle growth. The mechanisms by which the product functions may be multifaceted. In pigs, palatability of spray-dried plasma, which increases feed intake, has been suggested as a mechanism for improved performance (Ermer *et al.*,

1994). However, findings from recent studies have reinforced the idea that the benefits of spray-dried plasma are derived from its immunological properties (Hansen *et al.*, 1993; Zhao *et al.*, 2008). The presence of immunologically active compounds in blood products such as immunoglobulins, specific blood proteins, and nucleotides have positive effects on weaned pigs and chickens receiving diets containing porcine blood by-products (De Rodas *et al.*, 1995; Godfredson-Kisic & Johnson, 1997; Bregendahl *et al.*, 2005b; Martinez-Puig *et al.*, 2007).

Although the antibacterial action of spray-dried plasma has not yet been confirmed, the literature has highlighted both the external benefits on the microbial community of the intestine as well as the internal effects on pigs. These effects could also be implemented in birds. The effects are mainly localized in the gut; however, some systemic effects have also been identified. The proposed theory is that immunoglobulins and glycoproteins exist in plasma products and have the potential to bind with pathogenic bacteria receptors and thus reduce their adhesion to the mucosal wall in the GIT (Coffey & Cromwell, 1995; Owusu-Asiedu *et al.*, 2002; Bosi *et al.*, 2004; Garriga *et al.*, 2005; Pierce *et al.*, 2005). Furthermore, antibodies that exist in spray-dried porcine plasma also have the ability to inhibit or reduce pathogenic colonization in the GIT (Owusu-Asiedu *et al.*, 2002). These theories could explain the greater efficiency of plasma in high-pathogen environments than in clean environments (Coffey & Cromwell, 1995).

Inclusion rates of spray-dried plasma products in research trials with pigs have ranged from 2 to 25% (Torrallardona, 2010). However, in poultry nutrition the inclusion rate has ranged from 0.25 to 4%. Dose-dependent studies have been conducted to determine the optimal inclusion rate of spray-dried porcine plasma (Kats *et al.*, 1994; Coffey &

Cromwell, 1995). The optimal inclusion level in pigs has been reported to range between 4 and 8% (Van Dijk *et al.*, 2001; Torrallardona, 2010). However, lack of knowledge due to an insufficient number of studies and their debatable and controversial results means the questions on what is the optimum inclusion level of plasma products in poultry diets remains unresolved. More studies are required to determine the optimal level of spray-dried plasma in the diets for the various growth stages of broiler chickens.

The effects of dietary blood by-products, more specifically spray-dried porcine plasma, on the intestinal integrity of animals have been observed. However, the effects are not always consistent. Longer intestinal villi have been observed after inclusion of spray-dried porcine plasma in broiler diets (Jamroz *et al.*, 2011; Jamroz *et al.*, 2012). Performance improvement (Pierce *et al.*, 2005; Nofrarias *et al.*, 2006), insulin-like growth factor stimulation, and development of the small intestine (De Rodas *et al.*, 1995; Jiang *et al.*, 2000b) have also been confirmed in pigs and pheasants. The tendency of spray-dried plasma to improve the absorptive capacity by increasing villus height and the villus height/crypt depth ratio has also been reported (Owusu-Asiedu *et al.*, 2003; Torrallardona *et al.*, 2003; Zhao *et al.*, 2007). In contrast, various studies carried out on pigs, have indicated that feeding dried plasma to the animals as a source of protein has little or no effect on the morphological aspects of the intestinal wall (Nofrarias *et al.*, 2006) or on villus height, crypt depth, and villus and crypt goblet cell density (King *et al.*, 2008). Furthermore, Jiang *et al.* (2000b) found no influence of spray-dried plasma on villus or crypt morphology or the cell proliferation index. Since the effects of spray-dried plasma on intestinal morphology are inconsistent, this is unlikely to be the mechanism underlying the improved performance elicited by the product.

Animal nutrition strategies have changed since the banning of antibiotics to control disease and promote growth performance of animals. No alternatives have yet been developed to replace antibiotics for disease prevention and growth promotion (Ferket, 2004). Some new natural components have been introduced to animal diets to support the growth performance and health of animals in unsanitary or conventional conditions. Some of these products belong to the class of nutraceuticals, which could be defined as raw materials (or part of a raw material) that can provide both medical and nutritional benefits to the animals, including the prevention and treatment of disease (Kalra, 2003). The use of certain nutrients to control the immune system seems to be a substitute for the use of antibiotics because dietary immunomodulators that improve humoral immunity and reduce immunological stress in chickens could positively improve their growth (Klasing, 1998).

In searching for the sources of bioactive substances which can naturally stimulate immunity and improve the general health status of animals, nutritional experts have focused their interest on the use of blood by-products as raw materials in animal nutrition (Orda *et al.*, 1988; Coffey & Cromwell, 1995). The positive influences of dried blood plasma on immunological reactions and intestinal wall functions of weaned pigs and other non-ruminant animals have been reported (De Rodas *et al.*, 1995; Godfredson-Kisic & Johnson, 1997; Nofrarias *et al.*, 2006; Campbell *et al.*, 2009). The activity of blood by-products has been associated with specific immunoreactive globulins and nucleotides that exist in the composition of blood products (Shahidi *et al.*, 1984; Pierce *et al.*, 2005; Rodriguez *et al.*, 2007; Moretó & Pérez-Bosque, 2009). An improvement in the growth performance and general health status of animals (pigs, poultry, calves, and pets) have

been observed due to spray-dried plasma feeding (Quigley & Drew, 2000; Coffey & Cromwell, 2001; Campbell *et al.*, 2003; Campbell *et al.*, 2004a; Campbell *et al.*, 2004b). Mobilization of the mucosa membrane and the expression of the activation of immunological areas have been observed after inclusion of spray-dried porcine plasma in broiler diets (Jamroz *et al.*, 2011; Jamroz *et al.*, 2012). Modulation of the immune system, antibody functions, inflammatory response, and modification in the intestinal morphology as responses to dietary SDP have also been confirmed in numerous studies carried out on young pigs (Cain *et al.*, 1992; Kats *et al.*, 1994; Coffey & Cromwell, 1995; Owusu-Asiedu *et al.*, 2002; Rodriguez *et al.*, 2007; Campbell *et al.*, 2009).

2.7 Conclusion

The timing and the form of the supplemented nutrients is necessary for posthatch development, and most emphasis has been placed on the early feeding especially of broilers. The digestive and immune system development of the young birds is a complex process. The transition of birds from yolk dependent to exogenous feed continues to represent a multi-factorial challenge to birds, nutritionists and producers. However, advances in both feed technology and our understanding of nutrition at a more cellular level have continued to highlight nutrition as a valuable and viable tool to assist the chicks in this situation. Researchers need to investigate the interactions between the quality of chosen diets and the integrity of the immune and digestive systems. Knowledge of these aspects is necessary for optimal exploitation of nutrient sources and to promote the development of young chicks. As a commercial process when dealing with poultry nutrition, the first attention is given to the cost of the ingredients used. Therefore our understanding of the role nutrition can play in the development of the young birds has

probably been restricted by our reliance on the use of growth-promoting substances and the most available and cheapest traditional feedstuffs. However, the quality of the feedstuffs and probably introduction of novel high-quality ingredients, particularly high quality protein products which have no competition with human nutrition is promising to have an important role for maintaining chickens production and profitability.

The feeding trials outlined in the following chapters were designed and conducted to examine two dietary high-quality protein products, from both plant and animal origins, in the starter diet and their influence on the growth performance, digestion physiology and immune development of broiler chickens. In intensive poultry production, broilers often being the dominant population in the production chain and often face greater challenges throughout the production period and therefore they may have more benefit from nutritional strategies or respond better to specific strategies than layers or other birds.

The research limitation indicated in this literature review will be addressed in four chapters (Chapters 3, 4, 5 and 6). The first two chapters (Chapters 3 and 4) will deal with inclusion of processed plant protein and spray-dried plasma in the starter diet on different grain bases, and their effects on performance, digestive process and histology of broiler chickens. In addition, the feeding trial outlined in the following chapter (Chapter 5) aims to determine the effect of level and feeding duration of spray-dried plasma protein on broiler performance and subsequent digestion and absorption process of broiler chickens. Furthermore the last chapter (Chapter 6) aims to assess the immunological benefits of spray-dried plasma to broiler chickens challenged with *Salmonella sofia*.

CHAPTER 3 Effect of processed soy product on growth and gut physiology of broiler chickens

3.1 INTRODUCTION

The first few d post-hatch currently represent a greater percentage of the broiler chicken's life (Behnke & Beyer, 2004). Chick growth and development take place at a very high rate in the first post-hatch week. Maximum growth rate of broilers occurs in this short period of time, which represents about 20% of the broiler's total growth (Noy & Sklan, 2002). Therefore, a large amount of nutrients are required to secure and maintain the trend of this growth, because during those few d of adjustment, chicks are making the metabolic and physiological transition from having all the required nutrients supplied by the egg to having nutrients supplied by compounded feed (Behnke & Beyer, 2004). However, at this time the chicks are anatomically complete, but still have undeveloped digestive and immune systems (Maiorka *et al.*, 2006). Digestion and absorption of nutrients early in life depend primarily on pancreatic enzyme activity (Nitsan *et al.*, 1991). Pancreatic enzymatic (trypsin, chymotrypsin, amylase and lipase) activities in the chick are weak at hatch (Ullah *et al.*, 2012). Therefore, the use of high quality raw ingredients that have high nutritive value and highly digestible nutrients, especially protein and amino acids, is very necessary in this critical period of broiler life for the earliest possible development of gastrointestinal tract and growth performance. A good start leads to a uniform flock of chicks with a good first week weight, which is positively correlated to the final weight of the birds (Kleyn & Chrystal, 2008)

Adequate protein availability in the pre-starter phase seems to be essential to increase muscle development in later phases (Hargis & Creger, 1980) . The chemical composition

of diets, the content of crude protein, amino acids and energy values are the factors that determine the bird's development in the early period of life.

Soybean meal (SBM) is a common protein source in animal feeds. In general, soybean meal is considered the best plant protein source due to its nutrient composition. Soybeans are an excellent source of protein and energy for poultry and swine. The high protein content with its well balanced and highly digestible amino acids makes SBM a valuable protein for human and non-ruminant animal feeding (Kocher *et al.*, 2002). However, SBM has a high concentration of anti-nutritional factors, which decrease its nutritive value (Marsman *et al.*, 1997; Mehri *et al.*, 2010) and limit its inclusion in broiler chicken diets, especially at the starter phase. Based on the fact that young animals are more susceptible to dietary anti-nutritional constituents that have a negative effect on digestion than older animals, only the best ingredients with low levels of anti-nutrients should be used in starter diets to achieve good health and higher growth rate (Dersjant-Li, 2002).

It has been recognized for many years that thermal processing of soybeans is needed to increase its nutritive value (Palacios *et al.*, 2004) mainly by destroying the anti-nutritive factors. Various processed soybean products have been used in animal and poultry feeding these include soybean protein concentrate (SPC), soybean protein isolates (SPI) and products in which the soybean was pretreated with enzymes and/or microorganisms. Replacement of SBM with these processed products in animal diets is believed to result in better growth performance. Jankowski *et al.* (2009) concluded that partial or almost complete substitution of SBM with SPC in turkey poult diets enhanced the body weight of 8-week-old turkeys. In the same experiment it was found that incorporation of SPI as a substitute for SBM significantly improved feed utilization. It has been found that 5%

replacement of soybean with Hamlet Protein (HP) (AviStart) in broiler starter diet resulted in an improvement in body weight and feed conversion ratio when the diet was fed for 7 d (Hamlet Protein B011, 2011). Compared to the control, similar improvements in body weight, mortality rate and feed conversion ratio of birds were found when the diet was fed for 10 ds (Hamlet Protein BO12, 2011). Processed soy protein is purified from ANF that may have negative effects on broiler growth performance and subsequent physiological activity. The objective of the present study was to investigate the effect of a processed soy protein, Avistart (Hamlet Protein) , on performance, digestive physiology and morphology of the small intestine of broiler chickens.

3.2 MATERIALS AND METHODS

3.2.1 Experimental design and bird management

In this study, an experiment was designed to investigate the effect of a starter level of processed soy product (PSP) on broiler performance and digestive physiology of broiler chicks at subsequent age. The product was obtained from Hamlet Protein (Horsens-Danmark). Four inclusion levels of PSP (0, 25, 50 or 100 g/kg in either maize- or wheat-based diets) were used in the starter diets which were fed from hatch to 10 d. The diets were identical in nutrient profiles and formulated to meet breeder specifications (Tables 3.1 and 3.2). After 10 ds, the birds were switched to commercial-type grower diets (11-24 d) and then a finisher diet (25-35 d). Titanium dioxide (TiO_2), an indigestible marker, was incorporated into the grower diet at a rate of 5g/kg diet for nutrient digestibility assessment. In a completely randomized design, four hundred and eighty (Ross 308) d-old male chicks (initial weight, 36.94 ± 1.091 g) were randomly assigned to 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 d, and then the birds were transferred to a metabolic cage room up to 35 d. Feed and water were provided *ad libitum*. The room temperature was gradually decreased from 33 °C to 24 °C ± 1 °C at 35 d. Eighteen hours of lighting were provided per d throughout the trial, excluding ds 1 to 7 during which twenty three hours of lighting were provided. On d 10, 24 and 35, the birds and feed were weighed to measure the body weight, feed intake and feed conversion ratio.

On d 10 and 24, two birds from each cage were randomly selected and killed by cervical dislocation. The abdominal cavity was opened and internal organs were subsequently removed. The visceral organs were weighed. At d 24, the whole pancreas and part of the jejunum were taken for digestive enzyme assays and ileal digesta were collected and placed in plastic containers and immediately frozen. 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 analysis of $TiO_{2,}$ gross energy, protein and dry matter.

3.2.2 Animal ethics

The experiment was approved by the Animal Ethic Committee of the University of New England (Approval No: AEC 12-054). 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, 1990).

Table 3.1 Nutrient composition of the PSP used in the study

Nutrient (g/kg)	
Dry matter	930.0
ME Poultry MJ/kg	10.86
Crude protein	556.0
Crude Fat	25.0
Starch	30.0
Amino acid (Total)	
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
Amino acids (Digestible)	
Arginine	38.9
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 (Total)	8.00
Chloride	0.625
Magnesium	3.5
Iron (mg/kg)	200.0
Manganese (mg/kg)	50.0
Zinc (mg/kg)	60.0

Table 3.2 Ingredient and nutrient composition of the starter diets used

	PSP Level g/kg							
Starter	**Wheat base**				**Maize base**			
	0	25	50	100	0	25	50	100
Ingredient g/kg								
Canola meal	40	40	40	40	46	50	50	50
Wheat	600.3	581.5	574.0	597.2	-	-	-	-
Maize	-	-	-	-	584.32	564.45	557.30	575.74
Soybean meal	229.3	236.0	227.0	168.9	263.0	265.5	256.0	192.0
Meat meal	40	15	-	-	40	15	-	-
Canola oil	52.7	57.4	59.5	55.0	27.8	33.7	36.0	31.8
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
Dical phos[1]	10.8	15.1	17.3	15.8	12.3	16.5	18.7	17.2
Na bicarbonate[2]	4.2	4.4	4.6	3.2	4.6	4.8	5.0	5.1
Limestone	10.2	13.4	15.5	16.6	9.5	12.7	14.8	15.8
Salt	0.63	0.90	1.04	2.00	0.63	0.87	1.04	0.92
L-Lysine	4.5	4.2	4.1	4.2	4.4	4.2	4.0	4.1
DL-Methionine	2.4	2.3	2.2	2.2	2.5	2.4	2.3	2.3
Threonine	2.3	2.1	2.0	2.0	2.0	1.9	1.8	1.7
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 composition								
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
Met+cys	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	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

[1] Dicalcium phosphate; [2]sodium bicarbonate; [3]Trace mineral concentrate 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. [4]Vitamin concentrate supplied per kilogram of diet: retinol, 12000 IU; cholecalciferol, 5000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamin, 16 µg; biotin, 200 µg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg. [5]xylanase+amylase+protease; [6]phytase.

Table 3.3 Ingredient and nutrient composition of the grower and finisher diets used

	Grower		Finisher	
Ingredients g/kg	**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
Canola 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 composition				
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
Met +cys	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] Composition as in Table 3.2; [3]xylanase+amylase+protease; [4]phytase.

3.2.3 Measurements and analyses

3.2.3.1 Growth performance

Feed intake (FI) and live weight (LW) were recorded at d 10, 24 and 35 for determination of average FI and LW. Mortality was recorded when it occurred and feed conversion ratio (FCR; feed intake/weight gain) was corrected for mortality.

3.2.3.2 Visceral organ weight

Body weight and the weight of the small intestine (with contents), proventriculus plus gizzard, liver, heart, spleen, pancreas and bursa of Fabricius of sampled birds were recorded at d 10 and 24. The relative organ weight was calculated as mass per unit of live body weight (g/100g of live body weight).

3.2.3.3 Carcass parts yield

Carcass weight and the weight of breast (boneless), thighs and drumsticks were recorded at d 35. The relative part weight was calculated as an indication of mass per unit of live body weight (g/100g live body weight).

3.2.3.4 Tissue protein and digestive enzyme analysis

To evaluate the activity 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 small pieces 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.p.m) for 30 s on an Ultra Turrax T 25 Basic Homogenizer (IKA® Works, Wilmington, NC, USA). After that, samples of homogenate were taken into Eppendorf tubes (Eppendorf South Pacific, North Ryde, Australia) and stored in a freezer (-20 °C) for digestive enzyme analyses. The pancreas 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 tissue was transferred to new tubes and centrifuged with rotor 20.1 at high speed (30 000 x g) (AVANTI J-E 369001, Beckman countler, USA) for 20 min at 5 ºC. Aliquots of supernatant was collected and used for various enzyme assays and total protein measurement according to (Nitsan *et al.*, 1974). The activities of jejunal and pancreatic enzymes were measured by incubation with fixed substrate concentrations as standardized for poultry by Iji *et al.* (2001b). Assays 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) were conducted on the jejunal homogenates. An assay was conducted on the pancreatic tissue for tissue protein and chemotrypsin amidase (EC 3.4.21.1). The enzyme activities were measured according to the methods previously described for other species (Holdsworth, 1970; Serviere-Zaragoza *et al.*, 1997). Tissue protein concentration in both jejunum and pancreas was measured using the Coomassie dye-binding procedure described by Bradford (1976).

3.2.3.5 Titanium dioxide analysis

The TiO_2 content of the ileal digesta and diet samples was measured according to the method described by Short *et al.* (1996). About 0.1 g of the freeze-dried digesta sample or 0.2 g of diet sample was ashed in a porcelain crucible for 13 hours at 580 °C and, upon cooling, was dissolved in 5 ml of 7.4 M sulphuric acid. The samples were then boiled for 30 min at 200 °C and another 30 min at 250 °C until completely dissolved. After cooling, the solution was poured through grade 541 filter paper (Whatman 541, hardened, ashless, 90 mm Ø Cat No. 1541 090, Whatman International Ltd Maidstone, England) into 50-mL flasks. After that, 10 ml of hydrogen peroxide (30 % v/v) was added to each flask and the contents diluted up to 50 ml with Milli-Q water and mixed properly through inversion to

avoid bubbles. A typical orange colour then developed, the intensity of which was dependent on the TiO_2 concentration. Aliquots of solutions obtained and of similarly prepared standards were analyzed using a Hitachi 150-20 UV spectrophotometer (Hitachi Science Systems Ltd., Ibaraki, Japan) by measuring the absorbance at 410 nm. The TiO_2 content, measured in mg/ml, was determined from the standard curve and converted to mg/g of the sample.

3.2.3.6 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 analyzed along with the indigestible titatnium dioxide marker. The nitrogen content of ileal digesta 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 analyzer (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 the sample in ultra-pure oxygen. To interpret the detector response as percentage nitrogen (w/w), calibration was conducted using a pure primary standard of ethylenediaminetetra-acetic acid (EDTA). The gross energy of diet and ileal digesta samples was determined on an IKA®- WERKE bomb calorimeter (C7000, GMBH & CO., Staufen, GERMANY). 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:

$$\text{Digestibility coefficient} = 1 - \frac{Digesta\ nutrient\ (\frac{g}{kg}DM)/Digesta\ TiO2\ (\frac{g}{kg}DM)}{Diet\ nutrient\ (\frac{g}{kg}DM)/DietTiO2\ (\frac{g}{kg}DM)}$$

3.2.3.7 Intestinal histomorphology

Tissue samples were collected from the proximal jejunum and flushed with buffered saline and fixed in 10% neutral buffered formalin for histomorphological analysis. Jejunal samples were stored in 10% buffered formalin solution. Samples were embedded in paraffin wax using an automatic tissue processer. Sectioning of samples, staining with haematoxylin and eosin and preparation of slides was done at the Central Private Laboratory at Shilan Private Hospital in Duhok, Kurdistan Region. Sample sections were captured at 10x magnification using a Leica DM LB microscope (Leica Microscope GmbH, Wetzlar, Germany) and morphometric indices were determined as described by Iji *et al.* (2001a) using computer-aided light microscope image processing analysis systems (Video Pro, Leading Edge, Bedford Park, SA Australia). Images were digitized and the villus height (from the tip of the villus to the villus crypt junction) and crypt depth (from the villus crypt junction to the muscular mucosa) were measured in 7-10 well orientated villi for each jejunal section. The basal and apical widths of the villi were also measured in order to calculate the apparent surface area, as described by Iji *et al.* (2001a)

3.2.4 Statistical analyses of data

All data collected were analyzed by linear regression of Minitab 16 (Minitab Inc., 1998). Data from the maize-based diets were analysed separately from those from the wheat based groups.Differences between mean values were determined by ANOVA followed by comparisons using Fisher's multiple range test.

3.3 RESULTS

3.3.1 Gross response

Feed intake up to 10 or 35 d on wheat-based diet was not affected by starter level of PSP. However, on maize-based diets, FI to 35d decreased ($P < 0.01$) with rising levels of PSP (Table 3.4). On the wheat-based diet, body weight at 10 ($P < 0.01$) and 35 ($P < 0.05$) d was increased by rising levels of PSP, while on the maize-based diets, body weight was increased, but was not significantly affected by the level of PSP throughout this period. On the wheat-based diet, PSP reduced ($P < .01$) FCR for the two periods assessed, while on the maize-based diet, there was no reduction in FCR between hatch and 10 ds, and FCR tended ($P < .08$) to improve between hatch and 35 d.

Table 3.4 Feed intake (g/bird), body weight (g) and FCR (g feed/g weight gain) of broilers between hatch and 35 d of age after placement on starter diets containing different level of PSP

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

a,b,c – Mean values on the same row not sharing a superscript are significantly different (*P < .05; **P < .01; ***P < .001).

3.3.2 Visceral organ weight

At d 10, there was no significant effect of dietary inclusion of PSP on visceral organ weight of birds on either the maize-based or the wheat-based diets. However, on wheat-based diets, there was a marginal increase in the weight of the small intestine ($P < 0.08$) by rising level of PSP. Similar trend was found for liver ($P < 0.07$) of the birds fed starter diet with the highest level of PSP (Table 3.5).

Table 3.5 Relative weight of visceral organs (g/100g of body weight) of broiler chickens at d 10 at various PSP inclusion levels on either maize- or wheat-based diet

Cereal		PSP levels g/kg				SEM
		0	25	50	100	
Maize	Small Int[1]	8.3	8.2	8.3	8.4	0.12
	Pro+Gizz[2]	3.7	3.7	3.5	3.7	0.07
	Heart	0.87	0.88	0.93	0.92	0.029
	Liver	4.0	4.1	4.2	4.4	0.08
	Spleen	0.08	0.08	0.10	0.07	0.007
	Bursa	0.16	0.16	0.17	0.19	0.011
	Pancreas	0.46	0.43	0.44	0.46	0.011
Wheat	Small Int[1]	7.9	7.4	6.9	8.3	0.29
	Pro+Gizz[2]	3.2	3.5	3.6	3.4	0.06
	Heart	0.88	1.00	0.91	0.94	0.030
	Liver	4.1	3.8	4.0	4.2	0.07
	Spleen	0.06	0.08	0.07	0.0	0.004
	Bursa	0.17	0.17	0.13	0.17	0.008
	Pancreas	0.40	0.43	0.40	0.43	0.016

[a,b,c] – Mean values on the same row not sharing a superscript are significantly different ; [1] small intestine with digesta; [2] Proventriculus and Gizzard; SEM= Standard error of mean

Up to 24 d, the relative weight of the small intestine decreased ($P < 0.003$) as the level of PSP rose in the maize-based diet (Table 3.6). For chickens on the wheat-based diet, the relative weight of the pancreas tended to decrease ($P< 0.056$) in birds receiving the highest level of PSP in comparison with the control and other experimental groups. The

weight of other visceral organs was not affected by dietary inclusion of PSP in the starter diets of chickens on either the maize-based or wheat-based diets.

Table 3.6 Relative weight of visceral organs (g/100g of body weight) of broiler chickens at d 24 at various PSP inclusion levels on either maize- or wheat-based diet

Cereal	Organ	PSP levels g/kg				SEM
		0	25	50	100	
Maize	Small Int[1]	4.9[a]	4.5[ab]	4.5[ab]	4.0[b]	0.09**
	Pro+Gizz[2]	2.0	2.1	2.1	2.0	0.04
	Heart	0.76	0.79	0.74	0.73	0.021
	Liver	2.7	2.7	2.7	2.7	0.07
	Spleen	0.08	0.08	0.08	0.08	0.003
	Bursa	0.14	0.14	0.16	0.17	0.008
	Pancreas	0.23	0.24	0.25	0.23	0.007
Wheat	Small Int[1]	4.4	4.6	4.1	4.0	0.14
	Pro+Gizz[2]	1.7	1.6	1.7	1.5	0.05
	Heart	0.83	0.89	0.80	0.75	0.019
	Liver	2.5	2.6	2.6	2.6	0.08
	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

[a,b.c] – Mean values on the same row not sharing a superscript are significantly different (**P < .01); [1] small intestine with digesta; [2] Proventriculus and Gizzard; SEM= Standard error of mean

3.3.3 Carcass parts yield

Inclusion of a starter level of PSP had no significant effect on carcass parts yield of chickens at 35 d either on maize or wheat based diet (Table 3.7). However, for chickens on the maize-based diets, there was a 1% increase in the carcass yield from the birds that received PSP in their starter diets. There was a marginal increase in the weight of breast and thigh with an increase in the level of PSP. For wheat-based diet groups, the carcass yield percentage tended to increase (P < .058) with an increase in the level of PSP. There was a 2% increment in the carcass yield for the birds that received the highest level of PSP compared to the control. The weight of breast tended to increase (P < .07) and there was a

similar tendency (P < .06) for the weight of thigh as a result of PSP inclusion in the starter diets of chickens. The birds that were fed on the medium PSP level included in the starter diet had higher carcass yield and carcass parts weights than the control and other experimental treatments.

Table 3. 7 Carcass yield and parts yield of broiler chicken at 35 d at various PSP inclusion levels on maize- or wheat-based diets

Cereal	Carcass part	PSP levels g/kg				SEM
		0	25	50	100	
Maize	Carcass yield %	75.9	76.7	76.8	76.0	0.002
	Breast (g)	674.5	714.2	739.1	694.4	9.70
	Thigh (g)	304.0	334.2	336.8	321.3	4.50
	Drumstick (g)	242.6	245.1	254.9	238.3	3.23
Wheat	Carcass yield%	75.6	75.9	76.5	77.1	0.002
	Breast (g)	655.2	638.2	703.7	694.4	9.67
	Thigh (g)	294.6	299.4	330.4	323.1	6.01
	Drumstick (g)	235.0	231.4	248.5	240.7	3.53

SEM= Standard error of mean

3.3.4 Nutrient digestibility

There was no significant effect of PSP on the ileal digestibility of protein, gross energy and dry matter in chickens on either the maize-based or the wheat-based diets at d 24 (Table 3.8). However, the digestibility of protein, gross energy and dry matter was marginally improved with a rising level of PSP in the starter diets.

3.3.5 Tissue protein content and digestive enzyme activities

At 24 d, tissue protein content, sucrase and alkaline phosphatase activities in the jejunum of chicks on in both maize-based and wheat-based diets were not significantly affected by PSP inclusion in the starter diet (Table 3.9). There was a significant increase (P < 0.04) in

maltase activity in chickens on maize-based diets as a result of PSP inclusion in the starter diet, while this effect was absent on wheat-based diet chickens.

There was no significant effect of PSP on pancreatic tissue protein content and lipase activity in 24d old chickens regardless of cereal base. However, chymotrypsin amidase activity increased ($P < 0.03$) with an increase in level of PSP in chickens on the wheat-based diets, while this response was not found in chickens fed on the maize-based diets.

3.3.6 Histology of jejunum

Including PSP in the broiler starter diets had a significant effect on the mucosal histology of the jejunum at 24 d (Table 3.10). On both grain-types chickens, villus height increased ($P<0.01$) with a rising level of PSP in the starter diets. Crypt depth decreased with inclusion of PSP in maize-based ($P<0.01$) and wheat-based ($P<0.05$) diets. . There was an increase in villus/crypt ratio of chickens with rising levels of PSP (up to 50g/kg diet) in the maize-based ($P<0.05$) and wheat-based diets. There was no significant effect of PSP on the villous surface area; however, villous surface area numerically increased in the birds that received starter diets containing PSP.

Table 3.8 Digestibility coefficient of ileal protein, gross energy and dry matter of broiler chickens at 24 d given different PSP starter levels

Cereal		PSP 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

SEM= Standard error of mean

Table 3. 9 Effect of different starter levels of PSP in maize- and wheat-based diets on tissue protein content and digestive enzyme activities in the jejunum and pancreas of broiler chickens at 24 d of age

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

[a,b.c] – Mean values on the same row not sharing a superscript are significantly different (*P < .05); [2]AP, Alkaline Phosphatase; [3]CA, Chymotrypsin amidase; [4]Protein (mg/g tissue); [5]Enzymes (ηmol/mg protein) SEM= Standard error of mean

Table 3. 10 Jejunal villus height (µm), crypt depth (µm), villus height/crypt depth ratio and villus surface area (mm^2) of chickens that received different PSP levels in their starter diets in both maize- and wheat-based diets

Cereal		PSP levels g/kg				SEM
		0	25	50	100	
Maize	Villus height	1796.0[c]	1801.1[bc]	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[b]	0.24*
	Villus SA[1]	0.72	0.63	0.83	0.73	0.01
Wheat	Villus height	1619.1[c]	1691.5[bc]	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[b]	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 < .05; **P < .01; ***P < .001); Villus SA[1] = villous surface area (mm^2); SEM= Standard error of mean

3.4 DISCUSSION

3.4.1 Gross response

The results of this experiment demonstrate that the growth performance of broilers was affected by the inclusion of PSP in the starter diet, regardless of cereal base. Addition of PSP to the starter diet was found to improve the body weight and FCR of broiler chickens up to 10 or 35 d on both grain-based diets. The supplement was more effective in the wheat-based diet than in the maize-based diets. Overall, including PSP 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 HP300 for SBM resulted in better growth performance of broiler chickens. Šiugždaité *et al.* (2008) observed the same positive effect on weaned pigs with the substitution of HP soy concentrate for fish meal.

3.4.2 Visceral organ weight

In the present study the relative weight of the visceral organs was not affected by the inclusion of PSP in the starter diets of young chicks on either maize- or wheat based diets. The results were similar to those of Tousi-Mojarrad *et al.* (2012) who tested a soybean product that was processed by a technique used by Hamlet Protein. The essence of

feeding high quality products in the starter period is to improve the development of digestive function. The product tested in this study did not appear to have an impact on gross weight of organs.

3.4.3 Meat yield

Inclusion of PSP 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 HP at a level of 5 % in broiler starter diet for 7 d improved carcass yield and breast weight in comparison with the control. A medium level of PSP in maize-based diet and a high level of PSP in wheat-based diet produced the best outcomes. This improvement may be due to the better performance of HP-supplemented groups, which may result in better protein deposition in muscle tissue than other groups. It may also be related to the heavier body weight of the HP-supplemented groups. There is a positive relationship between live body weight and carcass yield and its parts weight.

3.4.4 Nutrient digestibility

In general, although non-significant, there was an improvement in the digestibility of protein, gross energy and dry matter due to the inclusion of PSP 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 improved in birds on diet containing HP compared to birds without the supplement. The supplement is able to improve energy and amino acid availability due to the reduction in anti-nutritive factors, such as trypsin-inhibitors and oligosaccharides, present in SBM (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).

3.4.5 Tissue protein and digestive enzyme activities

In the current study, PSP 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 a rising level of PSP, 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 PSP in diets for turkey. The supplement is not an important source of simple carbohydrates of the type that could stimulate terminal carbohydrases.

Inclusion of PSP in the broiler 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 PSP level on the wheat-based diets and the same was found for chickens that received a medium level of PSP in the maize-based diet. This may have a positive effect on the use of nutrients. The cost of visceral tissue regeneration and nutrients may be re-directed to muscle deposition in the rest of the body.

There was a marginal increase in the activity of chymotrypsin amidase enzyme in the birds that received the highest and the medium levels of PSP 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 (PSP) in the intestinal lumen. Although the SBM that was included as the main source of protein was processed, protease inhibitors are not completely eliminated from such meals. The test supplement has lower levels of protease inhibitors than native SBM

3.4.6 Jejunal histomorphology

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 PSP in the starter diet significantly improved the morphological traits of the small intestine, as measured at the jejunum. Birds that received PSP in the starter phase had significantly longer villi, less crypt depth, higher villus height:crypt depth ratio and higher villus surface area at 24 d 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 found that the villus height and villus:crypt ratio were increased in the duodenum and jejunum of egg-laying hens on diets supplemented with processed (fermented) soybean meal compared with those receiving 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 PSP-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 PSP would greatly equip the intestinal structure 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 antinutritive factors, including lectins have been shown to cause morphological changes in the intestine by combining with the specific receptors of the epithelial cell surface in the small intestine wall, destroying the brush border mucosa structure of the intestine (Qin, 2003). This will adversely affect nutrient digestion and absorption.

3.5 CONCLUSION

The results of the present study indicate that pre-treatment with enzymes and yeast could improve the nutritive characteristics of SBM, and inclusion of PSP to the starter diets of chickens could improve broiler performance. Carcass yield and weight of carcass parts were better in the birds that received PSP in their starter diets. Moreover, there were also significant positive physiological responses of chickens to the dietary inclusion of PSP in the starter diets of both maize- and wheat-based diets. In terms of nutrient digestibility and digestive enzyme activities, the digestibility of protein and energy was higher in birds on PSP-supplemented diets. The activities of digestive enzymes, such as jejunal maltase, sucrase, and pancreatic chymotrypsin amidase, were significantly improved by dietary inclusion of PSP to the starter diets. The changes in mucosal morphometry equate to those that could have an impact on nutrient digestion and absorption. It can be concluded that the inclusion of PSP in the starter diet could have beneficial effects on broiler productivity through its effect on digestive function. The product was effective in both wheat- and maize-based diets. The mode of action of this specific plant protein product on broiler

chickens productivity needs to be more precisely explained by further investigation. For better understanding the mechanisms behind the different sources of high quality protein products gross effects on broiler production, more investigation are going to apply by supplementing protein products from animal origin in broiler starter diets.

CHAPTER 4 Subsequent growth performance and digestive physiology of broiler chickens fed starter diets containing spray-dried porcine plasma

4.1 INTRODUCTION

Feeding the broiler chicks during the first 7 d of life represents a challenge to nutritionists and flock managers. From both physiological and nutritional perspectives, the newly hatched chick has yet to develop (Kleyn & Chrystal, 2008). The digestive system of young chicks is immature; therefore, its nutrient assimilation depends on the chemical and physical properties of feed (Moran, 1990; Noy & Sklan, 1998b; Sklan & Noy, 2000). Early maturation of the gut has been shown to be an important factor in raising a healthy chick, as the physiological development of birds is directly related to the digestion and nutrient absorption in the small intestine (Aptekmann *et al.*, 2001). It is widely accepted that in the immediate post-hatch period nutritional supplements help in the development of the digestive system, which improves the nutrient utilization, growth and overall development of chicks (Henderson *et al.*, 2008). Providing early feed supplements has been shown to improve the digestive system, which ultimately is reflected in increased body weight (Noy & Sklan, 1999). However, the impact of providing feed early is more than simply giving birds a head start over those where feeding is delayed by a day or two. What is consumed in the first few d following hatching can play a vital role in achieving the genetic potential of the bird for body weight, muscle yield and immune capability (Dibner & Knight, 2003). Broiler chicks have been shown to benefit from immediate access to feed. Although the focus of early nutrition has been on the provision of energy, chicks benefit from a more balanced nutrient profile, particularly regarding the inclusion

of amino acids. Blood products such as spray-dried whole blood, plasma or red cells have been documented as a source of proteins with a good amino acid profile and digestibility, and are therefore considered to have high nutritional value, and have been used as ingredients in farm animal diets for many years (Castelló *et al.*, 2004). Careful handling of the raw materials and utilization of the spray-dried process have dramatically improved the quality and subsequent use of blood protein products in the feed industries (Campbell, 1998). Spray-dried porcine plasma (SDPP) and similar products have been used by the pig industry to support piglets prior to and after weaning (Kats *et al.*, 1994; Coffey & Cromwell, 2001; Van Dijk *et al.*, 2001). As a high-quality animal protein source with an adequate supply of essential amino acids, SDPP increases diet palatability (Ermer *et al.*, 1994) and improves immunocompetence (Coffey & Cromwell, 1995), and is usually considered to be an essential ingredient in the diet of early weaned pigs (Thacker, 1999).

Positive effects have been observed in many studies due to the incorporation of porcine blood byproducts, particularly SDPP, in the diets of piglets. These effects include immune system modulatory effect, antibody functions, inflammatory response and changes in intestinal morphology (Owusu-Asiedu *et al.*, 2002; Rodriguez *et al.*, 2007; Campbell *et al.*, 2009; Moretó & Pérez-Bosque, 2009), small intestinal growth and activation of insulin-like growth factor (De Rodas *et al.*, 1995; Jiang *et al.*, 2000b), and performance improvement (Mazurkiewicz *et al.*, 1990; Pierce *et al.*, 2005; Nofrarias *et al.*, 2006). These results have increased the interest in the application of this high quality protein product in the poultry industry too and particularly in the starter phase. Spray-dried porcine plasma contains a diversity of functional proteins that may enhance broilers growth performance and intestinal development in the early age. The objective of this

study is to evaluate the effect of SDPP inclusion in starter diet on subsequent broiler performance, and digestive development and function.

4.2 MATERIALS AND METHODS

4.2.1 Experimental design and bird management

This experiment was designed to investigate the effect of a starter level of SDPP on broiler performance and digestive physiology up to 35 d of age. Four inclusion levels of SDPP (0, 5, 10 and 20g/kg substituted for meat and bone meal in either maize- or wheat-based diets) were used in the starter diets which were fed from hatch to 10 d of age. The diets were identical in nutrient profiles and formulated to meet breeder specifications (Aviagen, 2007). After ten d, the birds were switched to commercial type grower (11-24 d) and finisher (25-35d) diets. In a completely randomized design, four hundred and eighty Ross 308 d-old male chicks (initial weight, 41.0 ± 0.92 g) were randomly assigned to 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 d, then the birds were transferred to metabolic cage room to 35 d. Feed and water were provided *ad libitum*. The room temperature was gradually decreased from 33 °C to 24 °C ± 1 °C at 35 d. Eighteen hours of lighting were provided per d throughout the duration of the experiment, excluding d 1 to 7 during when twenty three hours of lighting were provided. Titanium dioxide (TiO_2) was incorporated to the grower diet as an indigestible marker at a rate of 5kg/ton diet for nutrient digestibility assessment. On d 10, 24 and 35, the birds and feed were weighed to measure the body weight, feed intake and feed conversion ratio.

On d 10 and 24, two birds from each cage were randomly selected and killed by cervical dislocation. The abdominal cavity was opened and visceral organs were removed and weighed. At d 24, the whole pancreas and part of the jejunum were taken for assessment of protein content and digestive enzyme activities. The ileal digesta were also collected into plastic containers and frozen immediately after collection for subsequent use to measure digestibility. After freeze-drying (Martin Christ Gerfriertrocknungsanlagen, GmbH, Germany), the ileal digesta samples were ground in a small coffee grinder and stored at 4 °C in an airtight container for chemical analyses of TiO_2, gross energy, protein, and dry matter.

4.2.2 Animal ethics

The experiment was approved by the Animal Ethics Committee of the University of New England (Approval No: AEC 12-054). 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 (NMHRC,1990).

4.2.3 Measurements and analyses

4.2.3.1 Growth performance

Feed intake (FI) and live weight (LW) were recorded at d 10, 24 and 35 for determination of average FI and LW. Mortality was recorded and feed conversion ratio (FCR: feed intake/weight gain) was corrected for mortality.

Table 4. 1 Nutrient composition (g/kg) of the 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

Table 4. 2 Ingredient and nutrient composition of starter diets

	SDPP Level g/kg							
Starter	**Wheat base**				**Maize base**			
Ingredient g/kg	**0**	**5**	**10**	**20**	**0**	**5**	**10**	**20**
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
Dical phos[1]	13.0	13.8	15.0	17.0	14.0	14.1	16.0	18.0
Sodium bic[2]	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 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 composition								
ME (MJ/kg)	12.67	12.73	12.71	12.67	12.70	12.72	12.71	12.72
Crude protein	219	220	220	222	213	214	215	216
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
Met+cys	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 P	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

[1] Dicalcium phosphate; [2]sodium bicarbonate; [3]Trace mineral concentrate 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. [4]Vitamin concentrate supplied per kilogram of diet: retinol, 12000 IU; cholecalciferol, 5000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamin, 16 µg; biotin, 200 µg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg; [5]xylanase+amylase+protease; [6]phytase.

4.2.3.2 Visceral organ weight

Body weight and the weight of the small intestine (with content), proventriculus plus gizzard, liver, heart, spleen, pancreas and bursa of Fabricius were recorded at ds 10 and

24. The relative organ weight was calculated as mass per unit of live body weight (g/100 of live body weight).

Table 4. 3 Ingredient and nutrient composition of grower and finisher diets

	Grower		Finisher	
Ingredients g/kg	**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
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 composition				
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
Met +cys	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] Composition as in Table 4.2; [3]xylanase+amylase+protease; [4]phytase.

4.2.3.3 Carcass parts yield

Carcass weight and the weight of breast (boneless), thighs and drumsticks were recorded at d 35. The relative part weight was calculated as an indication of mass per unit of live body weight (g/100 live body weight).

4.2.3.4 Tissue protein and digestive enzyme analysis

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). As for the pancreas was the entire tissue was homogenized in a similar way to the jejunum except that Milli-Q water (Millipore Australia, North Ryde, Australia) was used in place of buffer and. Details of these methodologies were described in Section 3.2.3.4.

4.2.3.5 Titanium dioxide analysis

The TiO_2 content of the ileal digesta and diet samples was measured according to the method of Short *et al.* (1996) as described in Section 3.2.3.5.

4.2.3.6 Ileal digestibility of nutrients

The concentrations of the TiO_2 marker and of nutrients in the feed and ileal digesta were used to calculate the digestibility coefficient of protein, gross energy and dry matter. Diets and ileal digesta were analyzed for protein, gross energy and dry matter as described in section 3.2.3.6.

4.2.3.7 Intestinal histomorphology

Tissue samples were collected from the proximal jejunum and flushed with buffered saline and fixed in 10 % neutral buffered formalin for histomorphological analysis as described in Section 3.2.3.7.

4.2.4 Statistical analyses

All data collected were subjected to analysis of variance by regression using Minitab 16 (Minitab Inc., 1998). Data from the maize-based diets were analysed separately from those from the wheat-based groups. Differences between mean values were determined by ANOVA followed by comparisons using multiple range tests according to Fisher.

4.3 RESULTS

4.3.1 Gross response

Feed intake either to 10 and 35 d, on either maize-based or wheat-based diets was not affected by the starter level of SDPP (Table 4.4). Rising levels of SDPP resulted in increased body weight at 10 and 35 d in birds on both the maize-based diet ($P < 0.01$, $R^2 = 0.331$ and $P < 0.05$, $R^2 = 0.173$) and the wheat-based diet ($P < 0.001$, $R^2 = 0.401$ and $P < 0.05$, $R^2 = 0.240$) respectively. On the wheat-based diet, SDPP reduced ($P < 0.001$, $R^2 = 0.504$ and 0.523) FCR for the two periods assessed, while on the maize-based diet, there was a reduction in FCR between hatch and 10 d ($P < 0.01$, $R^2 = 0.322$) and between hatch and 35 d ($P < 0.05$, $R^2 = 0.226$).

4.3.2 Visceral organ weight

On the wheat-based diets the relative weight of the proventriculus plus gizzard at 10d of age was increased ($P < 0.01$, $R^2 = 0.264$) by SDPP, especially in birds that received the highest level of SDPP (Table 4.5). 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 in chickens on the maize-based diet. At 24 d of age, the relative weight of visceral organs was not affected by the starter inclusion levels of SDPP on either wheat-based or maize-based diets (Table 4.6).

Table 4. 4 Feed intake (g), live weight (g) and FCR (g feed/g weight gain) of birds between hatch and 35d after placement on starter diets containing different levels of SDPP

		Age	SDPP levels g/kg				
Cereal		**(d)**	**0**	**5**	**10**	**20**	**SEM**
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
	Body 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
	Body 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***

a,b,c – Mean values on the same row not sharing a superscript are significantly different (*P < .05; **P < .01; ***P < .001).

Table 4. 5 Relative weight of visceral organs (g/100g of body weight) of broiler chickens at 10 d of age at various SDPP inclusion levels on either maize- or wheat-based diet

Cereal		SDPP levels g/kg				SEM
		0	5	10	20	
Maize	Small Int[1]	8.1	7.8	7.9	8.5	0.15
	Pro+Gizz[2]	3.5	3.6	3.4	3.7	0.06
	Heart	0.82	0.87	0.87	0.83	0.025
	Liver	3.9	4.1	3.8	4.0	0.08
	Spleen	0.08	0.06	0.09	0.07	0.003
	Bursa	0.15	0.16	0.19	0.13	0.007
	Pancreas	0.40	0.47	0.44	0.38	0.011
Wheat	Small Int[1]	8.4	7.9	7.8	7.9	0.22
	Pro+Gizz[2]	3.5[b]	3.6[b]	3.4[b]	4.2[a]	0.08**
	Heart	0.88	0.93	0.88	0.87	0.028
	Liver	3.8	4.1	3.7	4.1	0.06
	Spleen	0.0875	0.08	0.09	0.09	0.003
	Bursa	0.16	0.17	0.14	0.17	0.007
	Pancreas	0.47	0.43	0.42	0.47	0.012

[a,b,c] – Mean values on the same row not sharing a superscript are significantly different (*P < .05; **P < .01; ***P < .001); [1] small intestine with digesta; [2] Proventriculus and Gizzard; SEM= Standard error of mean

4.3.3 Carcass parts yield

In general, inclusion of starter levels of SDPP had no significant effects on carcass yield and the weight of carcass parts from either wheat-based or maize- based diets at 35 d. However, dressing percentage was marginally increased with a rising level of SDPP in the starter diets of broiler chickens (Table 4.7).

4.3.4 Nutrient digestibility

There was no significant effect of dietary inclusion of SDPP in starter diets, with either wheat or maize, on digestibility of ileal protein, gross energy and dry matter at 24 d. However, chickens that were fed low SDPP levels (0.5%) on maize-based diet tended ($P < 0.09$, $R^2 = 0.124$) to have a better protein digestibility. On wheat-based diets, nutrient digestibility was slightly increased in birds that received SDPP in their starter diets

compared to the control. Chicks that were fed on starter diet that contained a medium level (1%) of SDPP had better nutrient digestibly (Table 4.8).

Table 4. 6 Relative weight of visceral organs (g/100g of body weight) of broiler chicken at d 24 at various SDPP inclusion levels on either maize- or wheat-based diet

Cereal		SDPP levels g/kg				SEM
		0	5	10	20	
Maize	Small int[1]	6.0	6.1	6.1	5.6	0.16
	Pro+Gizz[2]	2.0	2.0	2.1	2.0	0.05
	Heart	0.74	0.83	0.77	0.76	0.016
	Liver	2.6	2.6	2.8	2.7	0.05
	Spleen	0.08	0.08	0.08	0.08	0.003
	Bursa	0.17	0.18	0.17	0.16	0.005
	Pancreas	0.24	0.26	0.24	0.24	0.007
Wheat	Small int[1]	6.0	5.5	5.7	5.5	0.19
	Pro+Gizz[2]	1.9	1.7	2.0	1.9	0.06
	Heart	0.84	0.73	0.76	0.86	0.020
	Liver	2.8	2.71	2.7	2.5	0.06
	Spleen	0.08	0.10	0.09	0.07	0.004
	Bursa	0.14	0.17	0.17	0.19	0.010
	Pancreas	0.20	0.20	0.20	0.22	0.006

[1]small intestine with digesta; [2] Proventriculus and Gizzard; SEM= Standard error of mean

4.3.5 Tissue protein content and activity of digestive enzymes

In the jejunum, there was no significant effect of SDPP on tissue protein contents of birds at d 24 on both wheat-based and maize-based diets (Table 4.9). On wheat-based diets, the activity of maltase was increased ($P < 0.009$, $R^2 = 0.269$) by rising level of SDPP and its more pronounced in the chickens fed on diet containing the highest level of SDPP. However, this effect was absent on maize-based diet chickens. The activity of sucrase increased significantly ($P < 0.006$, $R^2 = 0.300$) on wheat-based diet chickens that received the highest level of SDPP, but this effect was absent in maize-based diet chickens. There was a significant increase ($P < 0.02$, $R^2 = 0.181$) in alkaline phosphatase activity of maize-based diet birds that received the highest SDPP level. However, in chickens on wheat-

based diet, there was no significant variation in alkaline phosphatase activity among experimental treatments.

Pancreatic tissue protein and chymotrypsin amidase were not affected by dietary inclusion of SDPP in starter diet on maize-based diet chickens at 24 d (Table 4.9). However, chickens on wheat-based diet showed a significant reduction ($P< 0.02$, $R^2 = 0.214$) in tissue protein with rising levels of SDPP, and a significant increase ($P< 0.01$, $R^2 = 0.226$) in chymotrypsin amidase activity. Dietary inclusion of SDPP had no significant effect on pancreatic lipase activity of chickens at 24 d on either wheat-based or maize-based diet.

Table 4. 7 Dressing percentage and the weight of breast, thigh and drumsticks of broiler chickens at d 35 at various SDPP inclusion levels on either maize- or wheat-based diets

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

SEM= Standard error of mean

Table 4. 8 Ileal protein, gross energy and dry matter digestibility of broiler chickens at 24 d given different SDPP starter levels

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

SEM= Standard error of mean

Table 4. 9 Tissue protein contents and digestive enzyme activity in the jejunum and pancreas of broiler chickens at 24 d on the diet containing different starter level of SDPP on maize-based and wheat-based diets

Cereal			SDPP levels g/kg				SEM
			0	5	10	20	
Maize	Jejunum	Protein[3]	26.9	26.7	27.0	26.2	0.57
		Maltase[4]	1.8	1.7	1.5	1.9	0.05
		Sucrase[4]	0.16	0.13	0.14	0.14	0.004
		AP[1,4]	0.09^{ab}	0.08^{b}	0.08^{b}	0.11^{a}	0.003*
	Pancreas	Protein[3]	21.8	21.8	25.2	23.5	0.95
		CA [2,4]	6.5	6.6	5.6	6.0	0.28
		Lipase[4]	1.10	1.16	0.97	0.94	0.055
Wheat	Jejunum	Protein[3]	27.7	27.0	26.6	28.2	0.78
		Maltase[4]	1.6^{b}	1.4^{b}	1.5^{b}	1.9^{a}	0.04**
		Sucrase[4]	0.13^{b}	0.11^{b}	0.13^{b}	0.16^{a}	0.003**
		AP [1,4]	0.07	0.06	0.06	0.07	0.003
	Pancreas	Protein[3]	26.7^{a}	25.4^{ab}	26.1^{a}	21.4^{b}	0.77*
		CA [2,4]	6.4^{b}	6.1^{b}	6.3^{b}	7.7^{a}	0.22**
		Lipase[4]	1.08	1.09	1.05	1.18	0.021

[a,b.c] – Mean values on the same row not sharing a superscript are significantly different (*P < .05; **P < .01;); [1]AP, Alkaline phosphatase; [2]CA, Chymotrypsin amidase;[3]Protein (mg/g tissue); [4]Enzymes (ηmol/mg protein/min) SEM= Standard error of mean

4.3.6 Histomorphology of the jejunum

Feeding SDPP to the birds from 1 to 10 d resulted in an increment in the height of villi of the jejunum at 24 d in birds fed both grain-based diets (Table 4.10). However, statistically it was only significant ($P < 0.01$, $R^2 = 0.31$) for the birds fed wheat-based diet. Crypt

depth in the jejunum was increased due to the inclusion of SDPP in the broiler starter diet; however, it was significant ($P < 0.03$, $R^2 = 0.25$) only for the birds on the maize-based diets. There was a decline in the villus:crypt ratio in birds fed starter diets containing different levels of SDPP on both maize-based ($P < 0.008$, $R^2 = 0.38$) and wheat-based ($P < 0.025$, $R^2 = 0.32$) diets.

Table 4. 10 Jejunal villus height (µm), crypt depth (µm), villus height:crypt depth ratio, and villus:crypt ratio of chickens that received different SDPP levels in their starter diets on maize- and wheat-based diets

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[ab]	2017.4[a]	1980.5[a]	15.69**
	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*

[a,b,c] – Mean values on the same row not sharing a superscript are significantly different (*P < .05; **P < .01; ***P < .001); SEM= Standard error of mean

4.3.7 Economic analysis of feeding SDPP

The economics of feeding SDPP to broiler chicks is shown in Table 4.11. 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 body weight of the birds. Feed conversion ratio also improved with rising level of SDP 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 dollars per bird, which equated to feed costs per kg body weight of about 0.72, 0.71, 0.89 and 0.67 dollar per bird.

Table 4. 11 Economic analysis of feeding SDP to broiler chicks on maize- and wheat-based diets.

		SDPP levels g/kg			
	Period (d)	**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
Body weight (g)	10	318.0	340.0	342.5	349.5
Body weight (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 ($/bird)	0-35	1.84	1.81	1.79	1.83
Cost/kg body weight ($/bird)	0-35	0.72	0.71	0.69	0.67

4.4 DISCUSSION

4.4.1 Gross response

The results of the current study clearly demonstrated the beneficial effects of SDPP addition to the starter diets of broiler chickens. Body weight and FCR were significantly improved by the SDPP in the starter diets of broilers on either wheat- or maize-based diets. It is worth noting that body weight and FCR continued to improve during the post-starter period when all birds were fed the same common grower and finisher diets. Therefore, the important factor to consider in broiler production is that getting birds off to a good start during the starter phase translates into better performance throughout the experiment period. These results are supported by the findings of Campbell *et al.* (2003);

(2004a) and Bregendahl *et al.* (2005a) who reported that rate and efficiency of growth of broiler chickens were improved when SDPP was included in their feed from hatch to market weight. In their investigations, Jamroz *et al.* (2012) reported significant increase in body weight at d 14, 28 and 30 when broiler chickens were fed on diets containing SDP for 28 or 30 d, compared to the control.Coffey and Cromwell (2001) and Van Dijk *et al.* (2001) also found a similar positive effect of SDPP consumption on growth of weaning pigs. However, in contrast to these results, Jamroz *et al.* (2011) did not observe any significant effect of SDPP on broilers. From a nutritive perspective, this improvement in the growth performance of the birds fed SDPP may be due to the product being a high quality protein with a good amino acid profile that can support gut development and rapid muscle growth. 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 diet that contained SDPP and control birds. This response is of significant economic importance since SDPP is an expensive product.

4.4.2 Visceral organ weight

In this study, the relative weight of visceral organs up to 10 or 24 d was not affected by inclusion of SDPP in the broiler starter diets on either wheat- or maize-based diets except for the proventriculus plus gizzard, which were enlarged at 10 d. It would appear that any changes in the contribution of the GIT would be functional rather than physical.

4.4.3 Nutrient digestibility

Regardless of some fluctuation between different treatments, nutrient digestibility was almost identical in all experimental groups. 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. It should be noted that SDPP was fed only in the starter phase (1-10 d) while nutrient digestibility was measured at 24 d of age.

4.4.4 Tissue protein content and activity of digestive enzymes

In this study, 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 body weight of birds. The activity 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 antinutritional 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.

4.4.5 Meat yield and quality

The results of the current study showed that SDPP inclusion in the starter diets had no significant effect on the meat yield in terms of weight of breast, thighs, drumsticks, wings and neck of broilers on either of the grain-based diets. Dressing percentage was improved to some extent due to SDPP feeding. The results were in line to those of Campbell *et al.* (2003) found that dressing% and breast yield % of broiler did not significantly affected by suppling SDPP to broilers in their drinking water. The results were in contrast to those of Bregendahl *et al.* (2005a) who stated breast meat yield (skinless and bomeless) increased when SDP was fed to broiler chickens.

4.4.6 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 the 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 of the control (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 the histological response to SDPP is difficult to explain from a purely nutritional effect. It may be mediated through improvements in intestinal health and barrier function. Supplementation of SDP 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 SDP 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).

4.4.7 Economic benefits of feeding SDPP

A number of feeding strategies has been investigated towards pre-starter feeding of broiler chickens. Such strategies have included assessment of different supplements, as was done in the current experiment. A major setback to the adoption of products is the cost. The current cost of SDP, for example, is around $8000 per tonne. Feeding the supplement to the birds from hatch to 42 d at 2 % of the diet would require about 100 g or $800 per chick. This is a major deterrent to producers. The results of the this experiment demonstrated that response could be obtained when the product is fed for only 10 ds, which coincides with when chicks still have a lower feed consumption. From the economic analysis conducted, the increase in feed cost is only present in the starter period.

Subsequently, there is an improvement in cost of production, particularly in term of weight gain per unit feed consumption.

4.5 CONCLUSSION

From the results of the present study, it can be concluded that blood by-products and particularly SDPP may introduce in the starter diets of poultry. Incorporation of SDPP in the starter diets of broilers causes a large and significant improvement in their performance. These responses may be due to changes in the activities of digestive enzymes. Feeding SDPP also stimulated the development of the small intestinal mucosa by increasing villus height and crypt depth in the jejunum. This could lead to an increase in the absorption of essential nutrients and increase their utilization for subsequent metabolic and other biological activities, including growth. It appeared from this study that inclusion of SDPP to the broiler starter diets as a replacement for meat meal at a level of 20g/kg could improve broiler productivity. The product was more effective in wheat-based diets than in maize-based diets.

CHAPTER 5 The effect of level and feeding duration of spray-dried plasma protein on growth performance, digestive enzyme activities, nutrient digestibility, and intestinal mucosal development of broiler chickens

5.1 INTRODUCTION

Processed blood by-products, especially plasma, can be used as high-nutritional feedstuffs and valuable sources of proteins and other essential nutrients for animals. The use of spray drying has drastically improved the nutritional quality of blood by-products and the response obtained when these products are fed to animals. Spray-dried bovine and porcine plasma are usually used as sources of highly digestible and palatable proteins in the pig industry, which has used these products in starter diets of weaning pigs to improve their performance over the stressful weaning period (Coffey & Cromwell, 2001; Van Dijk *et al.*, 2001). In the past 15 years, the feeding of newly weaned pigs has been reformed by the introduction of spray-dried plasma products to their diets, particularly of pigs weaned before 18 d of age. Products such as spray-dried porcine plasma, where used, generally enhance performance through increased feed intake and feed efficiency in the immediate postweaning period (De Lange *et al.*, 2010).

The beneficial effects of dietary blood by-products, and more specifically spray-dried porcine plasma, on the immunity and intestinal integrity of young pigs has been confirmed in many experiments (Cain *et al.*, 1992; Kats *et al.*, 1994; Coffey & Cromwell, 1995; Owusu-Asiedu *et al.*, 2002; Rodriguez *et al.*, 2007; Campbell *et al.*, 2009). Performance improvement (Pierce *et al.*, 2005; Nofrarias *et al.*, 2006), stimulation of insulin-like growth factors, and development of the small intestine (De Rodas *et al.*, 1995; Jiang *et al.*, 2000b) have also been observed in pigs and pheasants. Similar to the effects observed in

pigs, improvements in feed intake, growth rate and feed efficiency have been reported in calves (Quigley & Wolfe, 2003), broilers (Campbell *et al.*, 2006b) , and turkeys (Campbell *et al.*, 2004a) in response to the consumption of dietary spray-dried plasma. Given the benefits of spray-dried plasma for pig production, its potential benefits for other production species are beginning to be evaluated. Positive findings obtained from the incorporation of plasma protein to the pigs' nutrition intensified the interest in the use of this source of animal protein and mineral elements in poultry nutrition. The product will become more appealing if it can be used in smaller volumes and/or over shorter periods of feeding with losing its benefits. The aim of the current study is to assess the performance response, development of digestion physiology, and growth and development of mucosal structures in broiler chickens given different levels of SDPP for different feeding durations.

5.2 MATERIALS AND METHODS

5.2.1 Experimental design and birds management

A 3 x 2 factorial experiment was conducted to investigate the effect of a starter level of SDPP fed over different durations on broiler performance and digestive physiology up to 35 d of age. Three inclusion levels of SDPP (0, 10 or 20 g/kg) were used in lieu of meat meal in starter diets, which were fed from hatch to 5 or 10 d of age. The diets were identical in nutrient profiles and formulated to meet breeder specifications. After 10 d, the diets were switched to commercial-type grower (11-24 d) and finisher (25-35d) type diets. Four hundred and eighty Ross 308 d-old male broiler chicks were randomly assigned to six treatments, each with six replicates, ten chickens per replicate. Chickens were reared

in wire floors with plastic mesh for the first week in multi-tiered brooder cages placed in a climate-controlled room. Feed and water were provided *ad libitum*. Titanium dioxide (TiO_2), as an indigestible marker, was incorporated into the grower diets at a rate of 5g/kg diet to enable assessment of nutrient digestibility. On d 10, 24 and 35, the birds and feed were weighed to measure the body weight, feed intake, and feed conversion ratio.

On d 10 and 24, two birds from each cage were randomly selected and slaughtered by cervical dislocation. The abdominal cavity was opened and internal organs were subsequently removed. At d 24, the whole pancreas and part of the jejunum were taken for digestive enzyme activity assays, and ileal digesta were collected into plastic containers for digestibility measurements and frozen immediately after collection. The ileal digesta samples were ground in a small coffee grinder and stored at 4 °C in airtight containers for analysis of TiO_2, gross energy, protein, and dry matter.

5.2.2 Animal ethics

The experiment was approved by the Animal Ethics Committee of the University of New England (Approval No: AEC 13-027). 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 1990).

5.2.3 Measurements and analyses

5.2.3.1 Growth performance

Feed intake (FI) and body weight (BW) were recorded at d 10, 24 and 35 for determination of average FI and LW. Mortality rate was recorded as it occurred, and feed conversion ratio (FCR; feed intake/weight gain) was corrected for mortality.

5.2.3.2 Visceral organ weight

The visceral organs (small intestine (with contents), proventriculus plus gizzard, liver, heart, spleen, pancreas, and bursa of Fabricius) of one bird per replicate were excised and weighed on d 10 and 24. The relative organ weight was calculated as mass per unit of body weight (g/100 of body weight).

Table 5. 1 Ingredients and nutrient composition of starter diets

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	0.5	0.5	0.5
L-lysine HCl	3.6	3.4	3.0
D,L-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 composition			
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] Composition as in Table 3.1. [3]xylanase+amylase+protease; [4]phytase.

Table 5. 2 Ingredients and nutrient composition of grower and finisher diets

Ingredients kg	Grower	Finisher
Wheat	612.3	627.4
Soybean meal	260	250
Meat meal	40	40
Oil	54	52
Choline chloride	0.5	0.5
Dicalcium phosphate	8.6	7.0
Sodium bicarbonate	2.0	2.0
Limestone	8.3	8.3
Salt	1.3	1.3
L-lysine HCl	2.0	2.0
D,L-methionine	2.8	2.0
Threonine	1.2	0.7
Titanium dioxide	0.5	0.5
Trace minerals[1]	0.75	0.75
Vitamins[2]	0.5	0.5
Avizyme[3]	0.5	0.5
Phyzyme XP[4]	0.1	0.1
Nutrient composition		
ME poultry (MJ/kg)	13.18	13.18
Crude protein	212.4	208.7
Lysine	11.2	10.9
Methionine	5.5	4.7
Arginine	13.0	12.7
Methionine + cystine	8.5	7.6
Threonine	7.4	6.8
Calcium	9.1	8.7
Available phosphorus	4.5	4.2
Sodium	1.7	1.7
Choline	1531	1431

[1,2,3,4] Composition as in Table 3.1.

5.2.3.3 Tissue protein and digestive enzyme analyses

To evaluate the activities of digestive enzymes and protein concentration, jejunal tissue was processed according to the method described by Shirazi-Beechey *et al.* (1991). The entire pancreas was also processed as described by Nitsan *et al.* (1974).The activities of jejunal and pancreatic enzymes were measured by incubation with fixed substrate concentrations as standardized for poultry by Iji *et al.* (2001b). Tissue protein

concentration in both the jejunum and pancreas was measured using the Coomassie dye-binding procedure described by Bradford (1976). (See section 3.2.3.4)

5.2.3.4 Ileal digestibility of nutrients

The TiO_2 contents of the ileal digesta and diet samples were measured according to the method described by Short *et al.* (1996). The crude protein (CP), gross energy (GE) and dry matter (DM) content of feed and freeze-dried ileal digesta samples were also measured along with the indigestible titanium dioxide marker. The ileal digestibility of these nutrients was calculated as described in section 3.2.3.6**.**

5.2.3.5 Mineral digestibility

The dried ileal digesta samples were prepared mineral analysis by grinding them with a stainless blade grinder. About 0.2 g of ground sample was placed into the digestion bottle. Two mL of a mixture of $HClO_4$ (70%) and H_2O_2 (30%) at volume ratio of 7:3 (v/v) were added to each bottle, which was then lightly sealed with a lid and stood overnight at room temperature to pre-digest. On the next d 1 mL of H_2O_2 was added and the bottles were tightly sealed and placed in an oven at 80 °C for 30 minutes. After cooling in a fume hood, a further 1 mL of H_2O_2 was added to the mixture, which was then capped tightly and placed in an oven at 80 °C for one hour. About 21 mL of deionised water were added to each bottle to make a sample weight of 25 g and samples were then filtered through Whatman No. 1 filter paper. Samples were stored at 4 °C to reduce adsorption onto plastic and growth of microorganisms. The samples were then analyzed for minerals using inductively coupled plasma spectroscopy (Anderson & Henderson, 1986).

5.2.3.6 Intestinal histomorphology

Tissue samples were collected from the proximal jejunum and flushed with buffered normal saline and fixed in 10 % neutral buffered formalin for histomorphological analysis. Samples were processed and histomorphological parameters were measured as described in section 3.2.3.7.

5.2.4 Statistical analysis of data

All data collected were analysed using the General Linear Models (GLM) procedure of Minitab version 16 for the main effect of SDP level, feeding duration, along with their interactions. Differences between mean values were determined using Fisher multiple range test.

5.3 RESULTS

5.3.1 Gross response

Feed intake up to 10 d of age was reduced by SDPP level but this was significant ($P < 0.01$) only for the diet containing (10 g/kg SDPP for short feeding duration) (Table 5.3). In general, feed intake was lower in diets containing SDPP than in the control group. Body weight at 10 d of age was also higher in chicks on diets containing SDPP and was significant ($P < 0.01$) for the diet containing 20g/kg SDPP and fed for 10 d (Table 5.3). Level and feeding duration of SDPP significantly ($P < 0.05$, $P < 0.001$) improved FCR up to 10 d of age. Chicks fed on the diet containing 20 g/kg of SDPP were the most efficient. The interaction between feeding duration and level of SDPP was significant ($P<0.05$) only for BW at 10 d of age.

When assessing over the entire production cycle (1-35 d), spray-dried plasma feeding duration, the level and their interaction did not significantly affect feed intake and body weight (Table 5.4). However feed intake was higher in the birds that consumed starter diets containing SDPP. The level of SDPP tended to have a significant (P<0.06) effect on body weight. Birds fed on starter diets that included SDPP also had higher body weight at 35 d of age than the control. The highest body weight was recorded for the chickens that received SDPP for the long feeding duration (10 d). Up to 35 d of age, FCR was improved by the SDPP feeding duration and the level, but it was only significant (P < 0.01, P < 0.001) in the birds that received SDPP for the long feeding duration (10 d). The interaction between feeding duration and level of SDPP tended to have a significant (P<0.077) effect on FCR. Livability and flock uniformity were not affected by the feeding duration nor by the SDPP level or their interaction.

Table 5. 3 Feed intake (g/bird), body weight (g) and FCR (g feed/g weight gain) and livability at 10 d of age of broiler chickens given SDPP for different feeding durations

Feeding duration	**SDPP Level g/kg**	**FI**	**BW**	**FCR**	**Livability**
5 d	**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 d	**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*
Source of variation					
Feeding duration		*NS*	*NS*	*<0.05*	*NS*
Level		*<0.01*	*<0.01*	*<0.001*	*NS*
Feeding duration × Level		*NS*	*<0.05*	*NS*	*NS*

a,b,c – Mean values in the same column not sharing a superscript are significantly different; NS= Non significant; SEM = Standard error of mean

Table 5. 4 Feed intake (g/bird), body weight (g), FCR (g feed/g weight gain) and livability of broiler chickens given SDPP for different feeding durations at 35 d of age

Feeding duration	SDPP Level g/kg	FI	BW	FCR	Livability	Uniformity
5 d	**0**	3755.0	2513.9	1.52[a]	0.93	91.04
	10	3892.5	2635.4	1.50[a]	0.93	90.88
	20	3778.0	2569.3	1.49[ab]	0.93	89.05
10 d	**0**	3755.0	2513.9	1.52[a]	0.93	91.04
	10	3871.4	2669.8	1.47[bc]	0.95	90.82
	20	3830.6	2676.4	1.45[c]	0.95	96.35
SEM		*36.34*	*24.21*	*0.004*	*0.009*	*0.93*
Source of variation						
Feeding duration		*NS*	*NS*	*<0.01*	*NS*	*NS*
Level		*NS*	*<0.06*	*<0.001*	*NS*	*NS*
Feeding duration × Level		*NS*	*NS*	*<0.077*	*NS*	*NS*

a,b,c – Mean values in the same column not sharing a superscript are significantly different; NS= Non significant; SEM = Standard error of mean

5.3.2 Visceral organ weight

Overall, there was no effect of SDPP level, feeding duration or their interaction on the relative weight of the visceral organs of chicks at d 10 ($P>0.05$) except for the pancreas which was significantly heavier ($P<0.05$) in the birds that received the highest level of SDPP for the long feeding duration (Table 5.5).

Up to 24 d of age, neither the SDPP feeding duration nor its level or their interaction significantly affected the relative weight of visceral organs (Table 5.6). However, chickens fed on diet that contained the highest level of SDPP had a relatively higher pancreas weight than the other chicken groups. Chickens that received the low level (10 g/kg) of SDPP over both feeding durations had marginally higher liver weight than the other experimental groups.

Table 5. 5 Relative weight of visceral organs (g/100g of body weight) of broiler chickens given SDPP for different feeding durations at 10 d of age

Feeding duration	SDPP g/kg	Gizz+pro	Smal.int	Liver	Spleen	Bursa	Pancreas
5 d	**0**	3.35	6.70	4.23	0.07	0.19	0.34[b]
	10	3.47	6.70	4.07	0.06	0.18	0.40[ab]
	20	3.53	6.73	3.85	0.07	0.16	0.41[ab]
10 d	**0**	3.35	6.70	4.23	0.07	0.19	0.34[b]
	10	3.47	6.43	4.06	0.09	0.18	0.43[ab]
	20	3.85	7.13	3.99	0.07	0.19	0.45[a]
SEM		*0.085*	*0.123*	*0.085*	*0.004*	*0.005*	*0.008*
Source of variation							
Feeding duration		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Level		*NS*	*NS*	*NS*	*NS*	*NS*	*<0.05*
Feeding duration × Level		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

a,b,c – Mean values in the same column not sharing a superscript are significantly different; NS= Non significant; SEM = Standard error of mean

5.3.3 Nutrient digestibility

At 24 d of age, SDPP feeding duration and the level had no significant effect on ileal digestibility of protein (Table 5.7). The level and feeding duration of SDPP significantly interacted ($P<0.05$), revealing the lowest protein digestibility in the birds that received the highest SDPP level and fed over the longer feeding duration. Gross energy digestibility was not affected by either factors but the interaction between SDPP feeding duration and level was significant ($P<0.05$). Feeding duration and level of SDPP also had no significant effect on the ileal digestibility of dry matter. However, a significant interaction was noticed between the two factors ($P< 0.001$).

Table 5. 6 Relative weight of visceral organs (g/100 of body weight) of broiler chickens given SDPP for different feeding durations at 24 d of age

Feeding duration	SDPP g/kg	Gizz+pro	Smal.int	Liver	Spleen	Bursa	Pancreas
5 d	**0**	1.88	4.74	2.51	0.09	0.21	0.18
	10	1.77	4.20	2.59	0.09	0.20	0.20
	20	1.94	4.94	2.51	0.08	0.18	0.23
10 d	**0**	1.88	4.74	2.51	0.09	0.21	0.18
	10	1.93	4.44	2.79	0.07	0.18	0.15
	20	1.77	4.32	2.41	0.09	0.21	0.20
SEM		*0.037*	*0.116*	*0.058*	*0.005*	*0.083*	*0.013*
Source of variation							
Feeding duration		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Level		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Feeding duration × Level		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

NS= Non significant; SEM = Standard error of mean

Table 5. 7 Ileal digestibility of protein, gross energy and dry matter at 24 d of age of broiler chickens given SDPP for different feeding durations

Feeding duration	SDP Level g/kg	Coefficient of digestibility		
		Crude protein	**Gross Energy**	**Dry Matter**
5 d	**0**	0.80^{ab}	0.73^{ab}	0.86^{a}
	10	0.79^{ab}	0.72^{ab}	0.84^{b}
	20	0.82^{a}	0.75^{a}	0.87^{a}
10 d	**0**	0.80^{ab}	0.73^{ab}	0.86^{a}
	10	0.81^{a}	0.74^{a}	0.86^{a}
	20	0.77^{b}	0.71^{b}	0.84^{b}
SEM		*0.005*	*0.005*	*0.002*
Source of variation				
Feeding duration		*NS*	*NS*	*NS*
Level		*NS*	*NS*	*NS*
Feeding duration × Level		*<0.05*	*<0.05*	*<0.001*

a,b,c – Mean values in the same column not sharing a superscript are significantly different; NS= Non significant; SEM = Standard error of mean

5.3.4 Amino acid digestibility

As separate factor neither SDPP level nor its feeding duration had significant effects on the ileal amino acid digestibility at d 24 of broiler age (Table 5.8). However except for

methionine a significant (P < 0.05) interaction between SDPP level and feeding duration has been detected for almost all chosen amino acids digestibility revealing the highest digestibility coefficients for the birds received high SDPP level over long feeding duration or low SDPP level over short feeding duration. Ileal amino acids digestibility was lower (P < 0.05) in birds offered diets contained high SDPP level for long duration than the other experimental groups.

Table 5. 8 Ileal amino acids digestibility of broiler chickens given SDPP for different feeding durations at 24d of age of chickens

	Feeding duration									
	5 d			**10 d**						
	SDPP Level g/kg							***Source of variation***		
Amino acids	**0**	**1**	**2**	**0**	**1**	**2**	***SEM***	**level**	**feeding duration**	**Level** x **duration**
Histidine	0.81^{ab}	0.80^{ab}	0.82^{a}	0.81^{ab}	0.83^{a}	0.79^{b}	0.005	*NS*	*NS*	*<0.05*
Serine	0.78^{ab}	0.78^{ab}	0.80^{a}	0.78^{ab}	0.80^{a}	0.76^{b}	0.005	*NS*	*NS*	*<0.05*
Arginine	0.85^{ab}	0.84^{ab}	0.86^{a}	0.85^{ab}	0.86^{a}	0.83^{b}	0.004	*NS*	*NS*	*<0.05*
Glycine	0.73^{ab}	0.73^{ab}	0.76^{a}	0.73^{ab}	0.75^{a}	0.71^{b}	0.005	*NS*	*NS*	*<0.05*
Aspartic.A[1]	0.75	0.74	0.77	0.75	0.76	0.72	0.006	*NS*	*NS*	*<0.07*
Glutamic.A[2]	0.87^{ab}	0.87^{ab}	0.89^{a}	0.87^{ab}	0.88^{a}	0.86^{b}	0.004	*NS*	*NS*	*<0.05*
Threonine	0.78^{ab}	0.77^{ab}	0.79^{a}	0.78^{ab}	0.80^{a}	0.75^{b}	0.005	*NS*	*NS*	*<0.05*
Alanine	0.78^{ab}	0.77^{ab}	0.80^{a}	0.78^{ab}	0.80^{a}	0.76^{b}	0.005	*NS*	*NS*	*<0.05*
Proline	0.82^{ab}	0.82^{ab}	0.84^{a}	0.82^{ab}	0.84^{a}	0.80^{b}	0.004	*NS*	*NS*	*<0.05*
Lysine	0.83^{ab}	0.83^{ab}	0.85^{a}	0.83^{ab}	0.85^{a}	0.82^{b}	0.004	*NS*	*NS*	*<0.05*
Tyrosine	0.82^{ab}	0.81^{ab}	0.84^{a}	0.82^{ab}	0.84^{a}	0.80^{b}	0.005	*NS*	*NS*	*<0.05*
Met	0.92	0.93	0.94	0.92	0.94	0.92	0.002	*NS*	*NS*	*NS*
Valine	0.79^{ab}	0.79^{ab}	0.81^{a}	0.79^{ab}	0.81^{a}	0.77^{b}	0.005	*NS*	*NS*	*<0.05*
Iso.Leu	0.82^{ab}	0.82^{ab}	0.84^{a}	0.82^{ab}	0.84^{a}	0.80^{b}	0.004	*NS*	*NS*	*<0.05*
Leucine	0.81^{ab}	0.81^{ab}	0.83^{a}	0.81^{ab}	0.83^{a}	0.80^{b}	0.005	*NS*	*NS*	*<0.05*
Phenyl.A[3]	0.83^{ab}	0.82^{ab}	0.85^{a}	0.83^{ab}	0.85^{a}	0.81^{b}	0.004	*NS*	*NS*	*<0.05*
Total AA[4]	0.80^{ab}	0.80^{ab}	0.83^{a}	0.80^{ab}	0.83^{a}	0.78^{b}	0.005	*NS*	*NS*	*<0.05*

a,b,c – Mean values in the same row not sharing a superscript are significantly different;[1]=Aspartic acid;[2]= Glutamic acid;[3]=Phenyl alanine; [4]=Total amino acids NS= Non significant; SEM = Standard error of mean

5.3.5 Mineral digestibility

The ileal digestibility of Zn was affected by the SDPP feeding duration, but it was only significant (P<0.05) in the birds fed on diet that contained 20 g/kg SDPP for long feeding

duration which had significantly higher Zn digestibility (Table 5.9). The coefficients of digestibility of Ca, Fe, K, Mg, Mn, and P were marginally increased in almost all SDPP-treated groups, particularly the groups fed on SDPP over the longer feeding durations compared to the control.

In general, up to 24 d of age, there was no significant effect of the SDPP feeding duration or level or their interaction on the ileal digestibility of minerals (Table 5.10). However, the digestibility of Mn, Zn and P was marginally higher in birds in the groups fed SDPP diets than in the control. Feeding SDPP at levels of 10 g/kg for 10 d, and 20 g/kg for 5 d marginally increased the digestibility of Ca, Fe, K, Mg, Mn, Zn, and P compared to the control and the other treatment groups.

Table 5. 9 Ileal digestibility of minerals at 10 d of age in broiler chickens given SDPP for different feeding durations

Feeding duration	**SDPP g/kg**	**Ca**	**Fe**	**K**	**Mg**	**Mn**	**Zn**	**P**
5 d	**0**	0.38	0.40	0.90	0.25	0.17	0.17^{ab}	0.55
	10	0.32	0.41	0.89	0.39	0.22	0.09^{b}	0.56
	20	0.46	0.38	0.91	0.36	0.20	0.13^{ab}	0.58
10 d	**0**	0.38	0.40	0.90	0.25	0.17	0.17^{ab}	0.55
	10	0.51	0.48	0.92	0.34	0.07	0.19^{ab}	0.64
	20	0.52	0.49	0.93	0.38	0.18	0.20^{a}	0.64
SEM		*0.025*	*0.020*	*0.005*	*0.023*	*0.030*	*0.002*	*0.015*
Source of variation								
Feeding duration		*NS*	*NS*	*NS*	*NS*	*NS*	*<0.05*	*NS*
Level		*NS*	*NS*	*NS*	*<0.09*	*NS*	*NS*	*NS*
Feeding duration × Level		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

a,b,c – Mean values in the same column not sharing a superscript are significantly different; NS= Non significant; SEM = Standard error of mean

Table 5. 10 Ileal digestibility of minerals at 24 d of age in broiler chickens given SDPP for different feeding durations

Feeding duration	SDPP g/kg	Ca	Fe	K	Mg	Mn	Zn	P
5 d	**0**	0.11	0.41	0.86	0.14	0.07	0.13	0.37
	10	0.11	0.40	0.86	0.11	0.12	0.13	0.38
	20	0.24	0.46	0.89	0.18	0.16	0.20	0.43
10 d	**0**	0.11	0.41	0.86	0.14	0.07	0.13	0.37
	10	0.18	0.43	0.89	0.15	0.16	0.19	0.40
	20	0.09	0.41	0.85	0.10	0.11	0.15	0.38
SEM		*0.022*	*0.012*	*0.007*	*0.017*	*0.019*	*0.016*	*0.017*
Source of variation								
Feeding duration		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Level		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Feeding duration × Level		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

NS= Non significant; SEM = Standard error of mean

5.3.6 Tissue protein content and enzyme activity

The SDPP feeding duration, inclusion level and their interaction had no significant effect on the jejunal tissue protein content measured at 10 d of age (Table 5.11). The activities of maltase, sucrase, alkaline phosphatase and amylase were not significantly affected either by the SDPP feeding duration or by the SDPP level or their interaction. However the activities of these enzymes in chick groups fed on SDPP diets was marginally lower than in the control groups. There was also no significant effect of SDPP feeding duration, level or their interaction on pancreatic tissue protein content, nor was there any effect on the activity of chymotrypsin amidase.

At 24 d of age, there was no significant effect of treatment in tissue protein content or enzyme activities in the jejunum (Table 5.12). However, SDPP inclusion tended increase ($P<0.06$) the activity of alkaline phosphatase compared to the control. The activity of maltase was marginally increased in chickens that consumed SDPP. Inclusion of the

supplement and feeding duration had no significant effect on pancreatic tissue protein content or chymotrypsin enzyme activity.

5.3.7 Jejunal histomorphology

At d 24, jejunal villus were significantly longer (P<0.001) in chicks on diets containing SDPP (Table 5.13). The differences in feeding duration was also significant (P<0.001) but only at the lower level of supplementation. The interaction between feeding duration and level of SDPP was also significant (P<0.001). There was no significant effect of the SDPP feeding duration, inclusion level or their interaction on the crypt depth of the jejunum. However, birds fed on diets that contained SDPP had deeper crypts than the control birds. Apparent villus surface area increased (P<0.001) with increase in level of SDPP and was larger (P<0.001) in birds fed SDPP over shorter duration than the longer duration.

Table 5. 11 Tissue protein contents and digestive enzyme activities of broiler chickens at 10 d of age given SDPP for different feeding durations

Feeding duration	SDPP g/kg	Jejunum					Pancreas	
		Protein[1]	Maltase[4]	Sucrase[4]	AP[2,4]	Amylase[4]	Protein[1]	CA[3,4]
5 d	**0**	19.65	2.45	0.147	0.09	0.61	42.38	2.11
	10	20.35	2.31	0.131	0.08	0.57	42.85	2.22
	20	20.74	2.08	0.126	0.07	0.57	43.13	2.36
10 d	**0**	19.65	2.45	0.147	0.09	0.61	42.38	2.11
	10	22.73	2.05	0.129	0.07	0.48	41.14	1.79
	20	21.35	1.98	0.123	0.06	0.53	46.23	1.76
SEM		*0.649*	*0.094*	*0.0068*	*0.005*	*0.031*	*1.129*	*0.107*
Source of variation								
Feeding duration		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Level		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Feeding duration × level		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

[1]protein (mg/g/tissue);[2]AP, Alkaline phosphatase; [3]CA, Chymotrypsin amidase; [4]Enzymes specific activity (ηmol/mg protein/min); NS= Non significant; SEM = Standard error of mean

Table 5. 12 Tissue protein contents and digestive enzyme activities of broiler chickens at 24 d of age given SDPP for different feeding durations

Feeding duration	SDPP g/kg	Jejunum					pancreas	
		Protein[1]	Maltase[4]	Sucrase[4]	AP[2,4]	Amylase[4]	Protein[1]	CA[3,4]
5 d	**0**	14.97	1.35	0.087	0.04	0.38	24.77	1.77
	10	15.90	1.48	0.093	0.07	0.42	26.83	1.65
	20	15.67	1.37	0.085	0.06	0.32	23.93	1.66
10 d	**0**	14.97	1.35	0.087	0.04	0.38	24.77	1.77
	10	16.29	1.43	0.078	0.07	0.37	24.00	1.90
	20	15.88	1.53	0.094	0.08	0.39	23.10	1.81
SEM		*0.616*	*0.081*	*0.0072*	*0.005*	*0.026*	*0.614*	*0.086*
Source of variation								
Feeding duration		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Level		*NS*	*NS*	*NS*	*<0.06*	*NS*	*NS*	*NS*
Feeding duration × level		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

[1]protein (mg/g/tissue); [2]AP, Alkaline phosphatase; [3]CA, Chymotrypsin amidase; [4]Enzymes specific activity (ηmol/mg protein/min); NS= Non significant; SEM = Standard error of mean

Table 5. 13 Jejunal villus height (µm), crypt depth (µm), villus height/crypt depth ratio and villus surface area (mm^2) of 24-d-old chickens given SDPP for different feeding durations

Feeding duration	SDPP Level g/kg	Response			
		Villus height	Crypt depth	VSA[1]	Muscle thickness[2]
5 d	**0**	1600.5[b]	185.0	5.73[b]	237.6
	10	1857.7[a]	202.8	6.01[b]	261.4
	20	1809.7[a]	265.5	7.52[a]	249.8
10 d	**0**	1600.5[b]	185.0	5.73[b]	237.6
	10	1538.2[b]	228.4	4.53[c]	226.5
	20	1776.7[a]	187.1	5.74[b]	248.1
SEM		*17.11*	*11.30*	*0.009*	*3.18*
Source of variation					
Feeding duration		*<0.001*	*NS*	*<0.001*	*<0.06*
Level		*<0.001*	*NS*	*<0.001*	*NS*
Feeding duration × Level		*<0.001*	*NS*	*NS*	*<0.07*

a,b,c – Mean values in the same column not sharing a superscript are significantly different ; [1] VSA = Villous surface area; NS= Non significant; SEM = Standard error of mean

5.4 DISCUSSION

5.4.1 Gross response

This study further confirms the benefits of feeding SDPP in the starter period. The inclusion of SDPP in the starter diets led to some saving in feed intake without detrimental effect on growth. Similar results were obtained in chapter 4 but contrast the finding of (Zhao *et al.*, 2007) who found that feeding SDPP-supplemented diets to early weaned pig significantly increased their feed intake to 10 d of age.

The improvement in body weight is in line with the findings of (Campbell *et al.*, 2005) who reported that the rate of growth efficiency of broilers was improved when SDPP was included in the diet over the production cycle. Jamroz *et al.* (2012) also reported a significant increase in broiler body weight at d 14, 28 and 30 when they were fed on diets containing SDPP for 28 or 30 d compared to the control. King *et al.* (2005) found that SDP consumption in the first 14 d of life improved the FCR of the broiler chickens in this particular period. It is worth to say that response in our studies continued for longer after the chickens were switched on the unsupplemented diets.

In general, SDPP inclusion in the broiler starter diets resulted in an improvement of the growth performance at an early age and continued to later age. This may be due to the protein utilization from SDPP (Jiang *et al.*, 2000b) which has been identified in weaner piglets as a causative mechanism. Some studies have linked this performance improvement to the immunological characteristics of SDP. The immunoglobulin contents of SDPP (Thomson *et al.*, 1994) may have a vital role in expressing the mechanism of action of SDPP.

5.4.2 Visceral organ weight

In the present study, there was a significant effect of the SDPP level on the relative weight of the pancreas in early age. There was an increase in the relative weight of the pancreas in broilers fed the highest level of SDPP. However, there was no significant effect of SDPP feeding duration, the level or their interaction on the relative weight of the proventricular+gizzard, liver, spleen, and bursa up to 10 or 24 d of broiler age. This is in line with the findings of our previous experiment (Chapter 4) in which no significant effect of SDPP was observed on the relative weight of visceral organs. It would appear that any changes in the contribution of the visceral organs would be functional rather than physical.

5.4.3 Nutrients and amino acid digestibility

The results of the present study demonstrate that the digestibility of nutrients and amino acids was affected by the interaction between the SDPP level and feeding duration at d 24 of broiler age. The digestibility of protein, gross energy, dry matter and amino acids was higher in the chicken groups that consumed the high SDPP level (20 g/kg, short feeding duration) and the low SDPP level (10 g/kg, long feeding duration) compared to the control and other experimental groups. Nutrient and amino acids digestibility was significantly lower in the birds that received the highest level of SDPP compared to the other groups which received the same product. In regards to nutrient digestibility, providing SDPP over short feeding duration is suggested by this study to obtain better nutrient digestibility and utilization. This could have benefits in term of improving nutrient digestibility on one hand and reducing the costs over using this expensive product for long feeding duration in

poultry diets on the other hand. It is worth to mention that SDPP was provided only in the starter diet whereas nutrient digestibility was measured in grower diets. The only explanation we speculate to this improvement in nutrient digestibility, is that perhaps when chicks have good initial growth performance, their metabolism may persist such that nutrient utilization is improved in subsequent stages of life. These results were supported by the findings of (Jamroz *et al.,* 2012), who demonstrated that SDPP supplementation of the broiler diets did not significantly affect the apparent ileal digestibility of protein and dry matter.

5.4.4 Mineral digestibility

Overall, it could be stated that the mineral digestibility was improved due to SDPP inclusion in the starter diets of broiler chickens and the improvement continued through the later stages. This could be due to the mineral profile enrichment of blood products which can positively improve the digestion process of these elements in whole diets. Blood by-products, especially plasma, can be processed and treated as a potentially beneficial source of proteins, amino acids and microelements for non-ruminant animals (Jamroz *et al.,* 2011). This is in line to some extent with the finding of (Jamroz *et al.*, 2011), who observed an improvement in the digestibility of Ca due to SDPP supplementation in the broiler diets.

It is worth to mention that there is no satisfactory method to measure the absorption and digestion of trace minerals due to the complexity of endogenous trace mineral excretion in the animal (Bao *et al.*, 2010). In fact the differences in the trace mineral absorption and excretion in the GIT are primary mechanisms for maintaining trace mineral homeostasis

(King *et al.*, 2000). Therefore, the changes in the digestibility of mineral profile found in the current study could be gut and body osmolarity regulation actions more than to be SDPP feeding effect.

5.4.5 Tissue protein and enzyme activity

Depending on the available results of the current study, we can propose that SDPP could exert enzymes regulatory effect at early age, thereby improving the development of enzyme producing cells and providing available precursor (amino acid) of enzymes which intern have a direct effect on the quality and the structure of enzyme itself and the production capacity and the amount of enzyme released by these cells. Furthermore it could be related to the form of substrate that SDPP could provide to facilitate enzyme function.

5.4.6 Jejunal histomorphology

In this study, light microscopic examination revealed a structural development of the histomorphology of the jejunal mucosa at d 24. Spray-dried plasma consumption at an early age resulted in a significant improvement in the structural development of the intestinal mucosa. This effect of SDPP on mucosal morphology could be due to the improvements in intestinal health and barrier function. Oral consumption of SDPP maintains gut barrier function and reduces or modulates the overstimulation of the inflammatory response (Campbell, 2011b). Increased epithelial permeability occurs during intestinal inflammation due to altered barrier function; therefore, supplementation of SDPP to animal diets can in part reduce wearing of the epithelial structure, thus improving the intestinal mucosal barrier function (Pérez-Bosque *et al.*, 2006). This may

increase the absorption capacity of the intestine and enhance nutrient uptake and improve nutrient utilization efficiency, which in turn has a positive effect on the rate of metabolic activity of birds. This could be reflected in an improvement in the performance and productive ability of the chickens by further increasing the rate and pattern of protein deposition in the body muscles and tissues.

These results are in agreement with the results of our previous trials (Section 4.3.6), namely, that SDPP has positive effects on the morphological properties of intestinal mucosa. Similar findings have been reported by (King *et al.*, 2005) and (Jamroz *et al.*, 2011; 2012). Owusu-Asiedu *et al.* (2003) also reported that intestinal villus height was increased due to SDPP supplementation of pig diets. The results of the current experiment imply that the interaction between the experimental factors is a key factor to effect jejunal histomorphology particularly villi height. Providing SDPP in the stater diets over short feeding duration, first 5 d, could lead to the targeted level of absorption and utilization of nutrients at starter and subsequent stages of broiler life, and longer provision of SDPP may have no additional effect on the jejunal histomorphology and further increase the absorptive area in the jejunum.

5.5 CONCLUSION

The results of the current study provide some evidence for the beneficial effects of the inclusion of SDPP in broiler starter diets. However, the extent of its effect and the optimum level that must be added to the diet of broiler chickens is still under debate. In terms of performance, SDPP feeding resulted in an improvement in body weight and FCR of birds. Nutrient digestibility and digestive enzymes activity were largely not affected by

SDPP. The outcomes achieved in this experiment suggest that SDPP could be beneficial in the early stage of broilers life where it effectively enhance early growth and development of the birds' bodily functions. The current study demonstrated that feeding SDPP for longer period (10 d) could have additional effects on broiler performance. Furthermore there was no additional response of birds to high level of this product. Therefore, economically it would be more beneficial to use a low level of SDPP over a longer period (first 10 d posthatch) without losing the beneficial effects of the product on bird growth or their physiological functions. Furthermore, SDPP is an expensive product, and therefore it is preferable and cost-effective for this type of product to be used at lower level and only at an early age, including use in the pre-starter diets, when a relatively small amount of feed is consumed by the birds

CHAPTER 6 Effect of dietary inclusion of spray-dried porcine plasma on performance, physiological and immunological responses of broiler chickens challenged with *Salmonella sofia*

6.1 INTRODUCTION

Contamination of poultry products by bacteria can lead to public health problems, and adverse media coverage of such occurrences has intensified consumer consciousness of food safety. *Salmonella sofia* is a geographically unique bacterial species that is consistently isolated in Australia, most commonly from poultry and poultry products (Sexton *et al.*, 2007; Eglezos *et al.*, 2008; Pointon *et al.*, 2008). According to the Australian Salmonella Reference Centre report, the serovars of 4,134 *Salmonella* isolates were obtained from broiler chickens all around the country in 2008. This report indicated that the majority of the isolated serovars were *Salmonella sofia*, which represented about 45.9% of all *Salmonella spp.* isolated. *Salmonella typhimurium* was the second most prevalent, accounting for about 9.4% of isolates (IMVS, 2008). *Salmonella enterica* serovar Sofia is often isolated from chickens in Australia. However, despite its high isolation rate from chickens and chicken meat products, *S. sofia* is rarely related to animal or human salmonellosis, probably because this serovar is avirulent in nature (Gan *et al.*, 2011). The dominance of *Salmonella sofia* on, and frequent isolation from, postprocessed chicken carcasses raises the possibility that this serovar has an enhanced ability to survive the hostile environment present within the poultry processing facility (Mellor *et al.*, 2010).

The rearing environment has a strong impact on animal growth and feed efficiency. Chickens are known to be very sensitive to *Salmonella* infections during the first week of

life because of delayed development of their intestinal flora. Immediately after hatching the gastrointestinal tract of chickens is colonized by a microfloral load, which forms an important barrier against colonization of potentially pathogenic bacteria, such as *Salmonella* (Dibb-Fuller *et al.*, 1997). Animal nutrition strategies have been changed after the banning of the use of antibiotics to control disease and promote growth performance of animals in some countries. Some new natural components have been introduced to animal nutrition to support the growth performance and health of animals in the conventional unsanitary conditions. Some of these products are termed nutraceuticals - raw materials (or part of a raw material) that can provide both medical and nutritional benefits to the animals, including the prevention and treatment of disease (Kalra, 2003).

In searching for bioactive substances which can naturally stimulate immunity and improve the general health status of animals, scientists have been interested in the use of blood by-products as raw products in animal nutrition (Orda *et al.*, 1988; Coffey & Cromwell, 1995). Positive effects of dried blood plasma on immune response and intestinal wall functions have been shown in weaned pigs and other non-ruminant animals (De Rodas *et al.*, 1995; Godfredson-Kisic & Johnson, 1997; Nofrarias *et al.*, 2006; Campbell *et al.*, 2009). The activity of blood by-products has been related to the specific immunoreactive globulins and nucleotides present in blood products (Shahidi *et al.*, 1984; Pierce *et al.*, 2005; Rodriguez *et al.*, 2007; Moretó & Pérez-Bosque, 2009). An improvement in the growth performance and general health status of animals (pigs, poultry, calves, and pets) has been observed due to feeding of spray-dried plasma (Quigley & Drew, 2000; Coffey & Cromwell, 2001; Campbell *et al.*, 2003; Campbell *et al.*, 2004a; Campbell *et al.*, 2004b).

The response to dietary SDPP has been found to be more pronounced in production conditions with high pathogen exposure than in clean rearing environment in pigs (Stahly *et al.,* 1994; Coffey & Cromwell, 1995). Similar observations have been reported for broiler chickens (Campbell *et al.*, 2003) and turkey poults (Campbell *et al.*, 2004a). Spray-dried plasma contains a diversity of functional proteins such as immuloglobulins, albumin, growth factors, and biologically active peptides (Borg *et al.,* 2002). These proteins are more efficient during animal exposure to environmental or immunological challenges. Coffey and Cromwell (1995) reported that pigs consuming SDP had greater efficiency and rates of growth when housed in a challenging environment than pigs housed in a clean environment. Similar responses to SDP have also been reported for broilers housed in different environments (Campbell *et al.*, 2003). Other studies reported that SDP consumption during *Escherichia coli* (Quigley & Drew, 2000; Bosi *et al.*, 2001) or *Cryptosporidium* (Hunt *et al.,* 2002) challenge improves health, recovery, and rate and efficiency of growth of animals.

Spray dried porcine plasma contains a diversity of functional proteins and biological active compounds that may enhance growth performance and stimulate broilers immune system while rearing in disease challenge conditions. This study aimed to assess the efficacy of spray-dried porcine plasma feeding in the starter phase and its effect on the growth performance and subsequent physiology and immunity of broiler chickens challenged with *Salmonella sofia*.

6.2 MATERIALS AND METHODS

6.2.1 Experimental design and bird management

Two levels of SDPP (10 g/kg and 20 g/kg) were substituted mainly in lieu of meat meal, and tested under disease challenge condition in the starter diets. A total of 420 d-old male Ross 308 chicks, vaccinated against Marek's disease, infectious bronchitis, and Newcastle disease were obtained from a local hatchery (Baiada hatchery, Kootingal, NSW, Australia) and randomly assigned to five dietary treatments, each with six replicates, 14 chickens per replicate. The birds were reared in floor pens (75 x 60 cm) in 2 rooms. The four starter diet treatments were the positive control (no supplement), diet supplemented with in-feed antibiotics, IFA (salinomycin 0.05% + zinc bacitracin 0.033%) and diets supplemented with SDPP at 10 g/kg and 20 g/kg diet. All 4 of these groups were challenged with *S. sofia* (5.9 x10^8 cfu/ml), while a fifth group was unchallenged and used as the negative control. The five treatments were arranged in a completely randomized design. The starter diets were fed from hatch to 14 d. The diets were identical in nutrient profiles and formulated to meet breeder specifications. After 14 d, the birds were switched to commercial type grower (14-24 d) and finisher (25-35 d) diets.

Feed and water were provided *ad libitum*. The room temperature was gradually decreased from 33 °C on d 1 to 24 °C ± 1 °C at 35 d. Eighteen hours of lighting were provided per d throughout the duration of the experiment, apart from ds 1 to 7 when 23 hours of lighting were provided. On d 13, 24 and 35, the birds and feed were weighed to measure the body weight, feed intake and feed conversion ratio. Mortalities were recorded as they occurred, and feed per gain values were corrected for mortality.

Table 6. 1 Ingredient and nutrient composition of starter, grower, and finisher diets

Ingredient g/kg	Starter			Grower	Finisher
	SDPP Level g/kg				
	0	10g/kg	20g/kg		
Wheat	606.7	614.6	621.4	612.3	627.4
Soybean meal	272.5	265.1	258.5	260	250
Meat meal	40	30	20	40	40
Oil	38.0	36.0	34.5	54.0	52.0
SDPP	-	10	20	-	-
Choline chloride	0.7	0.7	0.8	0.5	0.5
Dicalcium phosphate	11.2	12.6	14.1	8.6	7.0
Sodium bicarbonate	3.0	2.4	2.2	2.0	2.0
Limestone	10.7	11.9	13.0	8.3	8.3
Salt	1.0	1.0	0.9	1.3	1.3
Titanium dioxide	5	5	5	5	5
L-Lysine	3.6	3.4	3.0	2.0	2.0
DL Methionine	3.7	3.5	3.3	2.8	2.0
Threonine	2.0	1.8	1.6	1.2	0.7
Trace minerals[1]	0.75	0.75	0.75	0.75	0.75
Vitamins[2]	0.5	0.5	0.5	0.5	0.5
Avizyme[3]	0.5	0.5	0.5	0.5	0.5
Phyzyme XP[4]	0.1	0.1	0.1	0.1	0.1
Nutrient composition					
ME poultry (MJ/kg)	12.66	12.65	12.66	13.18	13.18
Crude protein	220	220	220	212.4	208.7
Lysine	12.7	12.8	12.7	11.2	10.9
Methionine	6.47	6.25	6.02	5.5	4.7
Arginine	13.33	13.24	13.2	13.0	12.7
Methionine +cystine	9.5	9.4	9.4	8.5	7.6
Threonine	8.32	8.33	8.34	7.4	6.8
Calcium	10.54	10.51	10.50	9.1	8.7
Available phosphorus	5.01	5.00	5.02	4.5	4.2
Sodium	1.86	1.86	1.92	1.7	1.7
Choline	1.61	1.60	1.61	1.53	1.43

[1,2,3,4] Composition as shown in Table 3.1

6.2.2 Infectious strain of *Salmonella sofia*

The strain of *S. sofia* was obtained from the Biotechnology Laboratory, RMIT University (Melbourne, VIC, Australia) and maintained in Luria Bertani (LB) broth with 30% (v/v) glycerol at -20°C. The strain was made rifampicin resistant as described by Eisenstadt *et al.* (1994) with some modifications as follows: 1) the gradient plate technique used

antibiotic agar containing rifampicin (95% HPLC, R3501-5G, Sigma–Aldrich, Castle Hill, NSW, Australia) at 80 μg/mL; and 2) to more accurately determine the level of resistance to rifampicin, the mutants were each streaked on several plates containing different concentrations of rifampicin, namely, 100 μg/mL, 110 μg/mL, and 120 μg/mL. The mutant strain was amplified by growth overnight at 39 °C in 1000 mL of LB broth. This challenge pre-mixture of broth bacterium solution was administered by oral gavage.

6.2.3 *S. sofia* challenge model

An infection dose rate of 5.9×10^8 cfu/mL was used. This follows the challenge models for *Salmonella* described by Bjerrum *et al.* (2003). The bacterial suspension was individually administered using a crop needle and a 10 mL syringe with a flexible tube attached. Chicks were given 2 mL of the bacterial suspension on d 8, d 10 and d 12. Birds in unchallenged groups received 2 mL of sterile LB broth. Unchallenged birds were always serviced first to reduce the likelihood of cross-contamination and all inoculation was completed inside the cages.

6.2.4 Sample collection and processing

Birds from the unchallenged group were processed first to minimize the likelihood of cross-contamination. Three birds per replicate on d 13 and 2 birds per replicate on d 21 were randomly selected, weighed, and euthanized by cervical dislocation. Thereafter, the birds were dissected to remove the small intestine. The contents of the ileum and caeca were collected by gently squeezing the digesta into plastic containers. Samples from each replicate were pooled together, and subsamples of each replicate (ileum and caeca) were transferred to separate plastic containers for pH measurement. The remaining ileal and

caecal contents were frozen immediately to -20 °C until further analyses were conducted. At d 13 and 21, blood samples were collected to measure the whole blood hematological parameters, and serum was harvested for serum biochemistry and ELISA assay.

6.2.5 Measurement and analyses

6.2.5.1 Growth performance

Feed intake (FI) and body weight (BW) were recorded on d 14, 24 and 35 for determination of average FI and BW. Mortality was recorded when it occurred and feed conversion ratio (FCR:feed intake/weight gain) was corrected for mortality.

6.2.5.2 Visceral organ weight

Body weight and the weight of the organs associated with immunity (liver, spleen, bursa of Fabricius and thymus) were recorded at d 13 and 21. The relative organ weight was calculated as mass per unit of live body weight (g/100 of live body weight).

6.2.5.3 Hematological and serum biochemical parameters

Blood samples (approximately 1 ml) from the jugular vein were collected in heparinized syringes for estimation of whole blood pH, Hb and minerals using blood gas machine (Radiometer ABL800 FLEX). Packed cell volume (PCV %) was measured in heparinized capillary tubes after centrifugation for 3 minutes at 3,000 rpm (Hawksley, microhaematocrite centrifuge, 1500, Enland). Subsequently, serum was harvested by collecting the blood into non-heparinized tubes and centrifuged at 3,000 rpm for 15 minutes using (Allegra, 6R centrifuge, Beckman, USA) and stored at -20 °C for analyses.

6.2.5.4 ELISA assays

Sandwich ELISA assays were used to determine the total antibody titre concentrations of IgG, IgM, and IgA at 2 time points: 1 d after the last *S. sofia* inoculation (d 13), and 7 d after the last *S. sofia* inoculation (d 21). On the designated ds, blood samples (one bird per replicate) were taken from the jugular vein into 7-mL serum tubes. Blood samples were then allowed to clot at room temperature for 2 h and subsequently centrifuged at 2,500 rpm, using (Allegra, 6R centrifuge, Beckman, USA) for 5 min to separate the serum from the cells. All serum samples were immediately frozen at -20 °C until antibody assays were performed. Serum IgA, IgG, and IgM concentrations were measured using chicken-specific ELISA reagents according to the instructions of the manufacturer (Bethyl Labs, Montgomery, TX). Serum samples were assayed in duplicate at dilutions of 1:50,000, 1:10,000, and 1:2,000 for IgG, IgM, and IgA, respectively. Antibody concentrations were derived from standard chicken reference serum samples included on each plate.

6.2.5.5 Intestinal pH

Intestinal pH was measured immediately after death and excision of viscera at d 13 and 21. The pH of ileal and caecal contents was determined by the modified procedure of Corrier *et al.* (1989). About 1 g of content was diluted in 9 mL of distilled water. The mixture was shaken and vigorously stirred with a stirrer, and the pH was measured with a pH meter (EcoScan 5/6 pH meter, Eutech Instrument Pte Ltd., Singapore).

6.2.5.6 Determination of concentrations of short-chain fatty acids

After thawing at room temperature, the concentrations of short-chain fatty acids (SCFA) and lactic acid of each digesta sample from the ileum and caeca were measured using gas chromatography (Varian CP-3800) according to the method described by Jensen *et al.* (1995). About 1–2 g of caecal/ileal digesta was weighed accurately in centrifuge tubes (placed on ice) and 1 mL of 0.01 M ethylbutyric acid (internal standard) solution was added. The mixture was vortexed and centrifuged at 5 ºC at 15,000 x g on a high speed centrifuge (AVANTI J-E 369001, Beckman countler, USA) for 20 min. Around 1 mL of supernatant was transferred to 8-mL vials (placed on ice). About 1 mL of standard SCFA mixture was transferred to 8-mL vials and 1 mL of H_2O (or culture medium/buffer) as blank to an 8-mL vial. Then 0.1 mL of 0.1 M ethylbutyric acid (internal standard) was added. About 2.5 mL of ether and 0.5 mL of concentrated HCl (36%) were added to the vials and tightly capped, vortexed for one minute and centrifuged at 3,000 rpm using (Allegra, 6R centrifuge, Beckman, USA) for 15 min at 5 ºC. About 400 uL of the supernatant (duplicate standard and blank) (using Eppendorf pipette) were transferred to a 2-mL GC vial and 40 uL MTBSTFA (50 ul MTBSTFA in the case of standards) was added. The vials were capped and were vortexed and put into an 80 ºC-heating block for 20 min. The vials were kept at room temperature for at least 48 hours and then run on the GC.

6.3 Animal ethics

The experiment was approved by the Animal Ethics Committee of the University of New England (Approval No: AEC13-129). 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 (NAHMRC, 1990).

6.4 RESULTS

6.4.1 Clinical symptoms of challenged birds and mortality

Within a few hours of the first inoculation with *S. sofia*, obvious clinical symptoms were observed, with the most obvious being in the challenged control group. Chicks were huddled in the corners of the cage, showing somnolence, loss of appetite and inhibition in drinking. They were generally depressed, sluggish and reluctant to move. Thin yellowish diarrhoea appeared in some chicks. The clinical symptoms were temporary and lasted for a few hours, then gradually disappeared, recovery being complete within 24 hours. None of the chicks died during the 48 hours after inoculation.

6.4.2 Gross response

Feed intake to 14 d was significantly lower ($p < 0.05$) in the unchallenged birds than in the challenged experimental groups. Up to d 24 and 35, feed intake was not affected by the treatments (Table 6.2).

At 14 d, body weight was higher ($p < 0.001$) in the birds fed the diet contained IFA and SDPP than in birds in both challenged and unchallenged control groups. Birds that consumed diets with IFA had significantly higher ($p < 0.001$) body weight compared to those that consumed the high level diet (20 g/kg) of SDPP; however, there was no significant difference in the body weight between the birds that consumed IFA and those that received the diet with low level of SDPP (10 g/kg). At 24 d, the body weight of

chicks in the challenged control birds was lower ($p < 0.01$) than that of the unchallenged and those that received SDPP and IFA in their starter diets. At 35 d, there was no significant difference in body weight between the experimental groups; however, the challenged birds that received SDPP and IFA had numerically higher body weight than the challenged control group.

Feed conversion ratio (FCR) was not significantly affected by treatment during the experimental period. However, birds that consumed SDPP and IFA had a better FCR under challenge conditions compared to challenged control birds. The treatments had no significant effect on the mortality of birds.

Table 6. 2 Effect of SDPP inclusion in the starter diets on the growth performance of broiler chickens challenged with *S. sofia*.

		Challenged groups				[1]UnC	
Response	**Age (d)**	[2]Pos.Control	10g/kg SDPP	20g/kg SDPP	[3]IFA	[4]Neg.Control	**SEM**
FI	1-14	585.5^{a}	588.8^{a}	571.3^{ab}	590.3^{a}	549.7^{b}	4.142*
(g/bird)	1-24	1525.4	1546.4	1497.5	1567.6	1532.2	15.226
	1-35	4235.4	4190.1	4086.4	4072.1	4116.8	48.76
BW	14	568.0^{bc}	583.0^{ab}	574.6^{b}	595.0^{a}	549.8^{c}	2.968***
(g/bird)	24	1293.6^{b}	1390.5^{a}	1361.6^{a}	1410.2^{a}	1394.1^{a}	8.507**
	35	2629.7	2676.7	2690.7	2693.3	2690.1	13.03
FCR	1-14	1.12	1.10	1.08	1.08	1.09	0.007
(g:g)	1-24	1.22	1.15	1.14	1.15	1.14	0.014
	1-35	1.64	1.59	1.55	1.54	1.55	0.017
Mortality	1-14	3.6	5.9	3.6	3.6	3.6	0.93
(%)	1-35	7.1	13.1	4.8	4.8	9.5	1.29

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); [1] UnC= unchallenged; [2] Pos.Control = Positive control; [3]IFA = in feed antibiotics.; [4] Neg.Control = Negative control; SEM= Standard error of mean

6.4.3 Organ weight

At 13 d, the relative weight of the bursa of SDPP-fed birds, when challenged was higher ($p < 0.05$) than that of the control groups (Table 6.3). Inclusion of SDPP to the broiler starter diets under disease challenge also marginally increased the weight of the other immune-related organs (spleen and thymus) at 13 and 21 d of broiler age.

Table 6. 3 Effect of SDPP inclusion in the starter diets on the relative weight of immune associated organs (g/100g body weight) of broiler chickens challenged with *S.sofia*

	Age (d)	Challenged groups				[1]UnC	
Response		[2]Pos.Control	10g/kg SDPP	20g/kg SDPP	[3]IFA	[4]Neg.Control	SEM
Liver	13	3.0	3.7	3.9	3.7	3.8	0.15
	21	3.0	2.9	2.9	2.8	3.1	0.04
Spleen	13	0.06	0.08	0.08	0.08	0.06	0.004
	21	0.07	0.08	0.07	0.08	0.08	0.003
Bursa	13	0.14^{c}	0.22^{a}	0.19^{ab}	0.16^{abc}	0.14^{bc}	0.008*
	21	0.17	0.18	0.18	0.19	0.22	0.007
Thymus	13	0.17	0.24	0.19	0.19	0.20	0.013
	21	0.17	0.13	0.17	0.16	0.17	0.006

[a,b.c] – Mean values on the same row not sharing a superscript are significantly different (*P < 0.05); [1] UnC = unchallenged; [2] Pos.Control = Positive control; [3]IFA = in feed antibiotics.; [4] Neg.Control = Negative control; SEM = Standard error of mean

6.4.4 Blood parameters

Hematological parameters of broiler chickens were not affected by the treatments. Inclusion of SDPP in the starter diets had no significant effect on the blood pH and PCV or concentrations of Hb and glucose of broiler chickens at 13 and 21 d (Table 6.4). However, birds that received the high level (20 g/kg) of SDPP had a numerically lower PCV value at d 21 followed by the birds in the unchallenged control group. In comparison with challenged groups, birds in the unchallenged group had lower blood Hb content at d

13 and 21. The challenged control group had marginally higher blood sugar (glucose) content than the other experimental groups.

6.4.5 Blood electrolytes

At 13 ds, the blood potassium content of the birds that received the low level (10 g/kg) of SDPP was lower ($p < 0.05$) than those of the other groups (Table 6.5). Broiler chickens that were fed starter diet containing the high level (20 g/kg) of SDPP had numerically higher blood sodium content than the other groups.

Table 6. 4 Effect of SDPP inclusion in the starter diets on some blood parameters of broiler chickens challenged with *S.sofia*

Response	Age (ds)	Challenged groups				[1]UnC	SEM
		[2]Pos.Control	10g/kg SDPP	20g/kg SDPP	[3]IFA	[4]Neg.Control	
pH	13	7.4	7.4	7.4	7.4	7.5	0.01
	21	7.3	7.4	7.3	7.4	7.4	0.01
PCV %	13	30.3	30.5	31.4	29.1	29.6	0.45
	21	36.0	36.7	32.1	35.3	32.4	0.67
Hemoglobin (g/dl	13	8.7	9.2	8.9	8.7	8.4	0.16
	21	10.2	10.0	9.4	10.2	9.2	0.18
Glucose (mmol/L)	13	12.8	12.7	12.1	11.1	12.9	0.39
	21	13.2	12.8	12.8	12.8	12.2	0.15

[1] UnC = unchallenged; [2] Pos.Control = Positive control; [3]IFA = in feed antibiotics.; [4] Neg.Control = Negative control; SEM= Standard error of mean

6.4.6 Immune response

In general, there was no significant effect of treatments on serum immunoglobulin content of broiler chickens at d 13 and 21 (Figure 6.1). However, the birds that consumed the lower level of SDPP (10 g/kg) had a lower IgA concentration than the other challenged bird groups. At d 13, IgG concentration tended to be lower ($p < 0.09$) in birds that received the low level of SDPP. At 13 and 21 d of age, disease-challenged birds that were

fed on starter diets that contained SDPP and IFA had lower serum IgG content than the control groups. At d 13, serum IgM concentration tended to be lower ($p < 0.08$) in the challenged birds that received the low SDPP level in their starter diets than the other groups. In general, IgM content decreased in the birds that consumed SDPP in their starter diets under disease conditions compared to other challenged bird groups.

Table 6. 5 Effect of SDPP inclusion in the starter diets on some blood electrolytes (mmol/L) of broiler chickens challenged with *S.sofia*

Response	Age (d)	Challenged groups				[1]UnC	SEM
		[2]Pos.Control	10g/kg SDPP	20g/kg SDPP	[3]IFA	[4]Neg.Control	
Potassium	13	4.40[a]	3.77[b]	4.35[a]	4.47[a]	4.57[a]	0.081*
	21	4.45	4.33	4.18	4.30	4.15	0.093
Sodium	13	145.2	146.7	176.2	145.5	146.3	0.304
	21	147.7	147.2	147.5	148.0	147.2	0.259
Calcium	13	1.30	1.30	1.27	1.30	1.24	0.010
	21	1.32	1.31	1.30	1.29	1.29	0.008
Chloride	13	109.0	107.7	109.0	108.8	111.7	0.363
	21	110.7	109.3	108.8	109.2	109.0	0.414

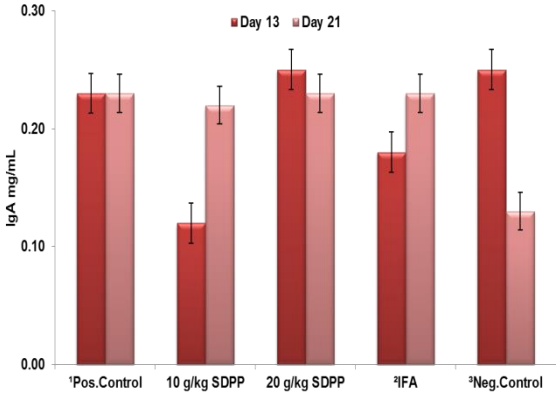
[a,b,c] – Mean values on the same row not sharing a superscript are significantly different (*P < 0.05); [1] UnC = unchallenged; [2] Pos.Control = Positive control; [3]IFA = in feed antibiotics.; [4] Neg.Control = Negative control; SEM = Standard error of mean

6.4.7 Serum biochemistry

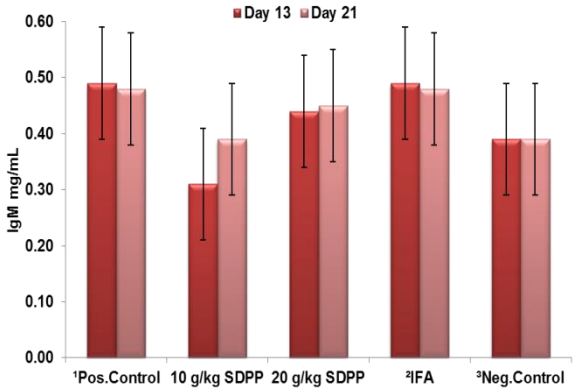
The inclusion of SDPP in starter diets had no significant impact in the serum biochemical parameters that were assessed, including total protein, albumin, globulin, albumin to globulin ratio, cholesterol, triglyceride, HDL and LDL at 13 and 21 d of age (Table 6.6). In general, the serum cholesterol content of the challenged chickens fed on diets that contained SDPP and IFA was numerically higher than the challenged control group. Serum HDL content was slightly higher in the SDPP-supplemented groups than the other

challenged groups. At d 13, birds that received the high level of SDPP in their starter diets had lower LDL than the other experimental groups.

IgA



IgM



IgG

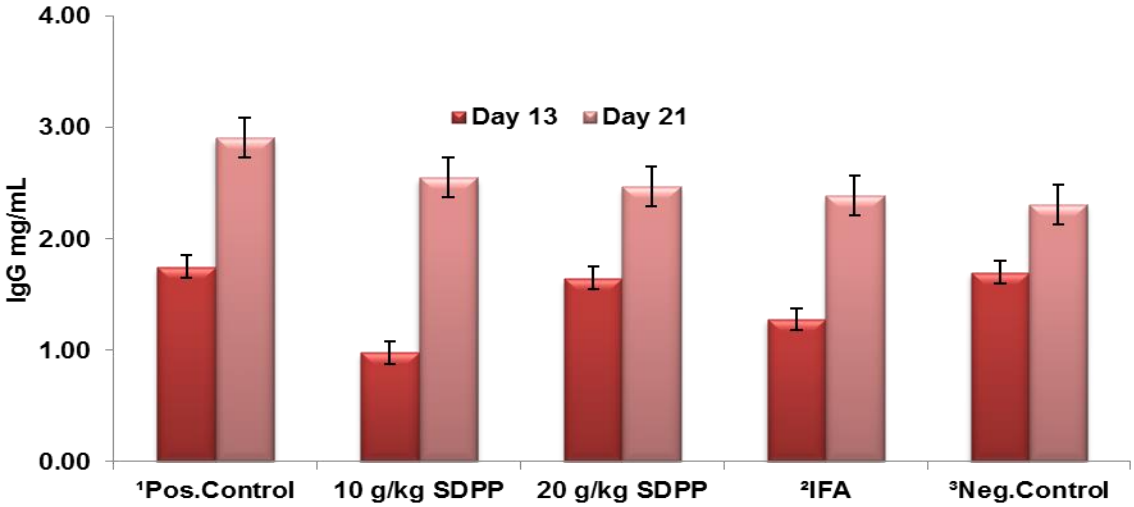


Figure 6. 1 Serum IgA, IgM and IgG concentrations (mg/ml) of broiler chickens fed diets containing SDPP and challenged with *S.sofia*

[1]=Positive control; [2]= In feed antibiotics; [3]= Negative control

Table 6. 6 Effect of SDPP inclusion in the starter diets on serum biochemistry of broiler chickens challenged with *S.sofia*

Response	Age (d)	Challenged groups				[1]UnC	SEM
		[2]Pos.Control	10g/kg SDPP	20g/kg SDPP	[3]IFA	[4]Neg.Control	
[8]**Total protein**	13	27.1	26.6	26.1	25.6	26.8	0.46
	21	28.5	29.3	29.2	30.2	27.5	0.38
[8]**Albumin (**	13	5.15	5.20	5.03	5.03	5.00	0.09
	21	5.45	5.68	5.61	5.32	5.75	0.08
[8]**Globulin**	13	21.9	21.4	21.0	20.5	21.8	0.39
	21	23.1	23.6	23.6	22.2	24.5	0.32
[5]**A/G**	13	0.23	0.25	0.24	0.25	0.23	0.003
	21	0.24	0.24	0.24	0.24	0.24	0.002
[9]**Cholesterol**	13	3.68	3.83	3.71	3.89	3.52	0.07
	21	3.72	4.05	3.93	3.97	4.20	0.06
[9]**Triglyceride**	13	1.84	1.69	1.78	1.83	1.64	0.07
	21	1.41	1.53	1.34	1.59	1.75	0.07
[6,9]**HDL**	13	2.66	2.82	2.86	2.88	2.55	0.08
	21	2.84	3.07	3.01	2.93	3.05	0.06
[7,9]**LDL**	13	0.65	0.67	0.50	0.65	0.65	0.029
	21	0.59	0.68	0.64	0.73	0.80	0.028

[1] UnC = unchallenged; [2] Pos.Control = Positive control; [3]IFA=in feed antibiotics; [4] Neg.Control = Negative control; [5]A/G = Albumin/globulin ratio; [6]HDL = High density lipoprotein; [7]LDL = Low density lipoprotein; [8]= g/dl; [9]= mg/dl; SEM = Standard error of mean

6.4.8 Concentration of SCFA of ileal content

There was no significant effect of the treatments on the ileal acetic acid concentration of broiler chickens at 13 and 21 d of age (Table 6.7). Lactic acid concentration was significantly affected by the treatment. At 13 ds, the ileal lactic acid concentration was higher ($p < 0.05$) in the challenged bird groups fed on starter diets that contained the high level of SDPP. The concentrations of succinic acid and formic acid in the ileal contents at 13 and 21 d were not affected by the treatments. However, birds that consumed the high SDPP level had a higher succinic acid concentration than the other experimental groups.

6.4.9 Concentration of SCFA in caecal content

There was no significant effect of the supplement or disease challenge on the concentration of acetic acid in the caecal content of broiler chickens at 13 and 21 d of age (Table 6.8). However, at d 13 the concentration of acetic acid tended to be higher ($p < 0.082$) in the challenged control birds than in the other experimental groups. Lactic acid concentration in the caecal contents was not affected by the treatments either age assessed. There was no significant difference in the formic acid concentration of the caecal contents between different experimental groups at d 13. However, the concentration of formic acid tended to be higher ($p < 0.075$) in the birds that received the low level of SDPP in their starter diets than in the other groups. At 21 d of age, formic acid concentration was also highest ($p < 0.005$) in the group fed on diet containing the high level of SDPP under disease conditions. In general, the concentration of formic acid was higher in the birds that received SDPP in their starter diets than in the other groups. Caecal propionic acid concentration was not affected by the treatments. However, at 13 d, the concentration of propionic acid tended to be higher ($p < 0.09$) in the unchallenged bird group than in the other groups. The concentration of butyric acid at 13 d was lower ($p < 0.01$) in the unchallenged bird group than in the challenged control group and SDPP-supplemented bird groups. At d 13, the challenged control group had significantly higher ($p < 0.01$) butyric acid concentration in the caecal contents than the group fed on IFA-supplemented starter diet. There was no significant effect of treatments on the caecal isobutyric acid concentration of broiler chickens at 13 and 21 d of age.

Table 6. 7 Effect of SDPP inclusion in the starter diets on short chain fatty acid concentration (μmol/g digesta) in the ileum of broiler chickens challenged with *S.sofia*

Fatty acid	Age (d)	Challenged groups				[1]UnC	SEM
		[2]Pos.Control	10g/kg SDPP	20g/kg SDPP	[2]IFA	[4]Neg.Control	
pH	13	6.80	6.84	6.82	7.04	6.82	0.04
	21	6.89	6.82	6.74	7.01	6.63	0.06
Acetic	13	1.66	1.94	3.08	1.33	2.71	0.22
	21	1.28	1.03	1.06	0.95	0.97	0.06
Lactic	13	32.4^{ab}	34.3^{ab}	50.9^{a}	17.7^{b}	19.3^{b}	3.24*
	21	18.8	13.6	21.5	12.0	12.7	1.29
Succinic	13	0.31	0.38	0.44	0.24	0.24	0.04
	21	0.17	0.17	0.19	0.08	0.12	0.01
Formic	21	0.18	0.16	0.13	0.15	0.19	0.01

[a,b.c] – Mean values on the same row not sharing a superscript are significantly different (*P < 0.05); [1] UnC = unchallenged; [2] Pos.Control = Positive control; [3]IFA=in feed antibiotics.; [4] Neg.Control= Negative control; SEM = Standard error of mean

Table 6. 8 Effect of SDPP inclusion in the starter diets on short chain fatty acid concentration (μmol/g digesta) in the ceca of broiler chickens challenged with *S.sofia*

Fatty acid	Age (d)	Challenged groups				[1]UnC	SEM
		[2]Pos.Control	10g/kg SDPP	20g/kg SDPP	[3]IFA	[4]Neg.Control	
pH	13	6.54	6.60	6.83	6.36	5.98	0.07
	21	6.78	6.72	6.71	6.81	6.51	0.03
Acetic	13	85.9	73.1	73.7	72.9	57.7	2.92
	21	75.4	96.7	75.8	71.9	75.2	4.44
Lactic	13	2.94	2.99	2.79	1.95	3.23	0.29
	21	0.61	0.91	0.85	0.22	0.28	0.10
Formic	13	0.21	0.98	0.20	0.20	0.33	0.10
	21	0.19^{b}	0.76^{b}	2.45^{a}	0.59^{b}	0.37^{b}	0.18**
Propionic	13	3.79	2.98	3.53	2.55	2.40	0.19
	21	4.65	6.35	5.46	5.03	5.73	0.43
Butyric	13	16.99^{a}	13.35^{ab}	15.30^{ab}	11.55^{bc}	8.31^{c}	0.74**
	21	16.46	19.38	15.77	17.49	14.23	1.01
Isobutyric	13	0.36	0.24	0.31	0.27	0.25	0.02
	21	0.45	0.62	0.54	0.46	0.53	0.03

[a,b.c] – Mean values on the same row not sharing a superscript are significantly different (**P < 0.01); [1] UnC = unchallenged; [2] Pos.Control = Positive control; [3]IFA = in feed antibiotics.; [4] Neg.Control = Negative control; SEM = Standard error of mean

6.5 DISCUSSION

6.5.1 Gross response

This experiment assessed the inclusion for 14 d of spray-dried porcine plasma in the starter diets of broiler chicks under *S. sofia* disease challenge. In the current study, it was observed that inclusion of SDPP in the starter diets of broiler chickens had positive effects on broiler performance in a conventional environment. Throughout the experimental period, there was an improvement in the body weight and FCR of broilers due to the consumption of SDPP at the starter phase while they were subjected to a pathogenic challenge. This response is a confirmation of observations in the previous two experiments on the supplement and has already been discussed.

The supplement has been tested under various disease and health conditions. Campbell *et al.* (2006a) found that dietary consumption of SDPP improved broiler performance during necrotic enteritis exposure. Henn *et al.* (2013) also reported that there was a positive effect of dietary SDPP on the growth performance of broilers reared under challenge conditions, particularly in the early stages of life. In pigs reared under unsanitary conditions, Zhao *et al.* (2007) observed a positive response to dietary SDPP in terms of growth performance.

The mortality rate in the current experiment was not a treatment-related issue. The improvement in growth response of broilers under disease challenge may be attributed to the immunological properties of SDPP. An immunologically active compound promotes the functions the immune system and may enhance resistance to diseases (Doyle *et al.*, 2001). Immunoactive compounds including immunoglobulins, albumin, growth factors, and biologically active peptides have all found in SDPP (Borg *et al.*, 2002). The

antimicrobial or immunomodulatory properties of these proteins are more efficient during exposure to environmental or immunological challenges. Thus SDPP may have the capability to reduce overstimulation of the immune system of broilers in such high-pathogen conditions, to maintain or promote the growth performance of birds. When the immune system is stimulated, metabolism is altered (Johnson, 1997; Klasing & Korver, 1997; Kelley, 2004) leading to a reduction in growth performance and protein accretion (Klasing & Austic, 1984; Kent *et al.*, 1996; Pérez-Bosque *et al.*, 2004). The nature of immune response to the disease is further discussed at section 6.5.5.

6.5.2 Organ weight

Feeding SDPP to the broilers under challenge conditions had a positive influence on the relative weight of immune-associated organs. The supplement improved the relative weight of bursa up to 13 d and further improved the weight of the immune-related organs (spleen, bursa and thymus) during and after disease challenge. These results are in agreement with the findings of Campbell (2004) who reported that bursa weight was enhanced in SDPP-fed broilers when compared to control birds. This could indicate the development of immunocompetence to counter disease agent(Campbell *et al.*, 2006a). However, King *et al.* (2008) found no significant effect of porcine and bovine plasma proteins on the weight of liver and spleen of weaned pigs.

6.5.3 Haematological parameters

The haematological parameters of broiler chickens were not affected by the treatments. Inclusion of SDPP in the starter diets had no significant effect on the blood pH, PCV, Hb, glucose and galactose of the chicks at to 13 or 21 d. This is in agreement with the results

of Jamroz et al. (2011; 2012) who stated that there were no significant changes in PCV and Hb content of broiler chickens fed SDPP contained diets. Similar results have been repoted by (Donkoh *et al.*, 1999) who incorporated sun-dried blood meal into the broiler chicken diets.

6.5.4 Blood electrolytes

In this study, feeding SDPP to broilers had some influence on blood electrolytes. Blood potassium content was reduced when measured in the birds that received the low level (10 g/kg) of SDP to 13 d of age, compared to other groups. Blood sodium content was higher in the birds fed on starter diet containing the high level (20 g/kg) of SDPP. The concentration of these two minerals may reflect their levels in SDPP. The other blood electrolytes measured were not affected by the inclusion of SDPP. There are no reports in literature describing the impact of SDPP feeding on blood electrolytes of broiler chickens.

6.5.5 Immune response and serum biochemistry

In the current study, no significant changes in serum immunoglobulin contents were detected due to SDPP inclusion in starter diets of broiler chickens which were then exposed to disease challenge. However, the concentrations of immunoglobulins IgA, IgG, and IgM were slightly lower in the challenged birds that consumed SDPP-supplemented starter diets compared to the challenged control groups. The current results may support the idea that immunoglobulin-containing compounds such as SDPP can reduce the overstimulation of the immune system and overall inflammatory responses of animals. Spray-dried porcine plasma has been used in weanling piglets as a source of IgG to modulate innate immune system activation through its effect on the expression of pro

inflammatory cytokines (Touchette *et al.*, 2002). The supplement has been shown to reduce the overstimulation of the immune response in animals, thereby conserving nutrient utilization for supporting the immune response and allowing nutrients to be utilized for productive purposes (Campbell, 2011b). The results of the current study are in line with the findings of Jamroz *et al.* (2011) who observed no significant effect of SDPP incorporation in the broiler diets on serum IgG concentration. Jamroz *et al.* (2012) also reported a minor reduction in the concentration of IgG as a result of SDPP inclusion in the starter diets of broiler chickens.

Incorporation of SDPP to the starter diets of broiler chickens challenged with *S. sofia*, had no significant effect on the serum biochemical content. The results were in line with those of Jamroz *et al.* (2012) who found no differences in the serum total protein content due to SDPP inclusion in the broiler diets.

6.5.6 Concentration of SCFA of ileal and caecal content

Although there were no major changes in concentrations of SCFA as a result of SDPP supplementation, the product may contain antibodies which could be active against some bacterial pathogens (Rump *et al.*, 1992; Owusu-Asiedu *et al.*, 2002). In studies on various animal species (swine and poultry), spray-dried animal plasma feeding reduced the mortality and morbidity of animals when they were challenged with pathogenic bacteria such as *E. coli*, *Salmonella* and *Pasteurella multocida* (Borg *et al.*, 1999; Campbell *et al.*, 2004b). Their results suggest that SDPP reduces attachment, adhesion, and replication of the organism (antigen–antibody interactions). Moreover, SDPP prevents growth retardation and clinical signs of disease in piglets orally challenged with different strains

of pathogenic *E. coli* (Van Dijk *et al.*, 2002; Bosi *et al.*, 2004; Yi *et al.*, 2005; Torrallardona *et al.*, 2007), a phenomenon which is, however, inconstantly associated with decreased circulation or excretion of pathogenic strains. This supports the theory of direct competition between SDPP and the intestinal receptors for pathogenic *E. coli*, more than a direct antimicrobial effect (Bosi *et al.*, 2004). It was not possible to analyse the obtained results due to the unavailability of such results in literature on poultry.

6.6 CONCLUSION

Incorporation of SDPP into the starter diets did reduce the concentration of serum immunoglobulins to some extent, which could be due to the presence of biologically active proteins in SDPP. This response could reduce the overstimulation of the immune system in the host animal and maintain body growth and health of the birds. The development of the immune organs also enabled the birds to withstand the disease challenge. The other variables were not as conclusive in explaining the mechanisms of the test product. The number of publications on the physiological effects of this feed supplement in poultry is minimal and the results are unclear. There is a need for further investigations into the use of SDPP in the starter diets for poultry.

CHAPTER 7 General Discussion, Conclusion and Recommendations

7.1 INTRODUCTION

There is intense interest in developing management and feeding schemes that stimulate gastrointestinal development and welfare in newly hatched chicks. This is aimed at improving growth and feed efficiency while minimizing the use of antibiotics. Great effort has been made to introduce high quality/purified protein ingredients into poultry diets. However, much more knowledge about the variation in their composition and nutritional value is needed. Available data on the physiological effects of high-quality protein products, particularly blood products, in poultry is small and the results are sometimes conflicting. In contrast to the relatively well-documented activity of blood by-products in piglets and weaner pigs (Nofrarias et al. 2006, Radomyski 2009), the results of investigations conducted on poultry are very scarce and do not give any clear information about the efficacy of the use of blood products in poultry diets (Mazurkiewicz et al., 1990).

Four experiments were conducted in the present study to assess the influence of early feeding of two high quality protein products of both animal and plant origins, including spray-dried porcine plasma and processed soy protein on early intestinal and immune development, survivability, and performance of broiler chickens.

7.2 TEST PROTEIN SOURCES

In this study, high-quality protein was from two different sources. Processed soy product was tested in one experiment (Chapter 3) while spray-dried porcine plasma was used in three other experiments (Chapters 4, 5 and 6). The two protein products were used separately because of the variation in their chemical composition and the large difference in their inclusion rates in the chicken diets. It should be noted that the main purpose of this study was to assess the responses of broiler chickens to the high quality protein products; therefore, the comparison between these two protein products was not considered. The most interesting results from this study were that both protein products had fairly similar effects on broiler chickens and they were both slightly more efficient with wheat-based diets.

7.3 GROSS PERFORMANCE

The inclusion of processed plant proteins (Chapter 3) and spray-dried porcine plasma (Chapter 4, 5, & 6) to the broiler starter diets did not increase feed intake at any point during the experimental period. The lack of increased feed intake may indicate that the products do not stimulate appetite. Indeed, the bioactive compounds in spray-dried plasma products (which enhance well-being and promote appetite), and the elimination of anti-nutritional factors from the processed soy product may be of less importance than the benefit of high digestibility of these proteins. It has been suggested that spray-dried plasma and similar products are more palatable than other raw materials; however, the findings in the current study support those of Torrallardona (2010) who concluded that the influence of spray-dried plasma products is not a palatability-mediated effect.

The present study demonstrates that chickens' performance, including LW and FCR, is supported by the inclusion of high-quality protein products in their starter diets. Offering high-quality proteins promotes superior 10-d performance, which is maintained throughout the production cycle of the broiler chickens. This study clearly shows that inclusion of processed plant and or blood products into poultry diets has the potential to further improve the productivity of broiler chickens. Inclusion of processed soy products improves the growth performance of broilers chickens (Chapter 3). This is in line with the results of trials on the same product conducted in Belgium and USA (van der Eijk, 2013). Jiang *et al.* (2006) also found that substitution of HP300 for SBM resulted in better growth performance of broiler chickens. This could be due to further purification of the highly digestible protein product and the reduction or elimination of poorly digestible ingredient and antinutritional factors (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.

Animal or blood byproducts are characterized by their high content of good quality protein and energy and reasonable essential amino acids profile. They are also free of crude fibre and other antinutritional factors (Konwar & Barman, 2005). Efficient processing methods in the form of spray drying have considerably improved the nutritional quality of blood by-products and improved the response obtained by feeding it to the animals. In this study, broiler growth performance was promoted by the inclusion of spray-dried porcine plasma in the starter diets (Chapters 4, 5 & 6). The improved performance may be due to the product being a high quality protein source with a good

amino acid profile that can support gut development and rapid muscle growth. The porcine blood products obtained by the modified spray-drying method can be treated and used as a potentially valuable source of proteins, amino acids, microelements, and some biologically active substances for non-ruminant animals (Jamroz *et al.*, 2011). The results of the present study support those of Campbell *et al.* (2003; 2004a) and Bregendahl *et al.* (2005b) who found that rate and efficiency of growth of broiler chickens were improved when SDPP was included in their feed.

7.4 MEAT YIELD

Inclusion of PSP in the starter diets of broiler chickens to some extent improved the meat yield and carcass parts weight, especially breast and thigh weights (Chapter 3). These results are in agreement with the findings of van der Eijk (2013), who reported that the inclusion of PSP at a level of 5% in broiler starter diet for 7 d improved carcass yield and breast weight. The present study also showed that SDPP inclusion in the starter diets could improve dressing percentage (Chapter 4).

This improvement may be due to the better performance of PSP- and SDPP-supplemented groups, which may result in better protein deposition in muscle tissue than occurs in other groups. It may also be related to the heavier body weight of the PSP- and SDPP-supplemented groups. There is a positive relationship between live body weight and carcass yield and its parts weight.

7.5 DEVELOPMENT OF IMMUNITY

Nutrition has a considerable effect on animal health and immunity, and it is a tool that can be exploited to advance the immune and digestive maturity of young birds. Introducing nutraceuticals to the broiler diets could be supportive from both nutritional and medical perspectives.

In this thesis, inclusion of SDPP to the starter diets of broiler chickens positively influenced the growth and development of immune-associated organs. Dietary supplementation of SDPP to starter diets improved the weight of the spleen, bursa and thymus of broiler chickens challenged a salmonella antigen (Chapter 6). The findings support those of Campbell (2004), who found that bursa weight was significantly heavier in SDPP-fed broilers than in the control. This could indicate the ability of SDPP to stimulate immune competence (Campbell *et al.*, 2006a). In contrast, King *et al.* (2008) found that feeding porcine and bovine plasma proteins had no significant effect on the weights of the liver and spleen of weaned pigs.

In the past, immunoglobulins were quantified by radial immuno-diffusion. Currently, enzyme-linked immuno-sorbent assay (ELISA) is the preferred method due to its high sensitivity, accuracy, and reproducibility (Swamy *et al.*, 2002). In the present study, a sandwich ELISA was developed to quantify serum immunoglobulins. In this study we found that there was a slight decrease in the concentration of serum immunoglobulins (IgA, IgG, and IgM) in the disease-challenged birds that consumed SDPP-supplemented starter diets compared to the challenged control groups (Chapter 6). The change in immunoglobulin concentrations is driven in part by the growth rate of birds. As the

growth rate and blood volume increase, the relative concentration of immunoglobulins are reduced (Curtis & Bourne, 1973). This may support the idea that immune-active substances of SDPP could exert their defensive action and prevent the overstimulation of the immune system and overall inflammatory responses of the animal. Supplementing SDPP as a source of immunoglobulin-G to weanling pigs under nonpathogenic or subclinical conditions appears to modulate activation of innate immune system because supplementation with SDPP reduces expression of pro-inflammatory cytokines (Touchette *et al.*, 2002). Spray-dried plasma reduces the overstimulation of the immune response in animals, thereby conserving nutrient utilization for supporting immune response and allowing nutrients to be utilized for productive purposes (Campbell, 2011b). The results of this research are in line with the findings of Jamroz *et al.* (2012), who reported an insignificant decrease in the concentration of IgG as a result of SDPP inclusion in the starter diets of broiler chickens.

The improved growth performance throughout the experimental period together with the improved growth of the immune-related organs and slight decrease in the concentration of immunoglobulins in chickens offered diets containing SDPP suggests that the chickens have a larger pool of nutrients available for growth (hence less energy expenditure by the immune system) and superior immune competence when challenged with disease. The results of the present study suggest that early feeding of SDPP can influence the long-term immune competence of chicks. It is logical to expect that the benefits of dietary supplementation of SDPP, which is rich in functional bioactive compounds, aids the growth of the immune system of young birds, to promote the immune competence. This effect could remain over an extended period, long after the supplement has been

withdrawn. It could be hypothesized that this may occur by reducing the susceptibility of chickens to immunological challenge in the grower-finisher phase. Providing dietary SDPP to chickens for a longer period may provide even greater immune responsiveness in grower and finisher phases, and warrants further investigation.

7.6 GASTROINTESTINAL DEVELOPMENT AND PHYSIOLOGY

7.6.1 Nutrient digestibility

Although non-significant, the results of the present study demonstrate that the digestibility of nutrients is improved to some extent by the inclusion of high-quality protein in the starter diets of broiler chickens. Chicks offered PSP showed higher protein, gross energy and dry matter digestibility (Chapter 3). The findings support those reported by van der Eijk (2013) that ileal nutrient digestibility improves in birds on diets containing PSP compared to birds on diets without the supplement. The supplement is able to improve energy and amino acid availability due to the reduction in antinutritive factors, such as trypsin inhibitors and oligosaccharides, present in SBM (Batal & Parsons, 2002, 2003). Trypsin inhibitors reduce the activities of trypsin and chymotrypsin, thus reducing the digestibility of dietary protein (Gallaher & Schneeman, 1986).

In the present study, the apparent ileal digestibility of nutrients was affected by the interaction between the SDPP level and feeding duration (Chapter 5). Chicks offered SDPP had improved ileal digestibility of protein, gross energy and dry matter.

It should be noted that PSP and SDPP were offered only in the starter phase (1-10 ds), while nutrient digestibility was measured at 24 d of age. This could be the reason for the

lack of effect on nutrient digestibility. However when chicks have good initial growth performance, the improved metabolic efficiencies could persist along with nutrient utilization in subsequent stages of life.

7.6.2 Mineral digestibility

In this study, the apparent ileal digestibility of minerals was positively affected by SDPP feeding duration and level (Chapter 5). In general, it can be said that the mineral digestibly was improved due to SDPP inclusion in the starter diets of broiler chickens and the improvement continued and extended through the subsequent stages of the broiler production cycle. This may have positive effects on the structure and development of bones. In addition, as its well-known trace minerals have vital roles in the biological and physiological activities inside the body. However, available data which analysed the relation between mineral composition of spray-dried plasma and its possible impacts on bone quality was not found in the available literature. This improvement in the mineral digestibility could be due to the high mineral content of blood products, which can positively improve the digestion process of these elements in whole diets. Blood by-products, especially plasma, can be processed and treated as a potentially beneficial source of proteins, amino acids, and microelements for non-ruminant animals (Jamroz *et al.*, 2011). This is partly in line with the finding of Jamroz *et al.* (2011), who observed an improvement in the digestibility of Ca and Cu due to SDPP supplementation of broiler diets.

7.6.3 Digestive enzyme activities

In this study, the application of high-quality protein products to the starter diets increased the activities of certain digestive enzymes in both the jejunum and pancreas (Chapters 3, 4 & 5). There are no reports in the literature on the effects of SDPP or similar products on basic digestive function. However, the presence of purified, highly digestible products such as SDPP and PSP would stimulate digestive enzyme activity. These responses would be complemented by those of rapid growth and increased development of the intestinal mucosa–longer villi and deeper crypts were observed in this study (Chapters 3, 4 & 5). There was an increase in the surface area of villi, which could improve nutrient absorption and utilization, and was reflected in rapid growth rate and body weight, which in turn, positively affected the normal development of enzyme-producing organs. The activity of pancreatic (Sklan & Noy, 2000) and intestinal mucosal (Uni *et al.*, 1999) enzymes are well correlated to the body weight of birds. The activity 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 activity 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 and concentrations of antinutritional factors such as soluble NSP. The differences in composition of basal diets may determine how SDPP and PSP functions although there does not appear to be much variation between the maize- and wheat-based diets tested in this study.

7.6.4 Concentration of SCFA in digesta contents

In this study, incorporation of spray-dried plasma to the starter diets had positive effect on the ileal SCFA profile of boiler chickens reared under disease challenge (Chapter 6). In the ileum, the concentrations of lactic and succinic acids increased in birds that consumed SDPP-supplemented diets. The effect of SDPP feeding under disease challenge on the concentration of SCFAs in the caecal content was variable for different acids, and the results at different ages were inconsistent. Changes in the SCFA profile could be due to the immunoglobulins that are present in animal blood by-products and which may contain some antibodies active against some bacterial species (Rump *et al.*, 1992; Owusu-Asiedu *et al.*, 2002), although the antibacterial action of spray-dried plasma has not been confirmed. In studies on various animal species (swine and poultry), spray-dried animal plasma feeding reduced the mortality and morbidity of animals when they were challenged with pathogenic bacteria (*E. coli*, *Salmonella*, *Pasteurella multocida*) (Borg *et al.*, 1999; Campbell *et al.*, 2004b). These results suggest that SDPP may reduce microbial populations, possibly by reducing attachment, adhesion, and replication of the organisms (antigen–antibody interactions).

7.7 GENERAL CONCLUSION AND RECOMMENDATIONS

Understanding the response of chickens to high-quality, processed protein products is essential to maximizing their use in diets for young birds'. The present study highlights the benefits of using both processed soy protein and spray-dried porcine plasma. From a nutritional perspective, these two products are different in their composition and source as well as in their inclusion rate in young chick diets; however, the present study shows that

their role in aiding growth performance, digestive and intestinal development is very similar.

The results reported in this thesis show that early feeding of high-quality, processed protein products can influence the growth performance, intestinal development and physiology, immune competence, and survivability at an early age and throughout the broiler production cycle. Early feeding of SDPP has important nutritional and non-nutritional roles in young developing chicks. The multiple protective properties of spray-dried porcine plasma present an exciting tool for poultry producers to improve boiler productivity.

Future research should consider combining high-quality, processed protein of both animal and plant origins to exploit both the nutritional and non-nutritional effects of these products, particularly SDPP. This may provide further benefits to the chicks at high risk of physiological and immunological challenge, and enhance the immune and digestive development of the young chicks.

Using these protein products throughout the broiler production cycle needs to be investigated. There is also a need to determine the optimal inclusion levels of these products in diets of starter and subsequent grower and finisher phases of broiler production.

More investigation into gut microflora and gastrointestinal immunity of broiler chickens on similar diets to those used in this study would be worthwhile. It would also be worthwhile to investigate the use of these products in layer diets to ascertain whether they have any effect on egg production.

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