APPLICATION OF EGG BY-PRODUCTS AS HIGH QUALITY PROTEIN, ENERGY AND BACTERICIDAL SUPPLEMENTS IN POULTRY AND SWINE NUTRITION.

A Thesis

Submitted to the Faculty

Of Graduate Studies

The University of Manitoba

by

Lisa D. Schmidt

In Partial Fulfillment of the

Requirements for the Degree

of

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FACULTY OF GRADUATE STUDIES

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Abstract

Egg-breaking facilities produce substantial quantities of technical albumen or whole egg by-products each year that are unsuitable for human consumption. Despite their excellent amino acid profile, energy content and the presence of anti-bacterial proteins (i.e., lysozyme), they have received little attention as animal feedstuffs. The objectives of the current study were (1) to determine the nutritive value of technical albumen and whole egg by-products, and (2) to investigate the effect of heat-treatment and gamma-radiation on the nutritive value of egg by-products and on induction of bactericidal activity of lysozyme against pathogenic bacteria. *In vitro* studies documented that the protein digestibility could be optimised through thermal treatment (moist heat) over the spray-drying process (from 50 to 80%). However, hot-room storage (dry heat) did not improve protein digestibility to the level observed in earlier experiments. In addition, *in vitro* studies have indicated the potential to induce the novel antimicrobial activity of lysozyme against both *Escherichia coli 0157:H7* and *Salmonella typhimurium266* by means of heat treatment and γ-radiation. In this study, survival rate of *E. coli* and *S. typhimurium*, were lowered from 87.3% and 80.9% when incubated with native lysozyme to 0.1 and 11.3% when incubated with thermally modified lysozyme (heated at 80°C for 20 min), respectively. Due to the promising results regarding the heat treatment of lysozyme, technical albumen was also thermally treated. No suppression in the growth of *E. coli* or *S. typhimurium* was noted. Gamma radiation was investigated as an alternative treatment to induce the novel activity of lysozyme. Radiation studies were conducted on both pure lysozyme and technical albumen. In the case of pure lysozyme the survival rate decreased to 11.4% and 16.6% for *E. coli S. typhimurium*, respectively when treated at 1.4 kGy. Antimicrobial activity of radiated (1.8

kGy) technical albumen reduced the survival rate of *E. coli* by 42.3%. To determine the nutritive value of egg by-products, seven *in vivo* animal trials were conducted. These studies included a rat trial, three early-weaned pig trials, two broiler chicken trials and the true metabolizable energy (TME_n) assay. In the rat trial, animals fed diets containing casein or spray-dried technical albumen (SDTA) performed better than animals fed diets containing spray-dried whole egg (SDWE). Furthermore, there was a negative effect of hot room storage on protein utilisation and growth of rats. Analysis of ileal *Enterobacteriaceae* levels showed that heat treatment (80°C, 20 min) or γ-radiation on SDTA showed some suppression of the gram-negative bacteria when compared to raw technical albumen.

The potential for spray-dried egg proteins to replace spray-dried porcine plasma (SDPP) in early-weaned pig diets was investigated in two 3-week performance trials. In the first experiment, average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) were lower ($P<0.05$) for SDTA, SDTA stored in a hot room (70°C) for 3 days and SDWE relative to the SDPP diet. The SDTA diet had numerically better performance parameters than the other diets containing egg proteins. Thermal treatment of the SDTA (i.e., hot-room storage for 3 days) negatively affected the quality of the product as documented by higher FCR. However, the reduction in animal performance was not reflected in amino acid digestibility data and the metabolizable energy content of the diets. Hot-room storage of SDTA augmented the antimicrobial activity of lysozyme against *Enterobacteriaceae* and lowered the bacterial levels below that seen in the control (SDPP) diet. SDWE, on the other hand, produced a significant increase in ileal bacteria levels. In the second experiment, the inclusion rate of SDTA was examined in pig diets. It was found that SDTA can replace from 25 to 50% SDPP without compromising pig performance. In the third experiment the impact of SDTA on

phase II growth parameters was examined. Animals fed a 3:1 blend of SDPP to SDTA during phase I of the experiment had better FCR in phase II despite the removal of SDTA and SDPP from the diets ($P<0.05$). In broiler chickens, similar body weight gains and feed conversion ratios were observed for diets containing SDTA and SDWE. Overall, diets containing SDWE had the best FCR, which was similar to that of the fish meal control and the SDTA diets. As was the case with the rat and early-weaned pig trials, hot room storage of SDTA negatively affected the nutritive value of this product. This was further substantiated by reduced TME_n content and amino acid digestibilities of SDTA following hot-room storage. Based on the results of the first trial, a long-term production trial was conducted. Five replicates of 60 birds were fed one of four wheat-soy diets: a positive control containing fish meal and antibiotic (PC), a negative control with no antibiotic added (NC), NC+SDTA and NC+SDWE. Diets containing SDWE performed the best as substantiated by an increased AME over the PC diet. In comparison to the PC diet, a trend towards reduced population of gram-negative *Enterobacteriaceae* was observed for NC+SDTA.

Foreword

This thesis was written in manuscript, according to the guidelines of the Department of Animal Science, University of Manitoba. The manuscripts to be submitted for publication are:

MANUSCRIPT 1. Schmidt, L.D., Slominiski, B.A.., Boros, D. and Blank, G. The nutritive value of egg by-products and their potential bactericidal activity: *In vitro* and *in vivo* studies. J. Nutr. (to be submitted).

MANUSCRIPT 2. Schmidt, L.D., Slominski, B.A., Boros, D., Campbell, L.D., Guenter, W. and Blank, G. The application of egg by-products as valuable protein supplements in broiler chicken diets. Poult. Sci. (to be submitted).

MANUSCRIPT 3. Schmidt, L.D., Nyachoti, C.M., Boros, D. and Slominski, B.A. The potential for egg by-products to replace spray-dried porcine plasma in early-weaned piglet diets. J. Anim. Sci. (to be submitted).

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1. Literature review

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List of Abbreviations

AA – Amino acid(s)

ADFI – Average daily feed intake

ADG – Average daily gain

AGE – Advanced glycosylation end-products

AME – Apparent metabolizable energy

ANF – Antinutritive factor

BBP – Biotin binding protein

BHI – Brain-Heart Infused broth

BV – Biological value

CFU – Colony forming unit

DE – Digestible energy

FCR – Feed conversion ratio

FFI – Furoyl-furanyl imidazole

HLz10 – Heat-treated lysozyme (10 min; 80°C)

HLz20 – Heat-treated lysozyme (20 min; 80°C)

HLz30 – Heat-treated lysozyme (30 min; 80°C)

IIP – Illinois ideal protein

IL-1 – Interleukin-1

LPS – Lipopolysaccharide

MOS – manno-oligosaccharide

NAG – N-acetylglucosamine

NAM – N-acetylmuramic acid

NC – Negative control

NLz – Native lysozyme

NPU – Net protein utilization

PC – Positive control

PER – Protein efficiency ratio

PWD – Post-wean diarrhea

RPER – Relative protein efficiency ratio

RS – Resistant starch

RTA – Raw technical albumen

SAA – Sulfur amino acid(s)

SBM – Soybean meal

SD – Swine dysentery

SDBM – Spray-dried blood meal

SDPP – Spray-dried porcine plasma

SDTA – Spray-dried technical albumen

SDWE – Spray-dried whole egg

sNSP – Soluble non-starch polysaccharide

TA – Technical albumen

TIA – Trypsin inhibiting activity

$TME_{(n)}$ – True metabolizable energy (nitrogen corrected)

TSA – Tryptic soy agar

WE – Whole egg

WFIP – Wang and Fuller's ideal protein

1. Introduction

Six metric tonnes of technical grade egg albumen and whole egg by-products are produced every month at the Inovatech egg-breaking facility in Winnipeg. Technical albumen is recovered from egg shells through centrifugation and may contain egg yolk. Both technical albumen and whole egg by-products are not recommended for human consumption as regulated by the Canadian Food Inspection Agency, as they may have come into contact with non-sterile equipment. Therefore they have a limited application and, at the present time, are being disposed to the environment. Avian eggs are composed primarily of proteins, lipids and carbohydrates. In addition, egg albumen is a good source of vitamins, minerals, maternal antibodies (i.e., γ-livetin) and the most plentiful source of lysozyme.

The nutrients in eggs are not all completely available because native proteins tend to be resistant to digestion. Heating or other means of denaturation improves the availability of egg nutrients by facilitating proteolysis. In studies on the effect of feeding different levels of raw egg white powder to rats, as little as 20% egg white produced measurable signs of toxicity, such as a 50% increase in the amount of urine excreted and a high output of urinary protein. Toxicity was largely prevented by heat denaturation of the egg white powder (Stadelman and Cotterill, 1977).

Currently, spray-dried porcine plasma (SDPP), a by-product of blood obtained from pork slaughter plants, is used as an appetite enhancer to improve

feed intake and gain in young pigs. It is believed that globular proteins in the plasma (antibodies) impart passive immunity to early-weaned pigs. Another advantage of SDPP is the high level of lysine, tryptophan, and threonine relative to other protein sources. The limiting amino acids in SDPP are methionine and isoleucine. A disadvantage of using SDPP is its relatively high cost. In a growth trial with early-weaned pigs, Owen *et al.* (1993) found that spray-dried egg protein could replace up to 3% SDPP. However, pigs consuming a diet in which 6% egg protein was substituted for porcine plasma had poorer average daily gain and feed conversion ratios as spray-dried egg protein was substituted for 3 to 6% porcine plasma.

Lysozyme, found in avian eggs, is an enzyme that can lyse the cell wall of certain gram-positive bacteria by cleaving the β-1,4 bond between *N*-acetylmuramic acid (NAM) and *N*-acetylglucosamine (NAG) of the cell wall peptidoglycan. Until recently, it was believed that pathogenic gram-negative bacteria (*i.e., Escherichia coli, Salmonella*) were resistant to lysozyme action because they contain only a small amount (5 to 10%) of peptidoglycan in their cell walls. Heat treating pure lysozyme at 80°C for 20 minutes results in conformational changes that increases its antimicrobial activity against gram-negative bacteria, with no detrimental effect against gram-positive bacteria (Ibrahim *et al.* 1996a, b; Ibrahim 1998). In this context, the development of value-added and probiotic type egg by-products could be novel alternatives to antibiotics used to control various diseases or used as growth promotants in livestock and poultry production. Natural, low risk alternatives to antibiotics by

bactericidal products would meet the demands of the feed industry, which is currently seeking opportunities to move away from antibiotics.

To recommend spray-dried egg albumen and whole egg as valuable supplements in animal nutrition, it is necessary to improve the protein availability (amino acids), energy and bactericidal activity of these products. The alternative of using components of the diet to manipulate the resident microflora, such that it more effectively excludes pathogens, is an intriguing possibility for egg albumen use. Therefore, the major objectives of this study were 1) to investigate the effect of heat on digestible protein content and induction of the bactericidal activity of egg albumen lysozyme, and 2) to evaluate the beneficial effect of heat treatment on protein, amino acid and energy utilization by young pigs and chickens fed egg albumen and whole egg by-products.

2. Literature Review

2.1. Introduction

The egg market has two key factions: consumers and processors. At present, approximately 20% of all eggs produced in Canada are sold to processing facilities. However, in Manitoba greater proportions of eggs (up to 50%) are processed at egg-breaking facilities. Due to lower feed costs within the province, Manitoba egg-producers are among the most economic in the country. For this reason the largest Canadian egg processor is located in Winnipeg, MB. During the egg-breaking process there are various problems that occur to downgrade an egg product. Technical grade egg albumen and whole egg by-products are produced in substantial quantities by many egg breaking companies in North America. Due to a limited market for downgraded egg by-products, egg processors have no choice but to discard these products. At present, the Canadian Inovatech facility in Winnipeg, MB produces approximately six metric tonnes of egg by-products each month. These products are recovered during processing and for various reasons are deemed inedible for human consumption by the Canadian Food Inspection Agency. As the egg-processing industry continues to grow, producing more value-added products, so will the amount of downgraded egg products increase. Currently, technical-grade egg-products have limited uses and are discarded to the environment, despite being an excellent source of protein, lipids and antimicrobial agents.

2.2. Chemical Composition of Avian Eggs

Eggs are one of the most nutrient dense food sources. They are an excellent source of protein (Table 1) in addition to vitamins, minerals (Table 2), and antimicrobial compounds (Table 3). Several factors affect the composition of eggs including breed, strain, age and diet of the hens. However, one of the greatest factors affecting the composition of an egg is the age of the hen. Hens tend to produce smaller eggs at the start of their laying cycle, which tend to have greater proportions of yolk relative to egg albumen. The majority of the egg is composed of water, accounting for approximately 70 - 80 % of the weight (Table 1). Proteins (10 - 13%) and lipids (7 - 12 %) are the primary nutrients in the whole egg. On a dry matter basis, protein and lipids constitute 45 - 55% and 35 - 45% of the egg, respectively. Looking more closely at egg albumen, it is composed primarily of protein (>85% on a dry matter basis), whereas the yolk is approximately two-thirds lipids and one-third protein (Burley & Vadehra, 1989). Overall, the whole egg represents a high quality protein and energy source in either human or animal nutrition. Eggs are excellent sources of vitamins and minerals. They contain phosphorous and calcium, which must be properly balanced in animal diets, in addition to iron, magnesium and zinc, just to name a few. Furthermore, eggs contain a plethora of B vitamins, including B6 (pyridoxine) and B12. They also contain riboflavin, thiamin, niacin, biotin, and folic acid (folate) (Burley & Vadehra, 1989).

TABLE 1. Percent composition of the hen's egg as reported by different sources.

Nutrient	Manitoba Egg Producers (2000)	American Egg Board (1998)	Burley & Vadehra (1989)	Stadelman & Cotterill (1977)
Water	80.4	75.85	74.13	73.7
Protein	10.3	11.95	10.5	12.9
Total Lipid	7.67	10.2	11.77	11.5
Carbohydrate	1	1.05	0.8	0.72
Ash	ND[1]	0.95	0.99	1

[1]Not determined.

TABLE 2. Protein composition of chicken egg white (percent of total protein content)[1]

Protein	Composition (%)	Function
Ovalbumin	54.0	Possible enzyme inhibitor and/or metal binder
Ovotransferrin	12.0	Iron binder
Ovomucoid	11.0	Protease Inhibitor
Lysozyme	3.4	Enzyme: bactericide
Ovomucin	1.5	Mucilaginous virus inhibitor
Ovoinhibitor	1.5	Protease inhibitor
Ovoglycoprotein	1.0	
Ovoflavoprotein	0.8	Vitamin binder
Ovomacroglobulin	0.5	Protease Inhibitor
Avidin	0.06	Biotin binder
Cystatin	0.05	Protease inhibitor
Ovoglobulin G2	4.0	Foaming agent
Ovoglobulin G3	4.0	Foaming agent

[1]Modified from Burley and Vadehra, 1989

TABLE 3. Selected vitamin and mineral content of liquid whole eggs (per 100 grams).

Nutrient	American Egg Board (1998)	Burley & Vadehra (1989)	Manitoba Egg Producers (2000)	Stadelman & Cotterill (1977)
Vitamins				
Vitamin A (IU)	525	846	792	1180
Vitamin D (IU)	-	43	87	50
Vitamin E (IU)	-	1.17	2.6	2.00
Thiamin (mg)	0.06	0.10	0.063	0.11
Riboflavin (mg)	0.46	0.303	0.453	0.3
Niacin (mg)	0.08	0.10	1.83	0.1
Vitamin B_6 (mg)	0.16	0.21	0.13	0.26
Folacin (ug)	73	14.6	70	5
Vitamin B_{12} (ug)	1.07	0.7	2.7	0.28
Pantothenic Acid (mg)	1.48	1.9	1.5	1.62
Minerals (mg)				
Calcium	59	56	42	54
Phosphorous	202	218	133	200
Magnesium	11	7.1	13.3	11
Iron	1.85	2.23	1.5	2.3
Zinc	1.38	1.4	1.5	1.4

Of the 13 significant proteins found in egg albumen, ovalbumen is the primary protein (Table 2). As the largest constituent of albumen, ovalbumen is the most likely protein to undergo non-enzymatic browning or the Maillard reaction, reducing the protein quality (see section 2.3.3.1). In addition to ovalbumen, there are two other proteins of interest: avidin and lysozyme. In animals, avidin-biotin complexes inhibit essential carboxylations in the tricarboxylic acid cycle (Kreb's cycle), thus inhibiting the production of adenosine triphosphate (ATP) and acting as an antinutritive factor (Knowles, 1989). The third protein of interest, lysozyme, is a known antimicrobial agent commercially isolated from egg albumen. In its native state it is effective primarily against gram-positive bacteria. However, it can be modified to significantly affect the growth of gram-negative bacteria (see section 2.7.3.4). Ovalbumen, avidin and lysozyme prevent bacterial infections in the developing chicken egg. However, in nutrition, these proteins pose an interesting dilemma: how to minimize the antinutritive effect of ovalbumen and avidin, while optimizing the antimicrobial effects of lysozyme.

2.3. Protein

2.3.1. Amino acid availability

The amino acid (AA) content and balance of proteins is indicative of overall protein quality. Various methods have been developed to better correlate

AA availability of proteins to an animal's AA requirement (McDonough *et al.*, 1990).

One *in vitro* assay developed by McDonough *et al.* (1990), uses three enzymes: trypsin, chymotrypsin and an intestinal peptidase. Maintaining a static pH, protein digestibility is then coupled to the amount of sodium hydroxide required to titrate the digestion solution. Although this method is reasonably accurate for most products, it tends to under-estimate the digestibility of egg protein due to the presence of protease inhibitors in the egg (McDonough *et al.*, 1990). In general, *in vitro* assays tend to under-estimate the protein digestibility of animal proteins, although they appear to correlate well with plant proteins.

Regardless of AA availability, a diet deficient or improperly balanced with respect to one AA will produce poor growth performance through reduced feed intake (Baker and Chung, 1992). As non-traditional protein feedstuffs become available, nutritionists will need to understand how the AA profile of novel feed constituents differs from a reference protein (Emmert and Baker, 1997). The ideal protein concept serves as a reference for research and practical diet formulation, relating AA requirements to that of lysine. The ideal protein concept uses lysine as a reference primarily because it is usually the first limiting AA in diet formulations (Chung and Baker, 1992c; Emmert and Baker, 1997). Further, protein accretion and maintenance represents the only need for lysine (Chung and Baker, 1992c; Emmert and Baker, 1997). In comparison, sulfur AA (SAA) often lost from the integument and epidermal structures, are obligatorily

precursors for other essential body metabolites (e.g. creatine, taurine, glutathione, catecholamines, carnitine) and experience obligatory oxidation (Chung and Baker, 1992c). In addition, lysine requirements have been extensively studied, firmly establishing levels for various dietary, environmental and body composition parameters. Also, lysine analysis in feeds is relatively simple and accurate (especially compared to SAA and tryptophan) (Chung and Baker, 1992c; Emmert and Baker, 1997). Finally, it is unlikely that any indispensable AA would be required in a concentration greater than lysine (Chung and Baker, 1992c).

There are several factors that must be considered in determining the ideal protein profile (IPP). The IPP can not be determined from the carcass AA composition. AA requirements based solely on carcass content would over estimate the requirement of slowly turned over AA and underestimate the required amounts of AA turned over at faster rates (Chung and Baker, 1992c). The turn over rates for individual AA must be considered to interpret the relationship between whole-body AA concentration and dietary AA requirements (Baker and Chung, 1992). For example, lysine is retained in tissues and thus has a slower turnover rate than either methionine or threonine. Diets devoid of AA with greater turnover rates, such as methionine, threonine or isoleucine, cause greater protein losses than diets devoid of lysine (Baker and Chung, 1992; Chung and Baker, 1992c).

There are several other factors that must be considered in determining the ideal protein values such as the ability of some AAs to act as precursors for essential AAs. Cystine, for example, can supply up to 50% of the requirement for SAA while tyrosine can supply up to 50 % of the phenylalanine requirement. Further glutamic acid is a more effective precursor for dispensable AA than are indispensable AA (Baker and Chung, 1992). Another factor that must be considered in diet formulation is the bioavailability of an AA. When supplementing diets, synthetic AA may contain the D-stereoisomer, which may have little biological activity (Baker and Chung, 1992). In addition to D-stereisomers, Maillard bound proteins, such as lysinoalanine and lanthionine, have little, if any, AA bioactivity (Baker and Chung, 1992).

Considering the AA requirements of monogastric animals, researchers must be aware of three distinct differences between poultry and swine. First, arginine is not synthesized by poultry. Second, SAA requirements of young chicks are higher while the tryptophan requirement is lower for poultry than swine. Finally, small quantities of glycine and proline are necessary in poultry diets (Baker and Chung, 1992). Despite the above difference there are two key unifying factors when using the ideal protein concept. First, the ideal protein should be equally applicable to animals of all ages. Second, high-energy diets necessitate higher AA concentrations. However, the energy to protein ratio required for optimal growth is still uncertain.

In poultry, the ideal protein concept has been used to address differences between AA requirements for different environmental, dietary and genetic factors. Emmert and Baker (1997) showed that the methionine + cystine, threonine, tryptophan, valine, arginine and isoleucine levels should be 75, 65, 18, 110 and 70%, respectively on a digestible basis, relative to the lysine content of the diet. Table 4 shows the ideal protein levels recommended by other research groups given differing environments, dietary and genetic factors. As with poultry, pigs have different AA requirements with different genetic potential (Tesseraud *et al.*, 1999; Baker and Chung, 1992). In addition, as the pig matures, the amount of essential AA increase relative to the lysine requirement (de Lange and Baidoo, 1997a). Table 5 gives three ideal protein models, the Illinois ideal protein (IIP), Wang and Fuller's ideal protein (WFIP) and the NRC requirements (1988). The NRC ideal protein failed to produce results comparable to the other ideal protein profiles, most likely due to lower values for leucine, valine, threonine, tryptophan, histidine and SAA (Chung and Baker, 1992c). The 1998 NRC values more closely approximate the values of the IPP or WFIP and therefore have not been included in Table 5.

Overall, the ideal protein concept allows researchers and industry to more specifically meet the nutrient requirements of young and growing animals. The

TABLE 4. Ideal Amino Acid profile for broiler chickens in different environmental conditions or ages

Amino Acid	Baker[1] (1996)	NRC (1994)	Austic[1] (1994)	Baker and Chung (1992)	
				0-21d	21-49d
Lysine	100	100	100	100	100
Methionine	36	45	38	ND[2]	ND
Methionine + Cystine	72	82	72	72	75
Threonine	67	73	62	67	73
Arginine	105	114	96	105	105
Valine	77	82	69	77	77
Isoleucine	67	73	65	67	67
Leucine	109	109	92	111	111
Tryptophan	16	18	18	16	17
Histidine	32	32	24	37	37
Phe+Tyr	ND	ND	ND	105	105

[1]As cited by Schutte, 1999; [2]ND, no data available.

TABLE 5. Comparison of Illinois Ideal Protein (IIP), Wang and Fuller's Ideal Protein (WFIP) and the NRC protein requirements, relative to lysine content for pigs (10-20kg) (modified from Baker and Chung, 1992)

Amino Acid	IIP	WFIP	NRC (1988)
Lysine	100	100	100
Arginine	42	42	42
Histidine	32	32	26
Tryptophan	18	18	15
Isoleucine	60	60	56
Leucine	100	110	74
Valine	68	75	59
Phenylalanine + Tyrosine	95	120	81
Methionine + Cystine	60	63	52
Threonine	65	72	59

more closely an animal's ideal AA requirements are met, the greater the nitrogen retention and the lower the environmental pollution. For example, pigs fed a 14.8% protein diet had 68% nitrogen-retention compared to 56% for animals fed a 20% protein corn-soybean meal-whey diet (Baker and Chung, 1992).

2.3.2. Optimizing protein digestibility

Several techniques have been utilized to optimize protein digestibility. The most popular is thermal denaturation. Heat treatment destabilizes both tertiary and quaternary protein structures, allowing for increased hydrolysis by proteolytic enzymes. In addition, heat denaturation has been used to destroy protease inhibitors, the most common example being the heat treatment of soybean meal to destroy trypsin inhibitors (Herkelman *et al.*, 1993). Although heating proteins may be advantageous, overheating may affect protein quality. Two common heat treatments used by the egg breaking industry include spray drying and dry heat.

2.3.2.1. Spray-drying. Spray drying is one of the best methods used to dry eggs, second only to freeze drying. However, due to high costs, freeze drying is only practical for research (Guardiola *et al.*, 1997). Although the drying chamber may reach several hundred degrees Celsius, evaporative cooling prevents heat degeneration even at comparatively high drying temperatures, as the temperature of the final product is only 65-70°C (Hayashi, 1989). Dehydration of thermosensitive materials, such as egg products can be optimized through several parameters including the distributor-atomizer distance, ensuring uniform

air flow (free stream of turbulence generated by the mesh) and using drying temperatures of 160 to 200°C and 65 to 70°C for the inlet and outlet temperatures, respectively (Kuts and Samsonyuk, 1989). If inferior drying processes are used protein denaturation, fat oxidation, destruction of vitamins, browning by aminocarbonyls and "off" flavors may occur. Kats *et al.* (1994b) noted that pig performance improved by feeding spray-dried blood meals compared to flash dried blood meals. They further speculated that improved pig performance was due to the more consistent, higher-quality protein source obtained through spray drying. Other researchers have noted that direct vs. indirect fired spray-dryers can significantly affect the protein quality due to oxysterol and Maillard product formation (Guardiola *et al.*, 1997). Both oxysterols and Maillard products are dependent on the inlet and outlet temperatures as well as the length of time the products are in the spray dryer. In addition, essential fatty acids can undergo oxidation further reducing the nutritive quality of the egg products. Although spray-drying has many advantages, it requires great attention to detail to limit oxysterol or Maillard product formation and to limit oxidation of essential fatty acids (Guardiola *et al.*, 1997).

2.3.2.2. Dry heat. The importance of heating egg proteins is two fold: first to reduce bacterial counts and second to denature proteins. To produce *Salmonella* free egg products, heating coupled with strict sanitation is the most commercially acceptable method. Whole egg or egg yolk can be heat pasteurized at 60-62°C for 3.5 to 4 minutes as a liquid. However, egg white

must first be stabilized or it may experience severe protein denaturation and loss of functional properties (Cunningham *et al.*, 1965). An alternative pasteurization for egg whites is dry heating following spray drying through hot-room storage at approximately 80°C (Kato *et al.*, 1990). Northolt *et al.* (1978) found that hot room storage for two weeks at 49°C was sufficient to reduce bacterial counts by 6 to 7 log units. In addition to reducing bacterial populations, dry heating induces several structural changes including protein denaturation by increasing the flexibility of the protein and reducing protein stability. Kato *et al.* (1990) found that dry heating egg proteins for 5 days increased the protein digestibility. However, the pH of whole egg is such that lysozyme, ovomucoid and ovalbumen are more heat stable (Cunningham *et al.*, 1965). Overall, heat denaturation of raw egg white powder can decrease the toxicity of raw egg albumen through reduction of bacterial counts and protease inhibitors (Peters, 1967).

2.3.3. Antinutritive proteins

The impact of antinutritive proteins has been studied for several years, most notably, their impact on feed intake (de Lange and Baidoo, 1997a). Several antinutritive proteins are present in eggs including Maillard products, avidin and protease inhibitors.

2.3.3.1. Maillard reaction. The Maillard reaction is common in mixed polymers, including eggs. The Maillard reaction, sometimes called the browning reaction, occurs when reducing sugars (glucose) and the amino group of certain AAs form covalent bonds. The new bonds are not susceptible to protease or carboxylase

digestion. The Maillard reaction affects AA availability, food characteristics and may also have physiological effects (Friedman 1996a, b).

The Maillard reaction is classified as a non-enzymatic, addition-elimination reaction of amines with reducing sugars. The Maillard reaction can be broken into two stages: the formation of Amadori compounds and their subsequent rearrangement to form advanced glycosylation end products (AGEs). There are two steps in the formation of Amadori products. First, a protonated amine group of a lysine residue electrophilically attacks the slightly positive carbonyl carbon of a reducing sugar. The rate of the addition is greatly affected by the bulk of the reducing sugar. For this reason pentoses have a greater reaction rate than hexoses followed by disaccharides (Buttner *et. al*, 1996). By far the slowest addition reaction between an amino group and a reducing sugar is in the presence of large polysaccharides such as starch or cellulose (Friedman, 1996a). Then the intermediate or addition compound rearranges. The nitrogen loses a proton while the oxygen gains a proton. The protonation of oxygen and subsequent loss of water is pH dependent. If the pH is too acidic, the addition reaction would be slowed down and the rate limiting elimination would be sped up. If the pH is too alkaline, the elimination reaction would be slowed significantly. The Schiff base is formed with the loss of another proton (H^+). The Schiff base can further undergo Amadori rearrangement (Fig. 1). Amadori products are the result of mild heating and in certain conditions can act as lysine source (Morales & van Boekel, 1996). However, once Amadori compounds

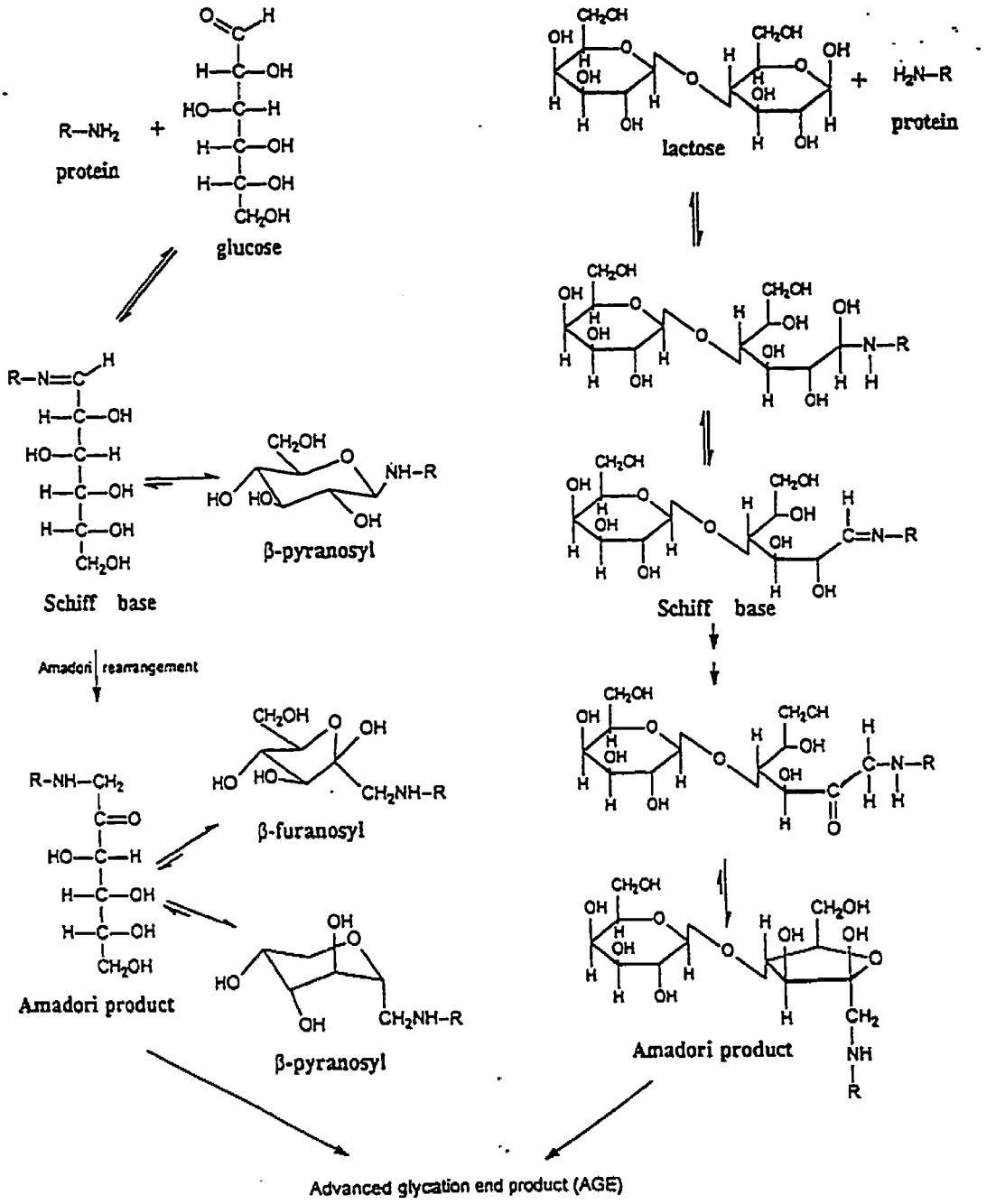


Fig. 1. Formation of Amadori products in the first stage of the Maillard reaction (modified from Friedman, 1996a).

undergo subsequent reactions to form advanced glycosylation end products, the reaction is irreversible. Some of the reactions the Amadori products can undergo include enolization, dehydration, aldol condensation as well as the Strecker reaction (Fig.2). The end result of any of these reactions is a cross-linked polymer, with an increased resistance to digestion.

One of the most obvious results of the Maillard reaction is the effect on food. Most foods damaged by heat start to show a typical brown colouration. For example, wheat gluten, when heated in bread, begins to form the typical dark brown crust at 215°C (Friedman, 1996b). A second effect seen in some foods is increased emulsification stability, due to the presence of Maillard products (Nakamura *et al.*, 1992). Nakamura *et al.* (1992) showed that conjugated protein-polysaccharide emulsifiers were 8 to 10 times more stable than commercial emulsifiers (Kato *et al.*, 1990).

Although the Maillard reaction affects food characteristics, it more importantly impacts on AA digestion and absorption. The main side effect of the Maillard reaction is growth inhibition. Although lysine has an intermediate heat tolerance, relative to the other AA, the effect of the Maillard reaction is amplified over that of other AAs as it is usually the first limiting AA in animal diets. A study by Friedman (1996b) demonstrated the significance of heat damage on casein heated alone or in combination with glucose or starch at four temperatures: 120°C, 180°C or 240°C for one hour and 37°C for 10 days (Table 6). As the temperature increased, the extent of protein damage increased and was

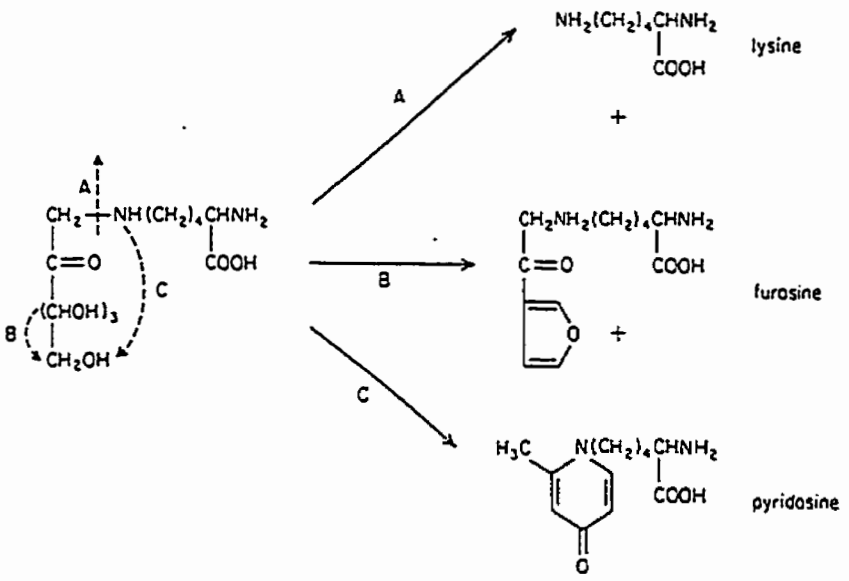


Fig. 2. Amadori rearrangement that can occur in the formation of advanced glycosylation end products (Maillard products) (modified from Furth, 1988).

TABLE 6. Weight gain, protein efficiency ratio (PER) and nitrogen digestibilities in rats fed heat-damaged casein and casein-carbohydrate mixtures.[1]

Protein Source	Weight Gain (g)	PER	Nitrogen Digestibility
Casein control (unheated)	102	3.22	93.7
		37°C for 10 d	
Casein	107	3.16	93.6
Casein + glucose	101	3.11	
Casein + starch	106	3.04	93.2
		120°C for 1 h	
Casein	99	3.08	93.1
Casein + glucose	116	3.37	93.4
Casein + starch	121	3.23	93.8
		180°C for 1 h	
Casein	63	2.5	72.9
Casein + glucose	41	1.86	84.6
Casein + starch	49	2.09	
		240°C for 1 h	
Casein	-17	-2.72	15.2
Casein + glucose	-17	-2.42	6.9
Casein + starch	-16	-2.54	14.9

[1]From Friedman, 1996b

reflected in poorer weight gains, protein efficiency ratio (PER), and nitrogen digestibility in rats relative to the unheated control diet. Even storage at moderate temperatures for extended periods of time (37°C for 10 days) caused a decrease in the PER and nitrogen digestibility, suggesting that even at low temperatures, significant damage can occur given sufficient time.

Maillard products also impact on digestive physiology, compounding the effects of limited AA availability. In a study by Oste *et al.* (1987), it became apparent that the presence of certain Maillard reaction products could inhibit protein digestion and absorption. In their study, 3 mg of radiolabelled protein was fed to 3 groups of 7 rats (Table 7). One group was fed a radioactive control diet while the other groups were fed either DL-2-formyl-5-(hydroxymethyl)pyrole-1-norleucine or 2-furoic acid. In each diet, free lysine residues were labeled with tritium (^{3}H) while protein-bound lysine was labeled with carbon-14 (^{14}C). The results of the trial are two fold. First, the reduction of ^{14}C serum levels suggests that there is an inhibition of protein and carbohydrate digestion. AGEs formed in the Maillard reaction inhibit protease activity including pepsin, trypsin, carboxypeptidase A and aminopeptidase N (Oste *et al.*, 1987, Pitotti *et al.*, 1994, Friedman, 1996a, b). However, Oste's *et al.* (1987) work also implies that the inhibition of these enzymes is not the only factor affecting protein digestion. Since the ^{14}C to ^{3}H ratio was not significantly different, other factors must affect both free and protein-bound lysine to the same extent. Therefore, AGEs probably interfere with the active transport of lysine. A more recent study by

TABLE 7. Radioactivity of plasma from rats fed experimental diets containing free (3H) and protein-bound (14C) lysine with or without the addition of single Maillard reaction products: DL-2-formyl-5-(hydroxymethyl)pyrole-1-norleucine (A) or 2-furoic acid (B).[1]

	^{3}H	^{14}C	$^{14}C/^{3}H$
control diet	29057 ± 1365	12646 ± 940	0.44 ± 0.02
Addition of A	26307 ± 2186	11589 ± 819	0.43 ± 0.04
Addition of B	27877 ± 1871	11860 ± 720	0.44 ± 0.03

[1]Modified from Oste *et al.*, 1987

Pitotti *et al.* (1994) suggests that protease inhibition is limited to trypsin and chymotrypsin. Moughan *et al.* (1996) concurred with Oste *et al.* (1987), in that absorption or at least utilization of unaltered lysine in heat damaged proteins was significantly reduced (by 59 ± 2.3%, mean ± SE). Although Moughan *et al.* (1996) did not offer an explanation for this observation, Friedman (1996a) believes that lysine derivatives interfere with lysine absorption through two mechanisms: (1) they can block the absorption site (as does difructosyllysine) or (2) they can compete for the absorption site (as is the case with monofructosyllysine). Other physiological effects of AGEs include changes in the liver, kidneys and stomach, altered mineral catabolism and altered allergenic responses (Friedman 1996a).

The Maillard reaction has a significant impact on protein quality in addition to physiological parameters. Therefore, it has become necessary to be able to quantify the degree of heat damage. If AA analysis were used, it would underestimate the formation of Maillard products, since early Maillard products are not stable under acid hydrolysis. Therefore, additional assays of stable AGEs are necessary. Typically, stable Maillard products should be used in these analyses such as lysylpyrraline, a good indicator of the degree of cross linking that has occurred in milk (Morales & van Boekel, 1996). Carboxymethyllysine is another indicator of the degree of advanced glycosylation (Buttner *et al.*, 1996). Another stable product that can be used to analyze the extent of heat damage is furosine. Furosine has conjugated double bonds, which can be easily detected using high

performance liquid chromatography (HPLC) analysis coupled to an absorbance detector (280 nm). To analyze for furosine using AA analysis, furosine would need to be reduced with sodium borohydrate to deoxyhexitol. The reduction of the free amino end prevents the furosine from reacting with ninhydrin during AA analysis. A third possible assay for AGEs involves furoyl-furanyl-imidazole (FFI). FFI is derived from glucose cross-links. The presence of an imidazole ring, a fluorescent chromophore, can estimate the difference in AGEs between heat treatments through absorption at 370 nm or fluorescence at 440 nm. Unfortunately, the majority of cross-linkages that occur are lysyl-glucosyl-lysyl and cannot be measured spectrometrically (Furth, 1988).

Another area of interest is the prevention of the Maillard reaction. Friedman (1996a) noted that increasing cysteine, N-acetyl-cysteine or glutathionine levels is one method to reduce the browning reaction. These compounds act as reducing agents or antioxidants in the presence of reactive oxygen species, lipid peroxides or strong nucleophiles that could induce cell detoxification. Other antioxidants could also be used to reduce the Maillard reaction, such as ascorbic acid. Acetylation of free amino groups would prevent addition reactions with reducing sugars or the reducing sugars could be eliminated through fermentation or replacement with non-reducing sugars. A final option to reduce the impact of the Maillard reaction would be to add microbial enzymes to breakdown Maillard reaction products in a process referred to as deglycation.

Although the Maillard reaction is essentially detrimental to protein quality, some Maillard products have beneficial effects, including antioxidant or antibacterial effects. Unfortunately, it is difficult to tell which Maillard products are helpful and which are harmful (Friedman 1996a, b). Therefore, in animal nutrition, it would be prudent to avoid feeding heat-damaged proteins.

2.3.3.2. Avidin. Avidin is commonly found in raw egg albumen. In sufficient quantities, it can effectively bind up both dietary biotin and biotin synthesized in the intestinal tract effectively creating a biotin deficiency (Cunha *et al.*, 1946). Biotin supplements or heat denaturation of raw egg albumen can decrease the toxicity of the powdered product (Peters, 1967).

2.3.3.3. Egg protease inhibitors. In addition to avidin, eggs contain protease inhibitors that impact on the ability of monogastric animals to digest egg whites. Key protease inhibitors present in egg albumen have trypsin inhibiting activity (TIA). Ovoinhibitor proteins and ovomucoids are such proteins found in the egg with TIA. Van Nevel *et al.* (2000) found 1 gram of egg powder inhibited 40mg of trypsin.

2.4. Protein quality and nutritional requirements

2.4.1. Rat

The biological value (BV), net protein utilisation (NPU) and the protein efficiency ratio (PER) are reliable means to determine the quality of feed ingredients. To determine the BV, total urine and faecal nitrogen must be collected and quantitated using metabolic cages. NPU is derived from the BV

and is determined by multiplying the BV by the true digestibility of a protein (Eggum, 1973). PER is another accepted method currently used to assess protein quality. In bioassays, male weanling rats are fed a test protein diet *ad libitum* for a 4-week period. The growth rates are divided by the amount of protein each individual rat ingested and compared to the casein control diet (Raiten *et al.*, 1998). In critical assessments of AA quality, test diets should be nitrogen deficient (Baker and Chung, 1992). The NRC (1978) recommends 12% protein for growth. Therefore, it is reasonable for researchers to use as little as 8% protein in PER assays (Sarwar and Peace, 1994; Sarwar, 1997). Sarwar (1997) reported that incorporating proteins at 10% instead of 8% would not significantly alter the observed results. When reporting PER, it is not uncommon to correct the diets so that the value of the casein control diet is 2.5 (Raiten *et al.*, 1998) or to report the relative PER (RPER) value (Sarwar and Peace, 1994). The RPER is the ration of the test diet PER to the control (casein) diet PER.

In addition to being correlated to the overall protein quality, PER is highly correlated with the content of sulphur AA (SAA), which is a reflection of the rat's response to higher SAA contents than other animals (Raiten *et al.*, 1998). Therefore, rat bioassays would be sensitive indicators of heat damage due to the reduced bioavailability of cystine in the formation of lanthionine in addition to the reduced bioavailability of lysine in the formation of Maillard products (Sarwar, 1997). Furthermore, as the PER decreases, the kidney weights tend to increase. In addition, as the level of dietary fat increases, PER decreases, most likely due

to lipid peroxidation. A fatty liver may indicate peroxidation reactions occurring in the liver (Jenkins and Mitchell, 1989).

When formulating the diets for rat bioassays, it is essential to ensure that decreases in PER are due to the protein quality and not a result of secondary nutrient deficiency. To this extent, the minimum linoleic acid and α-linolenic acid requirements of 12 and 2 g/ kg diet, respectively, must be met. In addition, rats require choline bitartrate in their diets. Reeves *et al.* (1993) recommended that fibre, choline bitartrate, a mineral mix (AIN-93G-MX) and a vitamin mix (AIN-93-VX) be included in the diet at a level of 5, 0.25, 3.5 and 1% respectively. Under standard laboratory conditions, biotin is not required in the diet since it is supplied by the intestinal bacteria. However, if diets contain raw egg whites, biotin should be added. According to NRC (1978), rats fed raw egg albumen at 10% require 2 mg of biotin per day (or 0.15 mg biotin/ kg diet). However, if raw egg albumen is increased to 20% of the diet biotin should be increased to 2 mg/kg diet to ensure adequate growth (Klevay, 1976; NRC, 1978).

Thomson *et al.* (1994) demonstrated that rodents are acceptable bioassay models for weanling-pigs, especially for novel protein supplements including spray-dried porcine plasma (SDPP). However, the animals fed SDPP showed an increase in liver weight over the control diet and higher metabolic energy losses. These effects may have resulted from either increased feed intake or the presence of growth factors in the blood products (Thomson *et al.*, 1995). Despite being acceptable models for weanling pigs, rats use AA differently than

pigs when antinutritive factors (ANF) are present. Therefore, caution should be used when extrapolating the results to pigs (Yu *et al.*, 1996). When extrapolating results of feed ingredients which contain ANFs (especially Maillard products) to chickens caution should also be exercised. A renal enzyme found in chickens improves the bioavailability of lysine incorporated into Maillard products. E-N-lysine acylase is not present in rats (Varnish and Carpenter, 1975). In addition, rats and pigs retain lysine better than threonine; chicks retain threonine as efficiently as lysine (Edwards *et al.*, 1999). Therefore, it is imperative to use caution when trying to extrapolate between the three species.

2.4.2. Chicken

Broiler chickens undergo several metabolic adaptations when shifting from embryonic yolk dependence to the utilisation of exogenous feed. Initially, fatty acids are taken up from yolk as efficiently as ingested feed. However, carbohydrates and AAs in exogenous feed are not absorbed efficiently until adequate enzymatic activity is present (Noy and Sklan, 1999).

Among feedstuffs, the energy from fat sources is better utilised, with no age dependency, than either carbohydrate or protein sources whose utilisation tends to improve with age. Given the improved energy utilisation, fat energy is preferable to carbohydrate energy in conserving nitrogen reserves in very young chicks (Sulistiyanto *et al.*, 1999). Although there is no statistical advantage as to the source of the dietary fat, poultry fat gives body weight gains that are numerically greater than birds fed corn oil at 3% (Peebles *et al.*, 2000). Perhaps,

the slight increase in body weight gain is due to the similarity in the composition of poultry (abdominal) fat and egg yolks (Sim, 1970). Overall, dietary fat levels influence metabolism due to their gross energy, free fatty acid content, ratio of saturated to unsaturated acids and saponification (Sulistiyanto *et al.*, 1999). However, increasing dietary protein levels can modulate the metabolic effects of dietary fat, shifting the flow of substrates from fat synthesis to glucose synthesis (Rosebrough *et al.*, 1999). In regards to fatty acid composition, chickens only require linoleic acid, which should be included at 1% of the diet (NRC, 1994).

Energy utilisation from lipids and carbohydrates does not limit the growth of young chicks. At 4 days post hatch, proteolysis is the primary factor limiting growth with only 78% protein digestibility. By 21 days post hatch, broiler chickens have increased protein digestibility to 90% (Noy and Sklan, 1995). Therefore, careful attention must be given to the AA requirements of young broiler chickens. Digestibility is a sensitive indicator of AA availability in dietary ingredients for poultry (Ravindran *et al.*, 1999). However, AAs measured in the terminal ileum are more accurate determinants of digestibility than excreta analysis due to substantial metabolism by hindgut microflora. Therefore, determination of AA digestibility by excreta analysis is not advised (Ravindran *et al.*, 1999). In diets it is advantageous to ensure arginine, lysine, methionine and methionine + cystine levels are 1.25, 1.1, 0.5, and 0.9% of the diet for 0 to 3 week old chicks, respectively (NRC, 1994).

The utilisation of limiting AA above maintenance levels is constant over a wide range of intakes. Although accretion of valine, lysine and threonine are 73, 79 and 82% respectively, greater oxidation of SAA produces a methionine accretion of 68% and total SAA accretion of 52% (Edwards and Baker, 1999). Despite the lower accretion rates, if methionine is given to broiler chicken in excess (*i.e.* > 1.25%) methionine toxicity can result in a 40% depression in weight gain (Katz and Baker, 1975). Lysine is the second limiting AA in poultry diets and supplementation of corn-soy diets is common (Emmert *et al.*, 1999). Lysine deficiency results in significant reductions in body weight, growth rate, feed intake, increased feed conversion rates and growth depression of the gastrocnemius, sartorius, or pectoralis major muscle (Tesseraud *et al.*, 1999).

Although lipids, carbohydrates and proteins compose the majority of the diets, it is important to meet broiler chickens requirement for vitamins and minerals. Due to the presence of avidin in eggs, it is important to ensure that biotin is included in broiler chicken diets at a rate of 0.15-0.20 mg/kg to ensure healthy birds (NRC, 1994).

2.4.3. Pig

As in chickens, pigs undergo several physiological changes at weaning including decreased villi length. Overall, sub optimal growth during the post-weaning period is largely due to the piglet's inability to consume and digest sufficient quantities of nutrients. To stimulate feed intake in newly weaned piglets various solutions have been investigated including altering the form of the

feed, to introduce wet or gruel feeding; using phase feeding, feeding multiple diet formulations to meet the piglets needs as they grow; or using feed flavouring reminiscent of the sow's diet at the time of suckling (de Lange and Baidoo, 1997b). However, to stimulate feed intake and maximize performance through increased diet complexity usually makes the diets more expensive (Dritz *et al.*, 1996). In order to minimize some of the costs associated with multiple diet formulations the minimum AA requirements of the piglets should be considered. For example, a 5 – 10 kg pig requires 0.54% arginine, 0.43% histidine, 0.73% isoleucine, 1.32% leucine, 1.35% lysine, 0.35% methionine, 0.76% methionine + cystine, 0.80% phenylalanine, 1.25% phenylalanine + tyrosine, 0.86% threonine, 0.24% tryptophan and 0.92% valine. In addition young pigs also require 0.1% linoleic acid and 0.05 mg/kg biotin (NRC, 1998). As the piglet increases in weight or reduces its lean growth potential, the lysine requirement decreases (de Lange and Baidoo, 1997a). Another AA that requires attention is methionine. Chung and Baker (1992b) found that 5 to 10 kg pigs require 0.255% digestible methionine. Assuming methionine has a digestibility of 89%, a 5-10 kg pig would require 0.29% methionine, not 0.35% as recommended by the NRC (1998). When piglets reach 10 to 20 kg, they require between 0.235 to 0.275% methionine. Grains incorporated into research diets are typically low in methionine; therefore, supplementing the diets with methionine results in a quadratic increase in the average daily feed intake and average daily gain of young pigs (Chung and Baker, 1992b). Excess methionine can be toxic to young

pigs; therefore, caution should be used when augmenting methionine above recommended levels (Owen *et al.*, 1995). Grains incorporated into diets often contain sufficient cystine levels. Young pigs require 0.58% SAA, of which, cystine can provide a maximum of 50% of the requirement (Chung and Baker, 1992a and 1992b). As the pig matures, its cystine requirement increases (Chung and Baker, 1992a).

The incorporation of spray-dried blood products to animal diets including spray-dried blood meal (SDBM) or spray-dried porcine plasma (SDPP) has been one approach used to improve post-weaning feed intake and weight gains. SDBM is an effective protein source in starter pig diets, despite being slightly deficient in methionine. Therefore, at higher inclusion rates, additional methionine may be necessary to optimise piglet performance (Kats *et al.*, 1994b). According to Owen *et al.* (1995), diets containing spray-dried blood products should contain 0.41-0.42% total dietary methionine to maximize growth 0-14 days post-weaning given a dietary lysine level of 1.6% and considering the ideal protein profile. For diets containing 1.3% lysine, methionine should be included at a rate of 0.34-0.35% (Owen *et al,* 1995). Another blood product, SDPP, has also been evaluated in early-weaned pig diets. SDPP can be included in swine diets up to 10% (Kats *et al.*, 1994a); however, the minimum required to increase early-weaned pig growth has been reported to be 6% (Coffey and Cromwell, 1995). In animals fed levels greater than 6% SDPP, poorer growth performance may be attributed to methionine deficiency (Kats *et*

al., 1994a). Despite improving animal performance in the first two weeks post weaning, animals fed SDPP diets do not perform as well during the third and fourth weeks post-weaning (Kats *et al*, 1994a; James *et al.*, 1999). Kats *et al.* (1994a) noted that animal performance decreased when they were switched to a diet that did not contain SDPP. In addition, Coffey and Cromwell (1995) observed a nursery environment and protein source interaction. It appears that the nursery environment alters the effectiveness of using SDPP as a protein source for early-weaned pigs. Bergstrom *et al.* (1997) also noticed that the response to dietary SDPP varies depending on the herd health status. To circumvent some of the above problems it is possible to use a combination of SDPP and SDBM to improve performance over that of either product used alone (Kats *et al.*, 1994a).

2.5. Use of eggs in animal diets

2.5.1. Monogastric growth studies

Through the years monogastric animals have been fed egg proteins for various research projects. Whether the studies evaluated protein quality or induced biotin deficiency, they are none-the-less relevant to the potential for spray-dried egg proteins to be used in the animal feed industry.

In rats, eggs have often been used as a reference protein. Overall eggs meet the minimum requirements of rats due to their higher concentration of SAA, sodium, potassium and chloride and low levels of phosphorous. Unlike casein

diets, there is no need to supplement the diets with methionine (Reeves *et al.*, 1993). In addition, the quality of soy protein could be improved by blending the protein with 70% egg white (Mori *et al.*, 1991). Egg-based diets were noted by Boyd *et al.* (1966) and Childs and Ostrander (1976) to affect the weights of the liver. Boyd *et al.* (1966) studied the effect of administering egg white solutions through oral canulas. They noted a decrease in the wet weight of the liver and kidneys of 17.8 and 15.2%, respectively, over the control diet. In contrast, Childs and Ostrander (1976) noted the opposite trend in the livers of rats fed dried whole egg. They saw an increase in liver weight per 100 g body weight relative to rats fed an egg yolk replacer, conducive to total lipid and cholesterol accumulation.

Unlike rats, few studies have been reported on the inclusion of egg proteins in broiler chicken diets. One study used chickens for a PER assay to assess various animal meals. In this study, Johnson and Parsons (1997) noted that spray-dried whole egg (SDWE) had a higher PER value than any other animal meal. However, Junqueira *et al.* (2000) noted that broilers fed a diet containing dried whole eggs (DWE) performed poorer than broilers on diets without DWE.

In pigs, egg proteins have been studied more extensively as a non-traditional protein supplement in an attempt to offset production costs. SDPP, used extensively in early-weaned pig diets, is an expensive protein supplement. James *et al.* (1999) suggested that the inclusion of egg proteins in diets could

reduce the cost over diets containing SDPP by 12-13¢ per kilogram. Nessmith *et al.* (1995) showed that SDPP (included in diets at 6%) could be replaced by SDWE up to a level of 50% without influencing the average daily gain (ADG) or the feed conversion ratio (FCR) in phase I diets. Other research groups found that inedible egg products were as valuable a protein supplement as SDPP (Owen *et al.*, 1993). Zimmerman (1999) found that inclusion of a 50:50 mix of inedible egg white and yolk (55.2% protein, 28.6% ether extract) replacing 0, 3, 6 or 9% of soybean meal resulted in a linear decrease in growth performance. Further, De La Llata *et al.* (1998) formulated four diets to evaluate the ability of spray-dried egg white to replace SDPP in early-weaned pig diets. The test diets contained either 5% SDPP, 7% spray-dried egg white or a mixture of 2.5% SDPP and 3.5% spray-dried egg albumen. They concluded that spray-dried egg albumen was not an effective replacement for SDPP.

Although eggs have a high nutrient content, their use in animal diets is limited. The exact reason for the reduction in pig performance when fed diets containing egg proteins is not clear. However, it is possible that some destruction or complexing of essential AAs occurs during the drying process (Zimmerman, 1999). Although early studies by Cunha *et al.* (1946) showed that raw eggs were digested as efficiently by mature hogs as cooked eggs, ANFs, such as advanced glycosylation end-products, avidin or protease inhibitors, may significantly affect early-weaned pig performance. In addition, egg albumen contains very little zinc. This could be critical, as the level of zinc required for

tissue growth (26 ppm) can not be met by plant proteins, due to the presence of phytate which decreases zinc bioavailability (Hankins *et al.*, 1985a,b). A third factor influencing performance of pigs fed egg diets is the fat availability. Eggs tend to be lower in linoleic acids and somewhat higher in saturated fats than soybean oil (Kratzer *et al.*, 1988). Therefore, Owen *et al.* (1993) theorized that the fat in egg proteins may be less available than in soybean oil, thus allowing egg products to only replace 6% SBM and 3% SDPP. Through an indirect study on lupin seeds, Van Nevel *et al.* (2000) examined the ability of egg proteins to inhibit lectins. They found that egg powder could inhibit 40 mg trypsin/g. However, the trypsin inhibiting activity was found to be too low to cause growth inhibition in the animals when egg powder was included in the diet at a rate of 5%. Egg proteins have the potential to be included in animal diets at low levels despite a poor understanding of the antinutritive effects associated with higher dietary inclusion rates.

2.5.2. Biotin concerns in animal diets containing raw eggs

One concern pertaining to the use of eggs in animal diets, as identified above, is the ability of egg whites to induce biotin deficiency syndrome. Cats, for example, can synthesize biotin indirectly through their intestinal microflora; therefore biotin is not required in their diet. However, biotin deficiency can be induced in cats by feeding raw egg white (Pastoor *et al.*, 1991). In livestock production, biotin requirements must be addressed. In a trout study, Castledine *et al.* (1978) documented the importance of meeting the biotin requirement.

Using egg whites they noted a marked repression of growth; however, exceeding the required amount of biotin did not increase feed intake, weight gain or feed to gain ratio. Studies with rats have been commonly used to better understand the aetiology of biotin deficiency syndrome. Biotin is involved in gluconeogenesis, fatty acid synthesis and AA catabolism as it serves as a co-enzyme for four carboxylase enzymes: pyruvate carboxylase, acetyl coenzyme A carboxylase, propionyl coenzyme A carboxylase and beta-methylcrotonyl coenzyme A carboxylase. In biotin deficient rats, ornithine transcarbamylase (OTC), a urea cycle enzyme, showed a 60% reduction in reactivity and mRNA levels. Despite the reduction in OTC activity, biotin deficiency could not limit the urea cycle, as it is not associated with the rate-limiting enzyme (Maeda *et al.*, 1996). To prevent biotin deficiency in rats fed a diet containing 20.0% egg white, Klevay (1976) recommended adding 2mg biotin/kg diet, contrary to preliminary studies suggesting that 200 mg biotin/kg diet were necessary for adequate growth during the first 40 days of a 120 day experiment.

In chickens, biotin in the yolk is associated with an easily denatured biotin-binding protein (BBP) which is denatured at temperatures lower than those required for avidin. Relative to avidin, BBP easily releases biotin at low temperatures (Kratzer *et al.*, 1988). Despite the presence of biotin in the yolk, the levels are inadequate to counteract the presence of avidin in the egg white. Therefore, unheated dried whole egg is biotin deficient. In a 29-day growth study by Kratzer *et al.* (1988), biotin deficiency syndrome affected the FCR for birds

fed a semi-purified diet containing dried whole egg, dried egg white but not dried egg yolk (2.86, 2.86, 1.47 respectively). To counteract the biotin deficiency Kratzer *et al.* (1988) recommended supplementing the diets with 500 μg biotin/kg diet.

There has been a larger focus on the impact of biotin deficiency syndrome in pigs than in other animals, since pigs are often used as a model for human studies. The response to biotin supplementation in pigs has been variable due to the fluctuations in available biotin content of feedstuffs (Kopinski *et al.*, 1989a, b). Biotin availability may be affected if it complexes to dietary components that are not hydrolyzed before reaching the terminal ileum (Kopinski *et al.*, 1989b). In general, availability of biotin from cereal grains is less than that from protein supplements for both pigs and poultry (Table 8). Of the feedstuffs assessed by Kopinski *et al.* (1989b), sorghum negatively affected dietary biotin availability, possibly through the formation of nonabsorbable biotin complexes. Small negative values, as in the case of Egret wheat, may be within experimental error (Kopinski *et al.*, 1989b).

To further understand biotin deficiency, several research groups have examined the site and extent of biotin absorption. The primary absorption site of vitamins is the central portion of the small intestine (SI). Kopinski *et al.* (1989a) showed that biotin content decreases from 112 μg/d in the stomach to 39 μg/d in the first quarter of the SI. There was a further decrease to 8μg/d in the last quarter of the SI. The authors used a highly available form of biotin and

TABLE 8. Availability of biotin in pigs and chickens fed various feedstuffs (%).[1]

Feedstuff	Biotin availability	
	Pig	*Chicken*
Wheat (var Banks)	6	11
Wheat (var Egret)	-3	-10
Sorghum	-123	-73
Barley	18	5
Meat Meal	82	69
Soya-bean Meal	12	28
Casein	95	75

[1](from Kopinski *et al.*, 1989b)

although it was absorbed in the upper portion of the SI, it does not negate the possibility that other regions of the SI are capable of biotin absorption. In the large intestine, biotin flows were similar for supplemented and unsupplemented animals with values ranging from 17-54 μg/d. This suggests that biotin is synthesized in the hindgut by microbes. Despite biotin absorption in the hindgut, the authors did not analyze for absorption of microbial biotin. In another trial, Kopinski *et al.* (1989d) orally dosed or caecally infused pigs with biotin to better understand its absorption. After 30 minutes 14.1% of the orally supplemented biotin was present in the plasma, opposed to 1.1% of the biotin administered through the T-cannula. Therefore, the post-ileal absorption of biotin was 7.7% as efficient as the absorption of biotin following oral dosing. As demonstrated above, orally supplemented biotin is effectively absorbed in the SI after only 30 minutes. Kopinski and Leibholz (1989) suggested that a dietary supplement of 50 - 100 μg/kg of diet was required to prevent biotin deficiency symptoms, when coprophogy is prevented. They further noted that 90 μg of biotin/kg diet was sufficient to meet the biotin requirements in diets containing autoclaved egg. In earlier studies, Cunha *et al.* (1946), using a modified paired feeding system, fed a purified diet containing 30% desiccated egg white for 6 weeks. Biotin deficiency was prevented in the pigs that received 100 μg biotin/d through intramuscular injection. The biotin deficient pigs required 50% more feed per pound of gain and showed a 45% decrease in their daily gain. Van Nevel *et al.* (2000) showed that feed intake was unchanged and ruled out biotin deficiency

caused by the presence of avidin in the egg powder. Kopinski *et al.* (1989c) also observed that biotin supplementation was not required for growth, citing that biotin recycling, via biotinidase, and absorption from the hind gut appeared adequate to meet the needs for growth. In conjunction with biotin recycling there is an increase in the enzyme activities of aspartate aminotransferase, lactate dehydrogenases and serum alanine aminotransferase to maintain normal synthetic processes and tissue respiration during biotin deficiencies. In trials in which biotin deficiency was induced by egg white diets, growth depression was due to protease inhibitors, such as ovoinhibitor and ovomucoids that have porcine trypsin-inhibitor properties. To further separate biotin deficiency from the impact of egg protease inhibitors, Kopinski *et al.* (1989c) fed 16 male Landrace-Large white pigs weaned at 2 days of age a biotin deficient diet that did not contain egg albumen or avidin. They found that there was no need to supplement diets with biotin to ensure normal growth rates. However, the authors speculated that if the trial was extended further there would eventually be an impact on growth due to decreased feed intake. Biotin may not be required for normal growth rates, but it is required to ensure proper carcass length and fat distribution (Kopinski *et al.*, 1989c). The same authors suggest that to prevent biotin deficiency symptoms, such as hoof and skin lesions, pigs require 100 μg biotin/kg diet.

2.6. Microflora of the intestinal tract:

Neighboring microbes can influence bacterial growth through a wide range of microbial factors including the production of lactic acid (lowers pH, carbon or energy sources), volatile fatty acids (inhibits some bacteria; is an energy and carbon source for others) or hydrogen sulfate (inhibits certain bacteria) to motility (allows movement to nutrients and away from toxins), microbial aggregation (inhibits access to adhesion receptors), adherence to epithelial or particulate surfaces (promotes colonization where luminal contents move quickly, provides continual inoculation of digesta, facilitates hydrolysis of fibrous material and growth within lumen), nutrient utilization (competitive exclusion, controls growth) and the production of bacteriocins or antibiotics (inhibits growth or kills sensitive bacteria). Environmental factors equally impact the microflora and include: oxygen levels (elevated levels inhibit strict anaerobes), temperature (regulates growth), pH (controls growth and survival), peristalsis (moves nutrients and microbes to distal areas), conjugated and unconjugated bile acids (detergents which can bind to cells and may be lethal), epithelial cell turnover (sloughing cells necessitates the replacement of adhering microbes), mucus (serves as microbial habitat), diet (fibrous materials provide a luminal habitat), the presence of phagocytic cells (destroys microbial cells), the host's age and genetics (subtle influence, not fully understood) and antibiotics (Miles, 1993). As public resistance to the use of antibiotics to modulate intestinal microflora increases, there is a greater urgency to understand how microbial and environmental factors can be used to modulate the natural gastrointestinal microflora. To this

extent, the above factors and how they impact animal nutrition are being manipulated in studies on rats, chickens and pigs, as discussed below.

2.6.1. Rat

In rats, two dietary components, fatty acids and inorganic calcium phosphate have been examined for their impact on microbial populations. Fatty acids and monoglycerides, liberated in the gastrointestinal tract, are generally inhibitors of gram-positive bacteria *in vitro*. However, *in vivo* evidence is limited. Sprong *et al.* (1999) investigated the effect of high or low milk fat diets against *Listeria monocytogenes* (gram-positive) or *Salmonella enteritidis* (gram-negative) infection. Rats fed high fat diets showed increased resistance to *L. monocytogenes* infection but not to *S. enteritidis*. The listericidal activity, of high fat diets, occurred primarily in the stomach and was enhanced by the presence of free fatty acids C10:0, C12:0 and C14:0 and monoglycerides C:12, C14:0 and C16:0. Therefore, it is possible that high milk fat intake may protect against gastrointestinal infections caused by fatty acid-sensitive (gram-positive) pathogens via enhanced bactericidal capacity of the stomach (Sprong *et al.*, 1999). Bovee-Oudenhoven *et al.* (1999) found that other dietary components reduced the translocation of *Salmonella* across the intestinal lumen into the systemic circulation. Dietary calcium phosphate (inorganic) reduces the luminal cytotoxicity towards gram-positive bacteria. Gram-positive bacteria, primarily *Lactobacilli*, can then exert their own antagonistic properties against pathogenic bacteria in the SI. They postulated that the adsorption of bile and fatty acids by

intestinal calcium phosphate and their subsequent precipitation would make the SI less hostile towards gram-positive bacteria. *Lactobacillus acidophilus*, for example, is more sensitive to the surface-active properties of bile and fatty acids than *Salmonella enteritidis*.

As demonstrated in the above two trials, dietary components can affect the endogenous microflora, which is an important variable in host defense against intruding pathogenic bacteria. Elimination of the protective intestinal microflora by dietary constituents or antibiotics in humans and animals can be accompanied by opportunistic infections.

2.6.2. Chicken

In chickens, control and prevention of enteric diseases is a key management strategy to maximise production. Enteric diseases have multiple aetiologies including: hypermotility, increased frequency of intestinal peristalsis leading to an increased digesta transit; an alteration in intestinal permeability where net secretions exceed absorption; hypersecretion, increased intestinal efflux of fluid and electrolytes into the intestinal lumen and finally malabsorption whereby the absorptive capacity of the intestines are altered usually a sequela of maldigestion (Mead, 2000).

To reduce the occurrence of enteric disease, modifications to the diet may be possible, as in rats. In a study by Danicke *et al.* (1999), enzyme supplementation, antibiotic supplementation and the type of fat used all impact on microbial growth. When diets were supplemented with xylanase (a

carbohydrase), the enterobacteria and total anaerobic microbes were reduced with similar trends for gram-positive cocci and enterococci. In rye diets supplementation with xylanase reduced the intestinal viscosity of broiler chickens. High intestinal viscosity may change the morphology of the host intestinal tissues by elongating epithelial villi, increasing cell turnover and thus increasing the colonization surface. Reduced viscosity in birds has the opposite impact, reducing the colonization surface, altering adhesion structures and decreasing luminal release of epithelial cells. Therefore, enzyme supplementation indirectly impacts microbial populations. Another means to improve performance in broiler chickens is to supplement the diet with antibiotics. Zinc bacitracin, for example, changes the pattern and activity of luminal and adhering microflora. It is believed that enzyme supplementation is the more significant than antibiotic supplementation in improving nutrient digestibility and shifting absorption towards the upper gastrointestinal tract.

2.6.3. Pig

The natural microflora of the pig's gastrointestinal tract, acquired during passage through the birth canal, consists of about 10^{14} microorganisms per gram digesta (Fuller, 1989). The population consists of approximately 400 different types of bacteria including *Bacteroides*, *Selenomonas* and *Fusobacterium*. The majority of bacteria are strict anaerobes found at levels of 10^{10} per gram of gut content (Durmic *et al.*, 1998a). As in chickens, hygiene, antibiotic therapy, diet and stress can influence the microflora present in the intestinal tract.

Stress, caused by early weaning, can induce several diseased states including swine dysentery (SD) and post-weaning diarrhea (PWD). SD is a mucohemorrhagic colitis induced by several bacteria (Hampson and Pethick, 1998; Durmic *et al.*, 1998a, b). Although, the exact pathogenesis of SD is unknown, researchers believe that resistant starch (RS) and soluble non-starch polysaccharides (sNSP) found in the diet, in addition to synergistic interactions between several bacteria contribute to the development of SD (Durmic *et al.*, 1998b; Pluske *et al.*, 1998). In general, pigs with SD tend to show increased numbers of gram-negative isolates associated with the colonic epithelium (Durmic *et al.*, 1998a, b). PWD is another class of diarrheal diseases. It develops 3-10 days after the withdrawal of sows' milk producing a yellowish-gray diarrhea leading to emaciation, depressed appetite, appearance of a potbelly and development of a rough hair coat. With an increased trend towards early weaning, there has been a parallel increase in PWD. A 10^3 to 10^5-fold increase in enterotoxigenic strains of *Escherichia coli*, which produce heat labile toxins, can induce secretary diarrhea (Hampson, 1994). Two specific forms of PWD include porcine intestinal spirochaetosis and post-weaning colivacillosis, which may be confused with SD. Porcine intestinal spirochaetosis is a chronic disease of weanling pigs caused by *Serpulina pilosicoli*; whereas, post-weaning colivacillosis is due to the proliferation of enterotoxic strains of *E. coli* (Hampson and Pethick, 1998).

One key factor that may exacerbate PWD is the physiological changes occurring after weaning. Several changes including increased pH of the gastric contents, reduction of the SI villi (by 25% in 24 hours), or changes in enzyme levels can stress the piglets leaving them more susceptible to bacterial attack. Other factors that influence the development of PWD are environmental temperatures such as cold or temperature fluctuations or rotavirus infection, which damages the intestine (villus atrophy) and produces an environment that favours colonization of haemolytic *E. coli*. To minimize the occurrence of PWD, weaner house temperatures should be 28-32°C with minimal temperature fluctuations and no drafts (Hampson, 1994). Antimicrobials and electrolyte replacement therapy have both been used to treat PWD. Genetic selection for pigs resistant to colonization is not common since an autosomal dominant gene controls the presence of the receptor (Hampson and Pethick, 1998).

One key function of intestinal bacteria is the digestion of complex carbohydrates from plants that escape digestion, including soluble non-starch polysaccharides, insoluble non-starch polysaccharides and resistant starch (Durmic *et al.*, 1998b). Researchers are trying to evaluate the ability of complex carbohydrates to shift the diversity of the microbes towards *Lactobacillus spp.* (Krause *et al.*, 1995). At present it is known that soybean meal in the diet may induce immunological responses that damage the intestine, while high levels of fish meal or sugar in weanling-pig rations predisposes animals to PWD by supplying nutrients for bacterial proliferation (Durmic *et al.*, 1998a, b).

Connections between the pig's diet and the presence and/or extent of proliferation of pathogenic enteric bacteria can limit the proliferation. Dietary components, such as soluble fiber and resistant starch, can manipulate the resident microflora to exclude pathogenic bacteria (Hampson and Pethick, 1998). The level and type of dietary fiber strongly influence bacterial counts in the colonic digesta (Durmic *et al.*, 1998b). Research has shown that fermentable carbohydrates have a role in the pathogenesis of SD. For example, slowly fermentable fiber, such as wheat bran, has a greater influence on the distal large intestine than rapidly fermentable fiber, such as oat bran or guar gum, which appear to be fermented in the ceacum and the proximal colon. Pluske *et al.* (1998) found that some combinations of carbohydrates can shift fermentation to the more distal parts of the colon, such is the case with guar gum and resistant starch (RS). In addition, the rapid fermentation of soluble non-starch polysaccharides (sNSP) facilitates colonization of the large intestine by *Serpulina hyodysenteriae* and enterotoxigenic *E. coli* in the SI (Hampson and Pethick, 1998). If hind gut fermentation is limited, the incidence of SD will decrease. Processing or the addition of enzymes to the diet can hydrolyze sNSP and reduce fermentable carbohydrates that enter the large intestine (Pluske *et al.*, 1998). Alternatively, feeding diets low in sNSP and resistant starch (RS) content can protect against SD in experimentally infected pigs. However, two separate experiments with similar diets could not produce similar anaerobic bacteria counts and resulted in different SD outcomes (Durmic *et al.*, 1998a). Overall,

diets with limited fermentation favour gram-positive bacteria and are protective against SD.

2.7. Antibiotic Resistance and Alternative Bactericidal Agents:

Antibiotics have been used as growth promotants since the 1950s. In recent years, pressure from the consumer is forcing us to closely evaluate the advantages of antibiotics in animal feed and actively search for alternatives.

2.7.1. Antibiotics:

The use of antibiotics in agriculture is two fold: to prevent or treat disease and to improve feed efficiency and rate of weight gain. Due to complex interactions, gastro-intestinal microbes have a significant influence upon the digestion and absorption of nutrients by the gut (Parker, 1992). As a result, controlling the levels or types of microbes in the gastrointestinal tract can significantly alter the feed conversion ratio.

There are three prime mechanisms through which antibiotics function. Antimicrobial mechanisms include inhibition of cell wall synthesis, as in penicillin; inhibition of protein synthesis, as in streptomycin; or inhibition of metabolite synthesis, as in sulfanilamide and inhibition of DNA or RNA synthesis (Hahn, 1977).

Antibiotics that inhibit cell wall synthesis are only effective on actively growing organisms. This group of antibiotics includes the β-Lacatams (cyclociliins or penicillin and cephalosporins), a group of peptidomimetic drugs,

analogous to D-AAs. To this end, antibiotics may be substituted into the peptide chain of peptidoglycan. Peptidomimetic drugs have an additional benefit. β-Lactam antibiotics compete with peptides for the PepT-1 and PepT-2 peptide transporters in the intestine and kidney. The presence of PepT1 and, to a lesser extent, PepT2 in the intestine helps to ensure the absorption of orally delivered antibiotics. PepT2 in the kidney helps to increase the half-life of the antibiotics *in vivo* (Ganapathy *et al.*, 1995, and Leibach & Ganapathy, 1996). Peptidomimetic antibiotics have a specific target (bacterial cell walls) and the ability to administer them orally makes them ideal to add to animal feeds to promote growth.

In Sweden, antibiotics were banned in 1986 (Gadd, 1997). Following the ban, Sweden saw an increase in post-weaning deaths (1.6%) and scouring (400%). From their experience, an outright ban on antibiotics would be harmful in North America. Gradually phasing out the use of antibiotics in animal production would allow producers to seek alternatives and the public to adapt to the higher costs of meat (Gadd, 1997). Knisley (1999) estimated that an antibiotic ban in North America would increase poultry, beef and fish costs to the public by $4.85 to $9.72 per person per year. One alternative that has been explored is the non-absorbable antibiotic, zinc bacitracin. Abdulrahim *et al.* (1999) assessed the benefits zinc bacitracin as a growth promotant in a production scale trial using four treatments: control diet with no antibiotics or probiotics, control + *L. acidophilus*, control + zinc bacitracin and the control + *L. acidophilus* and zinc bacitracin. The results showed that zinc bacitracin could

not improve the FCR and, in fact, was poorer than the control. However, when zinc bacitracin was used in conjunction with *L. acidophilus* there was a statistical improvement over control diet or the addition of *L. acidophilus* alone. Zinc bacitracin and *L. acidophilus* are believed to suppress competing microflora (ex: *Clostridium*) and allow colonization of the gut by *L. acidophilus*. Overall, the benefits of zinc bacitracin have been inconsistent.

2.7.2. Antibiotic resistance

Bacteria have decreased susceptibility and even resistance to several common antibiotics. Once resistant to antibiotics bacteria can confer resistance to other bacteria through transformation, conjugation or transposons. Antibiotic resistance occurs when bacteria can use alternate pathways to synthesize metabolites, chemically inactivate antibiotics, modify the structures to which antibiotics bind or can prevent the entrance of antibiotics (Travis, 1994).

2.7.3. Alternatives to antibiotics

The livestock industry is being unjustly blamed for the problems of antibiotic resistance, despite low and dropping use of antibiotics in animal production. Currently antibiotics are used to ensure safe and wholesome food from healthy animals, reduce human exposure to zoonotic pathogens, promote health and well being of animals and reduce the cost of food production. As feed technology improves, the agricultural industry has been able to market animals earlier each year. According to Goransson (1994), in the poultry industry, the reduction in the time to market has reduced the use of antibiotics by

approximately 10%, last year alone. In light of a European ban on most antibiotics, research has turned towards natural products to modify the microflora of the gastrointestinal tract. As producers adapt to life without antibiotics, some groups claim that antibiotics are no longer essential. However, antibiotics are still highly regarded in the North American market. Overall, antibiotics used as growth promoters reduce wastes disposed into the environment and prevent explosive diarrhea, dehydration and death due to necrotizing fibrinous enterocolitis. *Salmonella spp.* can be controlled through competitive exclusion, oligosaccharide use, addition of organic acids to water supply, or management (high quality feed and water, proper sanitation, low environmental stresses, *etc.*); however, none of these are 100% effective (Newman, 1996).

To circumvent the potential crisis antibiotic resistance poses, several alternate antimicrobial agents are currently being investigated. Novel antimicrobial agents include probiotics, mannan-based oligosaccharides, antibodies and lysozyme.

2.7.3.1. Probiotics. Probiotics, or direct fed microbials, have been defined as "a culture of specific living micro-organisms which implants in the animal to which it is fed and ensures the effective establishment of the intestinal populations of the beneficial organisms" (Jin *et al.*, 1997). The live microbial feed supplement beneficially affects host animal by improving its intestinal microbial balance (Hasler, 1998). Historically, researchers have focused primarily on *Lactobacillus* and *Bifidobacterium*, long used in food fermentation (Dicks, 1993; Goldin, 1998).

Generally, bacteria used as probiotics are capable of exerting beneficial effects on the host animal, are non-pathogenic and non-toxic, are present in viable cell counts, can survive and metabolize in the gut environment, are stable and are capable of remaining viable for long periods under storage and field conditions (Fuller, 1989). As most direct fed microbials lack pathogenic bacteria, they have a "generally recognized as safe" designation (Goldin, 1998).

In domestic animals, restricted contact between the mother and offspring may prevent the development of a complete microflora, leading to increased susceptibility to bacterial infections. The use of probiotics aims to complete the natural microflora of young domestic animals (Fuller, 1989). A complete microflora helps to create a restrictive physiological environment, increases competition for bacterial receptor sites and nutrients as well as secretes bactericidal compounds such as organic acids, hydrogen peroxide or bacteriocins (Spring, 1995; Jin *et al.*, 1997). Bacteriocins are water soluble, flavourless, non-carcinogenic proteins, that do not cause allergic reactions and can be degraded by proteolytic enzymes (Dicks, 1993). In chickens, the early establishment of mature microflora leads to increased volatile fatty acid and lactate concentrations, lowering the intestinal pH and creating unfavourable environment conditions for many enteropathogens (Miles, 1993; Spring, 1995). Other modes of action include antagonistic activity, competitive exclusion for adhesion sites or nutrients, increase digestive enzyme activity, stimulation of the

immune system, altered ammonia production and entertoxin neutralization (Jin *et al.*, 1997).

In chickens, stresses, such as transportation or overcrowding, can create a microbial imbalance. However, birds supplemented with an undefined competitive exclusion culture show a reduced number of birds that test positive for *Salmonella* by 50% over the control diet. In the birds that did test positive for *Salmonella*, the ceacal counts were 10^3 times lower than the untreated birds Newman, 1996).

Competitive exclusion cultures can either be defined (i.e. contain a limited number of known bacteria) or undefined. However, there are safety concerns associated with undefined cultures. Therefore, researchers are trying to develop a defined probiotic culture. It is unlikely that one single strain or a mixture of a limited number of strains could be as effective as an undefined culture as the exclusion of some beneficial bacteria would make the intestinal microflora less competitive against enteropathogens (Spring, 1995). In this regard, Spring (1995) showed that defined cultures reduce the risk of *Salmonella* infection to the same extent as lactose (Table 9). In comparison to undefined cultures, defined cultures had 32% more birds testing positive for *Salmonella*, with 10 times the number of colony forming units as Newman's (1996) undefined culture.

Probiotics, in theory, have the potential to replace antibiotics. However, researchers can not consistently show benefits to their use as growth promoters or antibiotic replacers. Jin *et al.* (1997) showed that body weight gain (BWG) in

TABLE 9. Effect of a defined competitive exclusion (CE) culture and lactose on *S. typhimurium* colonization in chicks[1].

Treatment	Log_{10} CFU/g cecal content	% positive birds
Control	6.26	97
5% dietary lactose	4.57	83
CE culture	4.13	87
CE culture + lactose	2.21	60

[1]Spring, 1995

broilers was significantly increased during the first 3 weeks of a growth trial but not during weeks 4 to 6. The researchers felt that the increase in BWG may have been partially due to an increased feed intake. In another trial, Cavazzoni *et al.* (1998) studied *Bacillus coagulans* for its ability to act as an alternative to antibiotics. The birds were fed either a control diet or a diet containing either virginiamycin or *B. coagulans*. They found that *B. coagulans* could be proposed as an alternative to antibiotics due to its growth-promoting, prophylactic action. Despite the positive results of probiotics, most trials have been carried out under hygienic conditions. Under large scale coordinated field trials, probiotics may not be as effective or contain the number of viable organisms that they claim (Fuller, 1989). In addition, legal restrictions to the marketing and negative public perception of bacteria will further limit the use of probiotics in animal feed (Sanders, 1998).

2.7.3.2. Prebiotics. Prebiotics are nondigestible food ingredients that beneficially affect the host and selectively stimulate the growth or activity of a limited number of bacteria in the intestinal tract (Gibson, 1998; Hasler, 1998; Roberfroid, 1998). The most common and successful prebiotics are sugar alcohols (lactulose) or oligosaccharides, containing xylose, mannose, galactose or soya maltose (Gibson, 1998). Prebiotics should be selected based on the ability to incorporate them into food vehicles, stimulate beneficial microflora while exerting anti-adhesive and attenuative properties, be administered in low doses, be derived from dietary polysaccharides, as well as being non-carcinogenic, having good

preservative and drying characteristics, and being able to regulate viscosity (Gibson, 1998). Chicory fructans, the prebiotic prototype, are fermented by bacteria in the large intestine and produce increased levels of lactic and short-chained carboxylic acids as end products. In humans, chicory fructans selectively stimulate the *Bifidobaceria* population. In addition, chicory fructans improve calcium bioavailability, due to the formation of soluble calcium and magnesium salts of short-chained acids (Roberfroid, 1998).

Prebiotic oligosaccharides are substrates for beneficial intestinal bacteria, can adsorb to certain enteric bacteria or block their adhesion site, modulate the immune system or bind mycotoxins (Spring, 1995; Spring and Privulescu, 1998; Gibson, 1998; Sanders, 1998). Many prebiotics claim to enhance the body's immune system or its natural defenses; however, there is a growing requirement to prove their efficacy (Young, 1998). One of the leading prebiotics is an oligosaccharide with mannose as the primary carbohydrate, referred to as manno-oligosaccharide (MOS). MOS is derived from yeast cell walls and have been shown to improve growth performance in both pigs (Table 10) and chickens (Table 11) when incorporated in diets as little as 1000 ppm (Spring, 1995). MOS exerts its protective abilities by blocking the attachment of certain bacteria to the intestinal wall. The addition of MOS to the diet has also led to reports of increased immunostimulatory responses in germ-free animals (Spring and Privulescu, 1998). In rats, MOS has been shown to stimulate phagocytosis and macrophage activity (Newman, 1995). Despite the relative success of MOS in

TABLE 10. Effect of Bio-MOS® on humoral immune response in germ-free and conventional piglets on selected immunoglobins in the blood serum[1]

Immunoglobulin	Piglet type	Control	Bio-MOS
IgA	germ free	163	364
	conventional	822	899
IgG	germ free	200	916
	conventional	1638	1619
IgM	germ free	-	109
	conventional	153	154

[1]modified from Spring and Privulescu, 1998

TABLE 11. Effect of manno-oligosaccharides (MOS) on *Salmonella* colonization in chicks.[1]

Treatment	Cecum		Organs	
	Colonized birds	*% colonization*	*Colonized birds*	*% colonization*
Control	38/50	76	42/50	84
MOS (1Kg/tonne)	9/50	18	14/50	28

[1]Spring, 1995

animal feeds, prebiotic products are not all equal. Therefore, research must differentiate between the various prebiotics (Sanders, 1998).

2.7.3.3. Immune system and passive antibodies. An animal's immune system consists of anatomical, physiological, complementary or phagocytic constituents. Anatomical constituents include the skin, mucous membranes, saliva, tears or mucous secretions containing antibacterial and antiviral compounds. Physiological defenses range from body temperature, pH (low gastric pH), oxygen levels to soluble proteins (lysozyme, lactoferrin, interferons and immunoglobulins such as IgA) predominant in external secretions (saliva, milk, tears, mucous secretions). The complement system is a group of serum proteins that circulate in an inactive state and provide controlled cascades, which are activated by membrane damage or cell lysis. Phagocytic defenses involve the ingestion of particles foreign to the body by lymphocytes. In addition, several trace minerals also play an important role in immunity such as zinc, iron, copper, and selenium. Zinc is required in DNA replication and synthesis and can depress immunological functions. Iron, copper and selenium are associated with antioxidant enzymes. Iron is required for myeloperoxidase found in neutrophils, which converts hydrogen peroxide to hypochlorous acid. It is also required for lymphocyte replication. Copper and selenium are also required for super oxide dismutase and glutathione peroxidase activity, respectively, which later protects the host from its own immune response (Newman, 1995).

Antibodies are responsible for the neutralization and precipitation of toxins or agglutination and lysis of bacteria. In animal production, vaccinating sows for specific strains of *E. coli* results in antibody secretion in the colostrum, offering passive immunity to the piglets. This immunity is only transient and all protection is lost after weaning. It may be possible to administer antibodies to piglets to extend their passive immunity using antibodies from bovine colostrum, animal blood, transgenic plants or microorganisms or egg yolk. Unfortunately, collecting antibodies from the colostrum of lactating mammals is impractical due to the short production period. Further, the antibody profile of animal plasma is dependent on the immunization and disease history of the animals from which the blood was collected and transgenic organisms are not well received by the public. Fortunately, egg yolks are a rich source of antibodies, containing approximately 150 mg of antibodies per yolk. Furthermore, laying hens can be induced to produce mammalian specific antibodies through hyperimmunization (Marquardt *et al.*, 1997 and Sim *et al.*, 2000).

Marquardt *et al.* (1997) have shown that egg yolk antibodies effectively offer passive immunity to piglets. In one study, Marquardt's research group challenged 20 piglets with *E. coli K88*. Ten animals were treated with antibody powder while the remaining pigs were untreated. In the control group there was a 30% mortality rate and on average lost 36 g. The antibody treated animals, on the other hand, gained 91 g, on average, over the three-day period with no

mortalities (Table 12), suggesting that antibodies imbibe passive immunity to early-weaned pigs for various strains of *E. coli*.

Although antibodies are a novel means to protect early-weaned pigs against PWD, the technology to produce monoclonal antibodies is expensive (Marquardt *et al.*, 1997). For this reason, it is not practical to add monoclonal antibody powder to animal feed on a daily basis, making antibody use limited to therapeutic applications only.

2.7.3.4. Lysozyme. Lysozyme, a ubiquitous enzyme with antimicrobial properties, is regularly consumed in eggs and added to pharmaceutical products including mouthwash, toothpaste and eye care products, as well as to food products. In cheese, lysozyme is added to prevent the formation of butyric acid during late blowing or gassing (Proctor and Cunningham, 1988). It is often added to meats, especially sausages to try to enhance the synergistic effects between lysozyme and other bactericidal agents such as nisin, or nitrates (Proctor and Cunningham, 1988). In Japan, lysozyme is used to lengthen the storage time of seafood and minimize bacterial spoilage, as it is non-toxic relative to other food preservatives (Carini *et al.*, 1985). Currently, various industries, including aquaculture, are examining the potential of lysozyme to control infection in animal populations. Modified forms of lysozyme may be one of the first lines of defenses towards bacterial infections in humans and animals (Gibbins and Losos, 1998).

TABLE 12. Response of newborn piglets challenged with different strains of Escherichia coli with or without antibody treatment[1]

Strain	Antibody Titer	Piglets with diarrhea/total			Dead/ Total
		Day 1	*Day 3*	*Day 5*	
K88	0	7/7	4/4	1/1	6/7
	2500	3/7	0/7	0/7	0/7
K99	0	4/4	0/0	0/0	4/4
	2500	3/4	0/4	0/4	0/4
987P	0	5/5	1/1	1/1	4/5
	2500	4/5	0/5	0/5	0/5

[1]modified from Marquardt *et al.*, 1997

Lysozyme is commercially isolated from domestic avian eggs; although, the highest concentration of lysozyme is found in tears (with an activity greater than 200 mg of hen egg-white lysozyme per mL of tears) (Proctor and Cunningham, 1988; Brightman *et al.*, 1991). It can also be found in several fruits and vegetables including cauliflower (27.6 μg/mL), broccoli (8.1 μg/mL) and papaya (7.9 μg/mL) (Proctor and Cunningham, 1988). The isolation of lysozyme from avian eggs uses adsorption chromatography and yields a recovery rate of 99% with fresh egg white. Lysozyme's greatest activity, as measured via lysis of *Micrococcus lysodeikticus*, is greatest when all four disulfide bonds are intact and the pH is between 3.5 to 7.0. Gram-positive bacteria, which have high concentrations of 1,4-linked NAM and NAG residues in their peptidoglycan, are more susceptible to lysozyme attack. However, in gram-negative bacteria, lysozyme has difficulty reaching the peptidoglycan layer due to a hydrophobic outer membrane.

Lysozyme, a 1,4-β-N-acetylhexosaminidase, catalytically cleaves N-acetylmuramic acid (NAG) and N-acetylglucosamine (NAG) residues preferentially in gram-positive bacteria (Proctor and Cunningham, 1988; Cunningham *et al.*, 1991). The mechanism of lysozyme is very specific. First it attaches to the bacterial wall by interacting with six exposed amino sugar residues connected by β–1,4 linkages joining the fourth and fifth rings of the polysaccharide chain. The fourth residue is distorted and the glutamate, residue 35 in the lysozomal active site, transfers a proton (H^+) to the glycosidic oxygen.

The resulting cleavage between the oxygen and carbon-1 atom of the fourth sugar residue creates a positively charged carbanion. This carbanion is stabilized by the negatively charged aspartic acid (52) side chain of lysozyme until it can combine with a hydroxyl ion that diffuses into position from the surrounding water. To complete the reaction, the solute replaces the hydrogen ion lost by glutamate (residue 35). Lysozyme then falls away, leaving a punctured bacterial cell wall (Proctor and Cunningham, 1988).

There are many factors that influence the activity of lysozyme including lipovitellin, temperature and water activity. Lipovitellin, an egg yolk protein, inhibits lysozyme. This inhibition is so strong that contamination of egg albumen by as little as 10% egg yolk will inhibit lysozyme activity. Temperatures greater than 60°C will also reduce lysozyme activity. Overall, lysozyme is more heat stable in phosphate buffers than in egg white probably due to the sulfhydryl groups of ovalbumen, which can reduce one or more of the disulfide bonds in the lysozyme molecule. One way to prevent thermal denaturation of lysozyme is to limit the water activity. Water migrates to the center of the lysozyme molecule causing the peptide to swell and eventually uncoil (Proctor and Cunningham, 1988).

Several research groups are trying to augment the antimicrobial activity of lysozyme especially against pathogenic gram-negative bacteria (Proctor and Cunningham, 1988, 1993; Yang and Cunningham, 1993; Ibrahim *et al.*, 1993, 1994a,b, 1996a,b). To this extent, lysozyme has been studied alone and in

combination with other antimicrobial agents. Proctor and Cunningham (1993) showed that synergistic interactions between lysozyme and other antimicrobials were species and system specific. For example, lysozyme in combination with nisin was not synergistic *in vitro* or in hamburger, but was in hotdogs. Yang and Cunningham (1993) found that EDTA in combination with lysozyme was effective against gram-positive and gram-negative bacteria. They believed that EDTA partially disrupts the outer membrane, thus allowing lysozyme to permeate and catalytically cleave the peptidoglycan layer. Other efforts to augment the activity of lysozyme against gram-negative bacteria have focused on chemical-modification of lysozyme's structure. Modification of lysozyme with hydrophobic moieties, such as palmitic acids to the lysyl residues (Ibrahim *et al.*, 1993), pentapeptides (Phe-Phe-Val-Ala-Pro) to the C terminus (Ibrahim *et al.*, 1994b) or modification with perillaldehyde (Ibrahim *et al.*, 1994a), allows improved passage of lysozyme through the lipopolysaccharide-rich outer membrane of gram-negative species. More recently, Ibrahim *et al.* (1996a, b) found that heating lysozyme in a 10 mM phosphate buffer for 20 minutes at 80°C produced a stable, irreversible conformational change, in which lysozyme retained 50% of its native enzymatic activity (Ibrahim *et al*, 1996a, b). When heat denatured lysozyme was incubated with gram-negative bacteria, it reduced survival by 30-60% over native lysozyme (Table 13).

Heat denaturation of lysozyme, at temperatures greater than 72°C, results in conformational changes associated with the cleavage or isomerisation of the

TABLE 13. Bactericidal action of native lysozyme (Lz) and thermally denatured lysozyme at 80°C for 20 minutes[1].

Bacteria	% Survival of bacteria	
	Native Lz	*Heat Treated Lz*
Gram-positive		
Staphylococcs aureus	0.9	0.8
Streptococcus mutans	18.6	0.6
Bacillus cereus	0.0	0.0
Gram-negative		
Escherichia coli	72.7	13.0
Proteus mirabilis	61.2	25.4
Salmonella enteritidis	97.5	59.2

[1]Modified from Ibrahim *et al.*, 1996a

disulphide bonds. Florescence spectroscopy (280 nm) in an aqueous solution confirmed the conformational changes, which were irreversible. Surface hydrophobicity studies showed an increase in hydrophobicity associated with the conformational changes, with the maximum change occurring after heating lysozyme for 20 minutes. It is believed that the change in surface hydrophobicity allows lysozyme to more easily permeate through the outer membrane of gram-negative bacteria (Ibrahim *et al.*, 1996a). In addition, conformational changes appear to increase the general affinity lysozyme has for membranes. For this reason, heat-treated lysozyme may act through an agglutination mechanism causing subsequent killing of bacteria through membrane disturbances (Ibrahim *et al.*, 1996b). In 1997, Ibrahim *et al.* proposed that lysozyme may remove divalent cations, Ca^{2+} or Mg^{2+}, from the outer membrane. Divalent cations are an integral component in stabilizing electrostatic interactions between lipopolysaccharides (LPS) and carboxyl groups of membrane proteins (Ibrahim *et al.*, 1996b). In sharp contrast to the theory proposed in 1997 (heat treated lysozyme competes with bacteria for divalent cations), Ibrahim (1998) proposed that heat treated lysozyme operates through a promoted binding affinity, and in fact competes with calcium (II) for binding sites on the outer bacterial membrane. Through studies with mutant T4 lysozyme and synthetic peptides, During *et al.* (1999) found that the amphipathic helical regions of heat denatured lysozyme insert into and destabilize bacterial membranes. Although a study by During *et al.* (1999) supported Ibrahim's earlier proposed mechanism, it did not eliminate a

third theory. The ability of lysozyme to interact with calcium binding sites, may induce the release of calcium from the bacterial membrane activating endogenous autolysins which in turn can hydrolyze the peptidoglycan (Ibrahim *et al.*, 1997).

Other means known to alter the antimicrobial effects of lysozyme included forming lysozyme-dextrin conjugates which increase lysozyme activity against both gram-positive and gram-negative bacteria (Nakamura *et al.*, 1990). Gamma radiation of egg white, on the other hand, is known to decrease lysozyme activity with increasing radiation doses (Proctor and Cunningham, 1988). Radiation causes polymerization through covalent bonds and was shown to inactivate a 0.3% lysozyme solution exponentially at pH 8 (Proctor and Cunningham, 1988). Despite this effect, radiation may offer yet another means to increase lysozyme's activity towards gram-negative bacteria, similar to the thermal denaturation described by Ibrahim *et al.* (1996a, b, 1997).

2.8. Regulation of microflora through natural dietary components

Researchers have long observed complex interactions between feed components, such as carbohydrates and natural proteins or enzymes, growth performance and the resistance to various bacteria. In some cases, carbohydrates such as β–glucans can decrease the amount of interleukin-1 (IL-1), a key cytokine in an animal's resistance to bacterial disease. Nutrients normally partitioned to produce IL-1 could then be put into growth. There is a fine balance between immune response and optimum growth (Dritz *et al.*, 1995).

If the immune system is compromised, to allow for increased growth rates, susceptibility to disease is increased. When considering carbohydrate sources in the diet, the inclusion of soluble non-starch polysaccharides and resistant starch should be kept to a minimum. Proteins and enzymes have also offered a potential means to improve the immuno-competence of animals. Proteins added may include egg yolk, an antibodies and nutrient source to prevent post-weaning diarrhea (Marquardt *et al.*, 1997), or may be more complex and utilize a combination of cationic antimicrobial peptides, which have a broad spectra of activity against gram-negative and -positive bacteria, fungi (including yeasts), parasites and viruses. These peptides, produced through exposure to proinflammatory cytokines, LPS or bacteria, initially interact with the polyanionic surface of LPS to competitively displace divalent cations and then insert into the membrane, effectively creating a channel through the cell wall to severely inhibit or kill the cells. Due to their mechanism, polycationic peptides often show synergy with conventional antibiotics and lysozyme against gram-negative and -positive bacteria. However, their therapeutic potential is limited due to the cost of production and the potential to be very toxic to mammalian cells (Hancock and Scott, 1997). One of the most promising alternatives to the use of antibiotics or polycationic peptides is the dietary inclusion of spray-dried egg whites that have been treated to induce the novel antimicrobial properties of lysozyme. Since eggs are an excellent source of nutrients and contain lysozyme, the potential to optimize lean growth and modify the gastrointestinal microflora is substantial.

3. Manuscript 1

THE NUTRITIVE VALUE OF EGG BY-PRODUCTS AND THEIR POTENTIAL BACTERICIDAL ACTIVITY: *IN VITRO* AND *IN VIVO* STUDIES.

3.1. Abstract

Technical grade egg albumen and whole egg by-products are produced in substantial quantities by many egg-breaking facilities across North America. The by-products are recovered during processing and, for various reasons, are not approved for human consumption. Both products are known to be rich in fat, maternal antibodies, protein (a well-balanced AA profile) and lysozyme (a bactericidal enzyme). The objective of this research was to explore the potential for egg by-products to be used as a valuable protein, energy and due to the presence of lysozyme, bactericidal supplement in animal nutrition. To optimize the conditions for improving the nutritive value of technical albumen and whole egg by-products, the spray-dried samples were heat treated at various temperatures. The effect of heat treatment on digestible protein content was determined *in vitro* using a pepsin-pancreatin digestion/dialysis system. The optimum protein digestibility value for technical albumen was found to approach 80% when heated at 105°C for 10 minutes. Only 50-55% of the protein was digestible for the spray-dried (unheated) products. Commercial heat treatments (i.e. storage of spray-dried albumen in a hot room) showed minimal improvements in digestible protein content (60%). The beneficial effect of heat treatments was further explored using pure lysozyme preparations. In this study, the survival rate of *Escherichia coli 0157:H7* and *Salmonella typhimurium 266*, when incubated with thermally modified lysozyme was lowered to 0.09 and 13.97%, respectively, compared to their survival rate when incubated with native lysozyme. In addition, γ-radiation of technical albumen produced a 60%

reduction in the population of *E. coli* as compared to the untreated sample. Feeding of raw, spray-dried technical albumen (SDTA) and SDTA subjected to radiation (1.8 kGy) or heat treatment (80°C) resulted body weight gain and protein utilization which were equal to or higher than that of the control. A negative effect of hot-room storage on protein utilization and rat growth was noted. In addition, feeding spray-dried whole egg resulted in poor performance of growing rats with body weight gain and protein utilization being lower than values observed for either the control or the SDTA diets. This was substantiated by poor digestibility of essential AAs including lysine, threonine and to some extent methionine as determined at the distal ileum of rats fed SDWE. Heat or radiation treatments of lysozyme also reduced *Enterobacteriaceae* counts *in vivo* when compared to unheated or non-radiated raw technical albumen indicating an enhanced antimicrobial effect.

3.2. Introduction:

Egg-breaking facilities produce substantial amounts of egg by-products each year that are unsuitable for human consumption as regulated by the Canadian Food Inspection Agency. Egg by-products such as technical albumen, recovered from egg shells through centrifugation that contains egg yolk, and whole egg have received little attention as animal feedstuffs despite their excellent AA profile, energy content and the presence of antimicrobial proteins (*i.e.*, lysozyme).

Based on the AA profile, egg by-products are an excellent source of SAAs. However, eggs contain antinutritive factors (ANF) that may affect the nutritive value of the egg products. It has been documented that thermal denaturation of the egg by-products could further optimize protein digestibility and reduce the impact of ANF (Herkelman *et al.*, 1993).

In addition to an excellent AA profile, egg albumen contains lysozyme. Lysozyme is composed of 129 AAs, cross-linked with four disulfide bonds (Cunningham *et al.*, 1991). Through its active site, lysozyme catalytically cleaves the N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) residues preferentially in gram-positive bacteria. Several studies have investigated the activity of lysozyme against gram-negative bacteria (Proctor and Cunningham, 1988, 1993; Ibrahim *et al.*, 1993; Yang and Cunningham, 1993; Ibrahim *et al.*, 1994a, b). More recently, Ibrahim *et al.*, (1996b) showed that thermally denaturing lysozyme invokes a novel antimicrobial mechanism, which is independent of its catalytic activity (native lysozyme). Heat-treated lysozyme is

believed to be able to better interact with the bacterial membrane of gram-negative bacteria, significantly reducing their survival *in vitro* (Ibrahim *et al.*, 1996a, b, 1997; Ibrahim, 1998).

The objectives of the current study were (1) to determine the nutritive value of technical albumen and whole egg by-products, (2) to investigate the effect of heat-treatment and γ-radiation on the nutritive value of egg by-products and (3) to induce the bactericidal activity of lysozyme against pathogenic bacteria through heat treatment and γ-radiation. To meet these objectives a series of *in vitro* protein digestibility trials, *in vitro* bacterial assays and an *in vivo* rat assay were utilized.

3.3. Materials and Methods

3.3.1. Laboratory prepared raw eggs

Raw eggs where obtained from the University of Manitoba layer hen barn. To prepare raw albumen samples, eggs were broken, separated, the yolk discarded, the albumen placed in a large tray and then frozen (-20°C) and freeze-dried. In preparation of the whole egg product, the eggs were broken, mixed and freeze-dried. All freeze-dried products were then ground with a mortar and pestle and thoroughly mixed to ensure homogeneity.

3.3.2. Commercially prepared egg by-products

Spray-dried technical albumen (SDTA) and spray-dried whole eggs (SDWE) were obtained from Canadian Inovatech Inc., Winnipeg, MB, Canada. Approximately 40 – 45 g of egg albumen and 35 – 40 g of whole egg were

evenly but loosely distributed, in glass petri dishes to a depth of 1 cm. Following autoclave heating (American Sterilization Co. model AS-OIT616G3E) for 10 minutes at temperatures of 100, 105, 110, 115, and 120°C, the protein digestibility was determined (Section 3.3.3.). The effect of hot-room storage on *in vitro* protein digestibility was also explored utilizing the resources of Canadian Inovatech Inc. Both SDTA and SDWE were heat treated in a hot room at 70°C for 1, 2, 3, 5 and 14 days. In addition, raw liquid TA was obtained from Canadian Inovatech Inc. and freeze-dried for incorporation in the rat assays.

3.3.3. In Vitro Protein Digestibility Studies

The raw, spray-dried and spray-dried hot-room stored egg products were analyzed for digestible protein content using the *in vitro* digestion technique described by Slominski *et al.* (1999). Samples (3 g) were incubated in an environmentally controlled incubator shaker with 50 mL of a 0.1M HCl/ 54 mmol NaCl solution containing 500 mg of pepsin (P-7000, Sigma, St. Louis, MO, USA) for one hour at 40°C. Following pepsin-digestion, the pH was adjusted to approximately 7.0 with 2.5 mL of 2.0 M NaOH. The pH was stabilized by adding 20 mL of a 0.1 M phosphate buffer followed by the addition of 250µL phosphate buffer solution containing 25 mg of pancreatin (P-7545, Sigma, St. Louis, MO, USA). The contents were then transferred into pre-soaked dialysis tubing (Spectrum, Houston, TX, USA) with a molecular cut off value of 12,000 – 14,000. The tubes were closed allowing for a small air gap in the tube to facilitate continued mixing of the contents. To simulate the environment of the small intestine and to minimize the effect of end product inhibition, pancreatin protein

digestion was performed using the digestion/dialysis unit filled with 0.05 M phosphate buffer (pH 7.0). Pancreatin digestion was conducted for 6h at 40°C. After incubation, replacing the buffer with ice-cold distilled water terminated enzyme activities and the contents were subjected to dialysis for 72 h, with continuous rotation of the tubes. The water was changed regularly at 12 h intervals. Following dialysis, the contents were frozen, freeze-dried and analyzed for crude protein (Kjeldahl N x 6.25). Digestible protein content was calculated as total sample protein minus protein retained in the dialysis tubing. Each sample was analyzed in triplicate.

3.3.4. *In vitro* bacterial assays

3.3.4.1. Maintenance of bacterial cultures. *Escherichia coli O157:H7* and *Salmonella typhimurium 266* were obtained from Dr. G. Blank, Department of Food Science, University of Manitoba, Winnipeg, MB. Cultures were maintained on trypticase soy agar slants (TSA, pH 7.0; BBL, Cockeysville, MD) at 4°C and were activated by transferring loop inocula into brain heart infusion broth (BHI, Difco Laboratories, Detroit, MI) and incubated for 16 h at 35°C.

In order to determine the time required to reach midlogarithmic growth each overnight culture (1 mL) was subsequently transferred to fresh BHI (99 mL) and a time course study (60 min intervals over 8-10 h) initiated. In this respect the increase in absorbency of the broth was related to time. Absorbancy was determined at 540 nm using a spectrophotometer (Pharmacia Biotech Ultrospec 2000). Sterile non inoculated BHI was used a blank.

3.3.4.2. Preparation of Cultures. Loop inocula were transferred from TSA slants and incubated at 35°C for 16 h in BHI (99 mL). Overnight culture (1 mL) was subsequently inoculated into fresh BHI (99 m) and incubated for 3-4 h at 35°C. Resultant midlogarithmic cultures were centrifuged (3000 x g, 7 min, 4°C) and the pellets suspended in sufficient sterile, sodium phosphate buffer (10 mM) in order to attain an absorbency of 0.1 at 675 nm (Pharmacia Biotech Ultrospec 2000). Viable plate counts using TSA (24 h, 35°C) indicated that this absorbancy corresponded to c. 10^6 colony forming units (CFU) / mL. The standardized cultures were subsequently used to assess the antimicrobial effectiveness of treated lysozyme and technical albumen.

3.3.4.3. Preparation of treated lysozyme and technical albumen

3.3.4.3.1. Thermal treatment. Lysozyme (1 mg; minimum of 22800 Shugar units/mg; Canadian Inovatech Inc. Abbottsford, BC) was dissolved in sodium phosphate buffer, pH 6.9 (100 mL, 10 mM) and heated at 80°C for 10, 20 or 30 min. Following heat treatment the lysozyme solutions were rapidly cooled in an ice bath. No lysozyme precipitate was formed as was reported by Ibrahim *et al.* 1996 a, b; 1997).

Spray dried technical albumen (SDTA), previously hot room pasteurized at 70°C for 0 (control), 3 or 14 d, was supplied by Canadian Inovatech, and prepared in phosphate buffer, pH 6.9 (100 mL, 10 mM). Based on the lysozyme content of the control (1.6 % w/w), it was calculated that 7 g would be required in order to yield a 1 mg/100 mL lysozyme solution (comparable to that of the pure lysozyme solution). However, due to the bulkiness (difficulty to dissolve

completely into solution) of SDTA, 4 g /100 mL solutions were prepared for each of the hot room pasteurized samples. This correspond to 0.64 mg/mL lysozyme for the control.

3.3.4.3.2. Gamma radiation. Pure lysozyme (2 mg /mL) was gamma radiated at dosages of 0, 0.8, 1.2, 1.4, 1.6 and 2.0 kGy (Gamma Cell 220, Acsion Industries, Pinawa, MB). Following treatment the lysozyme solution was further diluted in phosphate buffer (1 mg/ mL). In the case of technical albumen two sources were employed: liquid and SDTA (non-hot room pasteurized). The lysozyme content of the samples was c. 3.50 and 2.83 mg/mL. In each case radiation was carried out at dosages previously given.

3.3.4.4. Antibacterial Activity Assay. Equivolumes (1.0 mL) of standardized culture and heat treated lysozyme solutions were combined in sterile test tubes, stirred gently and incubated at 35°C. After 60 min incubation samples were withdrawn from each heat treated solution, rapidly cooled and serially diluted using saline (0. 85%). Survivors were determined following direct plating using TSA and incubation at 24 h for 35°C. Results are expressed as % survival rate. A similar protocol was employed for the technical albumen samples with the exception that sampling was performed at 0, 20, 40, 60, 80, 100 and 120 min. A similar protocol was used for radiated treated samples at 35°C. Plates were examined for homogeneity of colonies. One colony was selected and aseptically streaked onto an agar slant. The agar slant was incubated for 24 hours at 35°C, and then stored at 4°C for a 1 to 2 month period.

3.3.4.5. Determination of catalytic activity. The catalytic activity of lysozyme was determined using the method described by Shugar (1952). Samples were dissolved to a concentration of 0.015 mg/mL in a 1 M phosphate buffer (pH 6.2). A *Micrococcus lysodeikticus* substrate was prepared in phosphate buffer to have an absorbance of 1.7 ± 0.1 at 450 nm. To 2.9 mL of the substrate (*M. lysodeikticus*), 100 µL of the standard or sample was added. The change in absorbance was recorded over the following three minutes, disregarding readings from the first minute to allow the test to equilibrate. The catalytic activity (lower detection limit of 100 units/mg) was then determined from the change in absorbance, and calculated based on sample weight, dilution factor, time (3 minutes) and Beer's constant (ε=0.001) using the following formula:

$$\frac{(\text{Absorbance}_{\text{initial}} - \text{Absorbance}_{\text{final}})(\text{Dilution factor})}{(\varepsilon)\,(\text{time})(\text{concentration of lysozyme solution})(\text{volume of lysozyme solution})}$$

3.3.5. Rat Study

3.3.5.1. Experimental design and housing

One hundred twenty weanling male Sprague-Dawley rats (initially 77.6 ± 7.3 g) were obtained from the Central Breeding Facility, University of Manitoba, Winnipeg, MB, Canada and were used to determine the protein efficiency ratio (PER) of several heat treated technical grade egg products. After a three-day adaptation period, rats were assigned by weight to one of twelve dietary treatments in a completely randomized block design, allotting 10 rats per treatment and one rat per cage. All rats were housed in an environmentally controlled room with an average temperature of 23°C. Rats were subjected to a

12-hour light-dark cycle with *ad libitum* access to feed and fresh water. The twelve experimental diets were formulated to meet AIN-93 requirements (Reeves et al., 1993) and fed over a 4-week period. The diets consisted of a casein control, casein + native lysozyme, casein + heat-treated lysozyme, raw technical albumen, spray-dried technical albumen (SDTA), SDTA heated for 20 min at 80°C, SDTA stored in a hot room (70°C) for 3 days, SDTA radiated (1.8 kGy), SDTA pelleted, SDTA stored in a hot room and pelleted, spray-dried whole egg (SDWE) and SDWE stored in a hot room (70°C) for 3 days. The diets contained 11.1% protein, 0.50% Ca, 0.27% phosphorous, 0.36% methionine, 0.84% lysine (Table 14). Selected diets were steam pelleted by Dr. D. Higgs of the Department of Fisheries and Oceans, Vancouver, BC, Canada, using a small-scale pelleter and a conditioner temperature of 80°C.

Rats were weighed three times a week on days 0, 3, 5, 7, 10, 12, 14, 17, 19, 21, 24, 26, and 28 to determine growth rates. Feeders were weighed on days 7, 14, 21, and 28 to determine feed intake. At the end of the experimental period, rats were euthanized using carbon dioxide asphyxiation, in a manner approved by the University of Manitoba Animal Care Committee, to determine liver and kidney weights and collect digesta from the distal ileum for bacterial and AA analyses.

3.3.6. Chemical and Bacterial analyses

3.3.6.1. *In vivo Enterobacteriaceae* determination. Ileal digesta was collected form the terminal 10 cm of the small intestine into sterile containers. Digesta (1g), pooled from 5 animals, was serially diluted using saline (0.85%) and plated

TABLE 14. Composition and calculated analysis of experimental diets used in the rat trial (Experiment 1).

	Mash Diets										Pelleted Diets	
	1	2	3	4	5	6	7	8	9	10	11	12
Ingredient (%)												
Casein	13	13	13									
Native lysozyme		0.007										
Heat-treated lysozyme			0.007									
Raw technical albumen				17.2								
Spray-dried technical albumen (SDTA)					17.2						17.2	
SDTA heat treated (80°C; 20 min)						17.2						
SDTA stored in a hot room (3d;70°C)							17.2					17.2
SDTA radiated (1.8 kGy)								17.2				
Spray-dried whole egg (SDWE)									25.6			
SDWE stored in a hot room (3 d; 70°C)										25.6		
Cornstarch	47.106	47.099	47.099	45.492	45.492	45.492	45.492	45.492	43.842	43.842	45.492	45.492
Dextrinized cornstarch	14.15	14.15	14.15	14.75	14.75	14.75	14.75	14.75	10	10	14.75	14.75
Sucrose	10	10	10	10	10	10	10	10	10	10	10	10
Soybean oil	5.5	5.5	5.5	2.5	2.5	2.5	2.5	2.5	0.5	0.5	2.5	2.5
Mineral mix[1]	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Vitamin mix[2]	1	1	1	1	1	1	1	1	1	1	1	1
Choline bitartrate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
BHT	0.014	0.014	0.014	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
Alphacell	5	5	5	5	5	5	5	5	5	5	5	5
Cysteine	0.18	0.18	0.18									
Chromium oxide	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Calculated Compostion												
Metabolizable energy (kcal/kg)	3993	3993	3993	4036	4036	4036	4036	4036	4321	4321	4036	4036
Protein (%)	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1
Fat (%)	5.5	5.5	5.5	5.15	5.15	5.15	5.15	5.15	12.1	12.1	5.15	5.15
Calcium (%)	0.50	0.50	0.50	0.53	0.53	0.53	0.53	0.53	0.56	0.56	0.53	0.53
Available phosphorus (%)	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.41	0.41	0.29	0.29
Cystine (%)	0.20	0.20	0.20	0.38	0.38	0.38	0.38	0.38	0.72	0.72	0.38	0.38
Methionine (%)	0.36	0.36	0.36	0.56	0.56	0.56	0.56	0.56	0.39	0.39	0.56	0.56
Lysine (%)	0.95	0.95	0.95	0.90	0.90	0.90	0.90	0.90	0.84	0.84	0.90	0.90

[1]Mineral premix provided, per kg complete diet: Ca, 0.5%; P, 0.156%; K, 0.36%; S, 0.03%; Na, 0.102%; Cl, 0.157%; Mg, 0.051%; Fe, 35 mg; Zn, 30 mg; Cu, 6 mg; I, 0.2mg; Mb, 0.15 mg; Se, 0.15 mg; [2]Vitamin premix provided, per kg complete diet: nicotinic acid, 30 mg; pantothenate, 15 mg; pyridoxine, 6 mg; thiamin, 5 mg; riboflavin, 6 mg; folic acid, 2 mg; vitamin K, 750 µg; D-biotin, 200 µg; Vitamin B_{12}, 25 µg; vitamin A, 4000 IU; vitamin D_3, 1000 IU; vitamin E, 75 IU.

on violet red bile agar (Becton Dickinson, Cockeysville, MD, USA) using a pour plate-overlay technique. Incubation was for 24 h at 35°C. Results were reported as CFU/g digesta (average of triplicate determination).

3.3.6.2. Chromic Oxide Analysis. Diets and ileal digesta were analyzed for chromium content using atomic absorption spectroscopy as described by Williams *et al.* (1962). Diet (1 g) or 0.3 to 0.4 g of digesta was weighed into silica crucibles and ashed for 12 h at 600°C. After cooling, 3 mL of 0.3% w/v manganese sulfate in 85% phosphoric acid was added to the ashed material, followed by 4 mL of 4.5% w/v potassium bromate solution to oxidize the chromic oxide to free chromium. The solution was then heated until the indicator (manganese sulfate/phosphoric acid solution) turned purple. The crucibles were then removed from the heat source and allowed to cool. The chromium solution was then rinsed into a 200 mL volumetric flask containing 25 mL of a 4000 p.p.m. calcium chloride solution, and brought up to volume with deionized water. Known volumes of a standard chromium solution were used to create a calibration curve for atomic absorption analysis.

3.3.6.3. Amino acid analysis. Ileal digesta and diets were analyzed for AA content to determine AA digestibility on an LKB Biochrom 4151 Alphaplus AA analyzer, using an ion-exchange column (Biochrom, Science Park, Cambridge, UK). Most AAs were analyzed using the standard hydrolysis procedure (see below), while cystine and methionine were analyzed using the oxidized hydrolysis procedure. Samples were not analyzed for tryptophan.

3.3.6.3.1. Standard hydrolysis procedure. Ground ileal digesta and diets (100 mg dry matter basis) were weighed into hydrolysis tubes. To each sample, two drops of 2-octanol and 4 mL of 6 M HCl were added. The hydrolysis tubes were capped and evacuated for 60 seconds. After tightly securing the lids, the hydrolysis tubes were placed in an electrically heated block for 24 h. After samples were cooled to room temperature, 4 mL of 25% w/v NaOH was added. The samples were allowed to return to room temperature before diluting with sodium citrate buffer (pH 2.2) to a total volume of 50 mL. After vigorously mixing the samples, 10 mL were filtered through Whatman #40 filter paper (Andrews and Balder, 1985). Samples (50 μL) were then loaded onto the ion exchange column.

3.3.6.3.2. Oxidized Hydrolysis Procedure. Two drops of 2-octanol was added to the pre-weighed samples (100 mg of ileal digesta or diets). Performic acid was prepared one hour in advance by combining 35% hydrogen peroxide and 88% formic acid in a 1:9 ratio. Performic acid (2 mL) was added to the samples, which were then placed in the refrigerator (4°C) for 20 hours. To stop the oxidation, 0.5 mL of 12 M HCl was added to the samples. After standing for 6 h at room temperature, with occasional mixing, an additional 2 mL of 12M HCl was added to the oxidation tubes. The tubes were then placed in the electrically heated block (110°C) for 16 h. The samples were removed from the block the following day and allowed to cool to room temperature before being neutralized with 2.5 mL NaOH (25% w/v). After neutralization the samples were diluted to 50 mL with sodium citrate buffer (pH 2.2), mixed vigorously, and filtered through

Whatman #40 filter paper (Moore, 1963). Samples (50 μL) were then loaded onto the ion exchange column for analysis.

3.3.7. Statistical Analysis

All collected data were subjected to analysis of variance (ANOVA) using the general linear model procedure of the Statistical Analysis System (SAS, 1998) and the procedures of Snedecor and Cochran (1980). Duncan's multiple range test was used to compare and separate treatment means (Duncan, 1955).

3.4. Results:

3.4.1. *In vitro* Protein Digestibility Study

In an attempt to improve protein digestibility, the actual temperatures achieved in processing the eggs were 100.5, 105.6, 108.5, 109.8, 112.5, 116.6 and 122.0°C. As shown in Fig. 3, protein digestibility of raw egg albumen averaged 39%. With mild heat treatment (spray drying), protein digestibility increased to 53%. A further increase in protein digestibility was observed for samples heat-treated at higher temperatures. No effect of heat treatment on digestible protein content was observed in whole eggs when compared to that of the spray-dried product (Fig. 3). The optimum protein digestibility value for technical albumen and whole egg by-products approached 80% when heated at 100 and 105°C, respectively.

As a consequence of improved protein digestibility of egg albumen heat treated at temperatures higher than that in spray drying (i.e., 50-60°C), commercially viable options of heat treatment were discussed with the representatives of Canadian Inovatech Inc. (Winnipeg, MB). Storage of

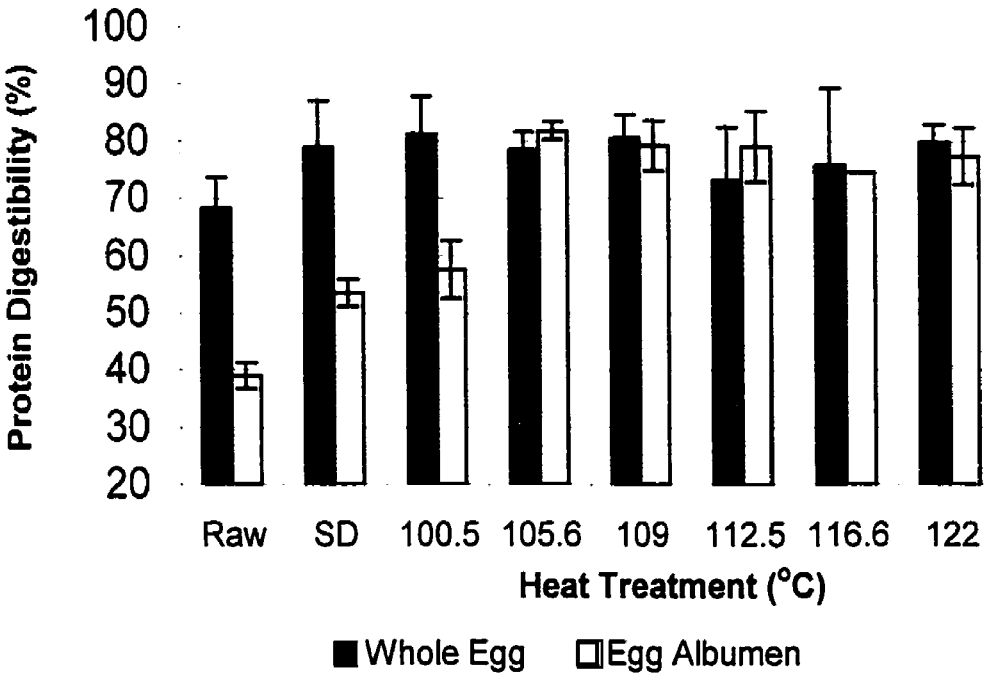


Figure 3. Digestible protein content of raw, spray-dried (SD) or SD and heated (various temperatures; 10 min) egg albumen and whole eggs. Bars represent SEM (n=3).

technical albumen and whole egg products in a hot room (70°C) for 1, 2, 3, 5 and 14 days was chosen as means of modifying the three-dimensional protein structure and thus improving digestibility. Following hot-room storage, the samples were subjected to *in vitro* protein digestibility analysis. No significant effect of hot-room storage on digestible protein content was noted (Fig. 4).

3.4.2. In vitro Lysozyme Studies

In addition to the effects on protein digestibility other beneficial effects of heat treatment were further explored using a pure lysozyme preparation.

3.4.2.1.Determination of midlogarithmic growth for *E.coli* and *S. typhimurium.* Midlogarithmic growth was determined at 540 nm in BHI broth. As shown in Fig. 5, both *E. coli O157:H7* and *S. typhimurium 266* reached midlog phase after incubation for 3 to 4 h.

3.4.2.2. Effect of heat-treatment and radiation on the bactericidal activity of lysozyme. As shown in Fig. 6, heating induced a novel bactericidal activity of lysozyme against both *E. coli* O157:H7 and *S. typhimurium* 266 with an optimum heating time of 20-30 minutes. In this study, the survival rate of pathogenic *E. coli* and was lowered from 87.3 and 80.9% when incubated with native lysozyme to 0.1 and 11.3% when incubated with thermally modified lysozyme, respectively. In addition pure lysozyme was also subjected to γ–radiation. As shown in Fig. 7, following radiation at 1.4 kGy there was a tendency for lysozyme to decrease the survival of *E. coli* and *S. typhimurium* to 11.4 and 16.4% relative to native lysozyme (control).

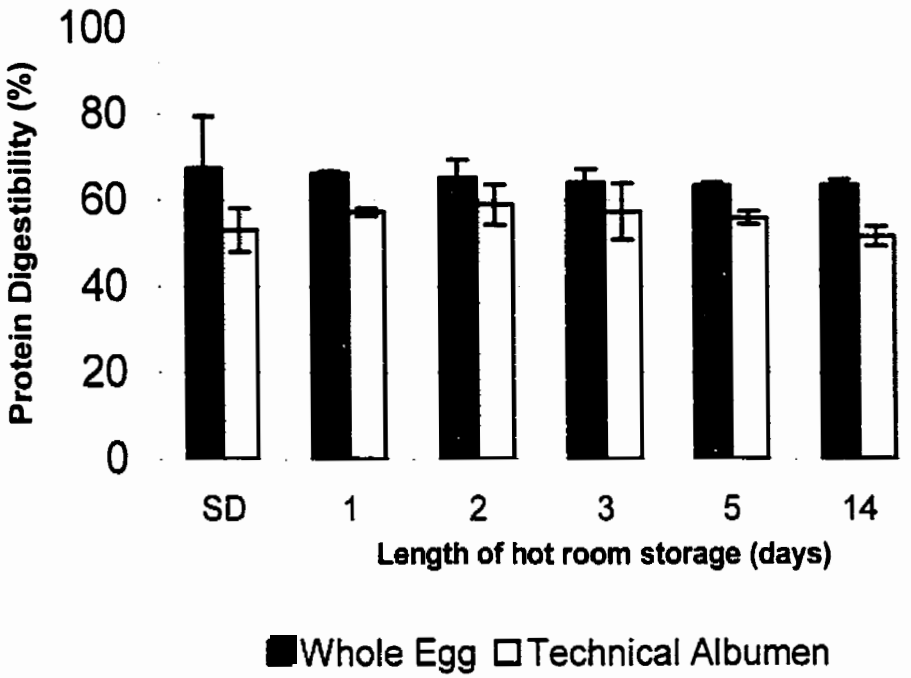
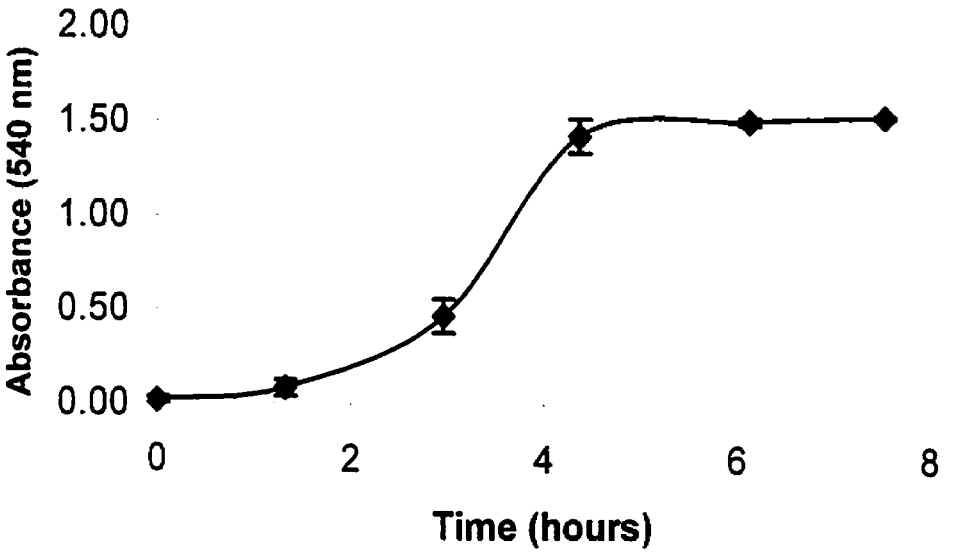


Figure 4. Protein digestibility of spray-dried (SD) technical albumen and whole eggs stored in a hot room (70°C) for various lengths of time. Bars represent SEM (n=3).

A. *Samonella typhimurium 266*



B. *Escherichia coli O157:H7*

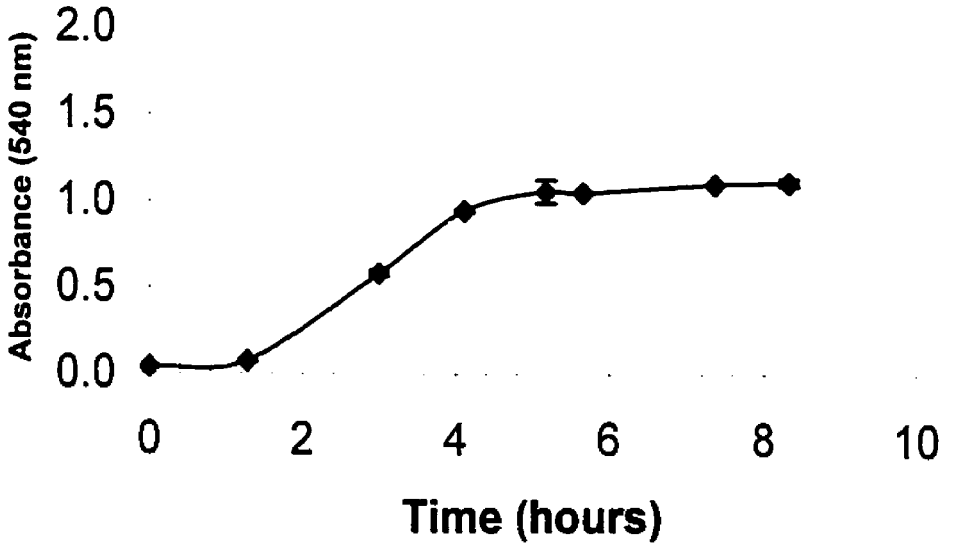


Figure 3. Determination of the midlogarithmic phase of *Salmonella typhimurium* 266 (A) and *Escherichia coli O157:H7* (B).

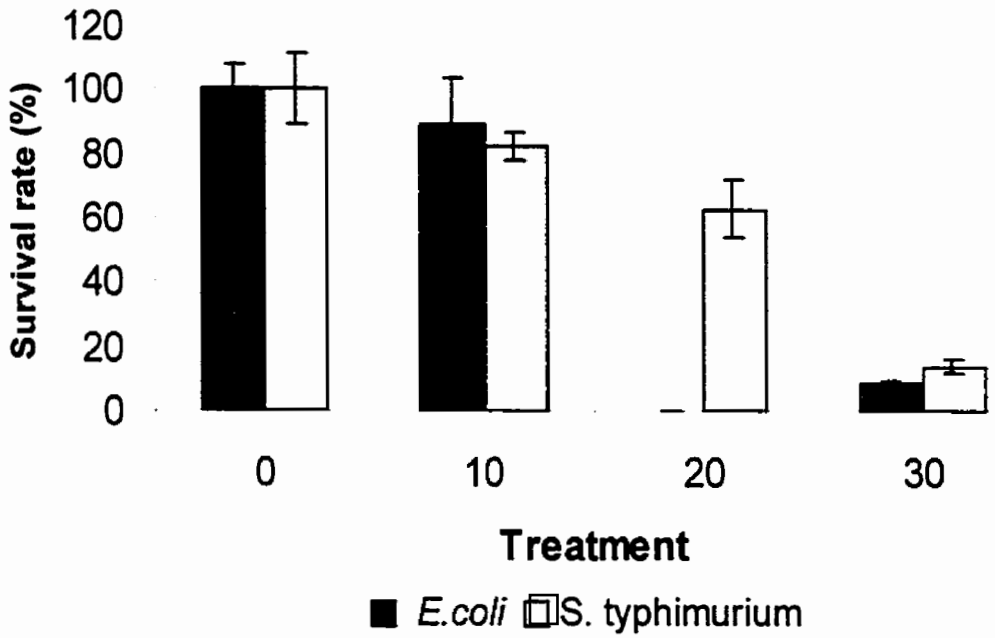


Figure 6. Percent survival of *Escherichia coli O157:H7* and *Salmonella typhimurium 266* following 60 min incubation in lysozyme previously heat treated for 10, 20 and 30 min at 80°C. Bars represent SEM (n=9).

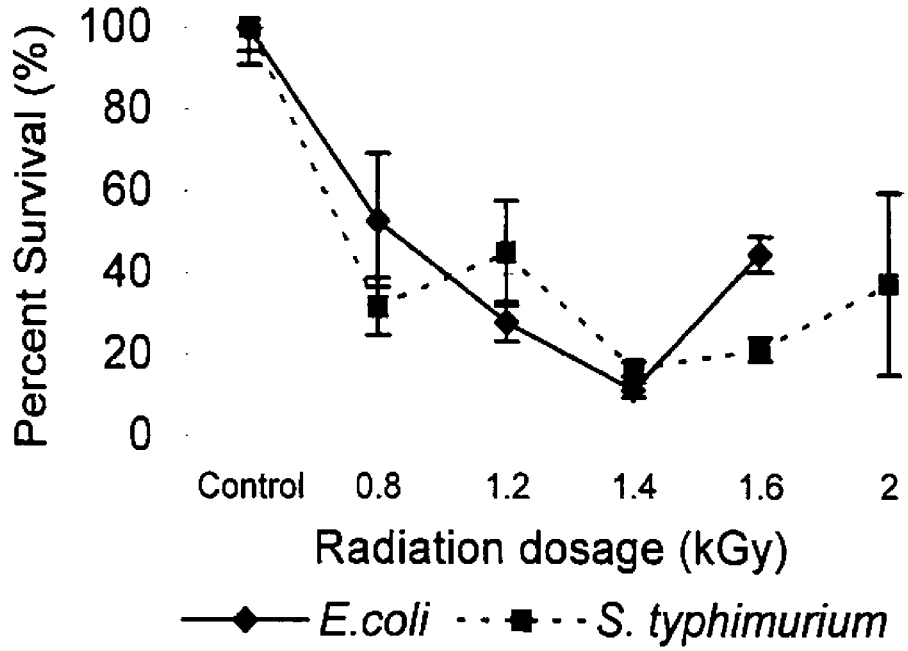


Figure 7. Percent survival of *Escherichia coli O157:H7* and *Salmonella typhimurium 266* incubated with γ–radiated lysozyme (1mg/mL solution). Bars represent SEM (n=9).

3.4.2.3. Effect of heat treatment and radiation on bactericidal activity of SDTA lysozyme. The presence of lysozyme in egg albumen offers a unique opportunity to regulate gastrointestinal microflora. In a culmination of the protein digestibility and the pure lysozyme study, the ability to modulate lysozyme activity *in situ* was evaluated. To this end, the catalytic activity of lysozyme was determined in the SDTA samples stored in a hot room. As shown, increasing the length of hot room storage reduced the catalytic activity of lsyozyme as determined by the *M. lysodeikticus* assay (Table 15). The reduction in catalytic activity was an indictor of the degree of thermal denaturation. To this extent, the ability of heat-treated SDTA was further evaluated in an antimicrobial time course study. Figure 8 shows the inability of SDTA to limit the growth of *E. coli* or *S. typhimurium* over the unheated SDTA sample. However, when subjected to γ-radiation, SDTA radiated as a liquid reduced the growth of *E. coli* up to 44.3% at 0.6 kGy. Furthermore, when radiated as a powder SDTA reduced the growth of *E. coli* up to 42.3% at 1.8 kGy (Fig. 9).

3.4.3. In vivo study with growing rats

Based on the results of the *in vitro* studies, several heat-treated and radiated products were selected to determine the growth rate, protein efficiency ratio (PER) and AA digestibility of SDTA and SDWE products, relative to the casein control. The diet intake (DI), body weight gain (BWG) and the PER were poorest in the SDWE diets and the diet containing SDTA stored in a hot room (70°C) for 3 days. The diet containing SDTA heated at 80°C for 20 min had similar BWG as the pelleted diets. However, with respect to PER values, it

TABLE 15. Lysozyme activity of hot room stored SDTA

Length of hot room storage (d)	Lysozyme Activity (Units/mg)
0	360.2 ± 9.1[1]
1	284.4[2]
2	242.4
3	191.5 ± 8.0
5	172.6 ± 4.2
14	BDL[3]

[1]Mean ± SD; [2]Single replicate only; [3]Below detection limit.

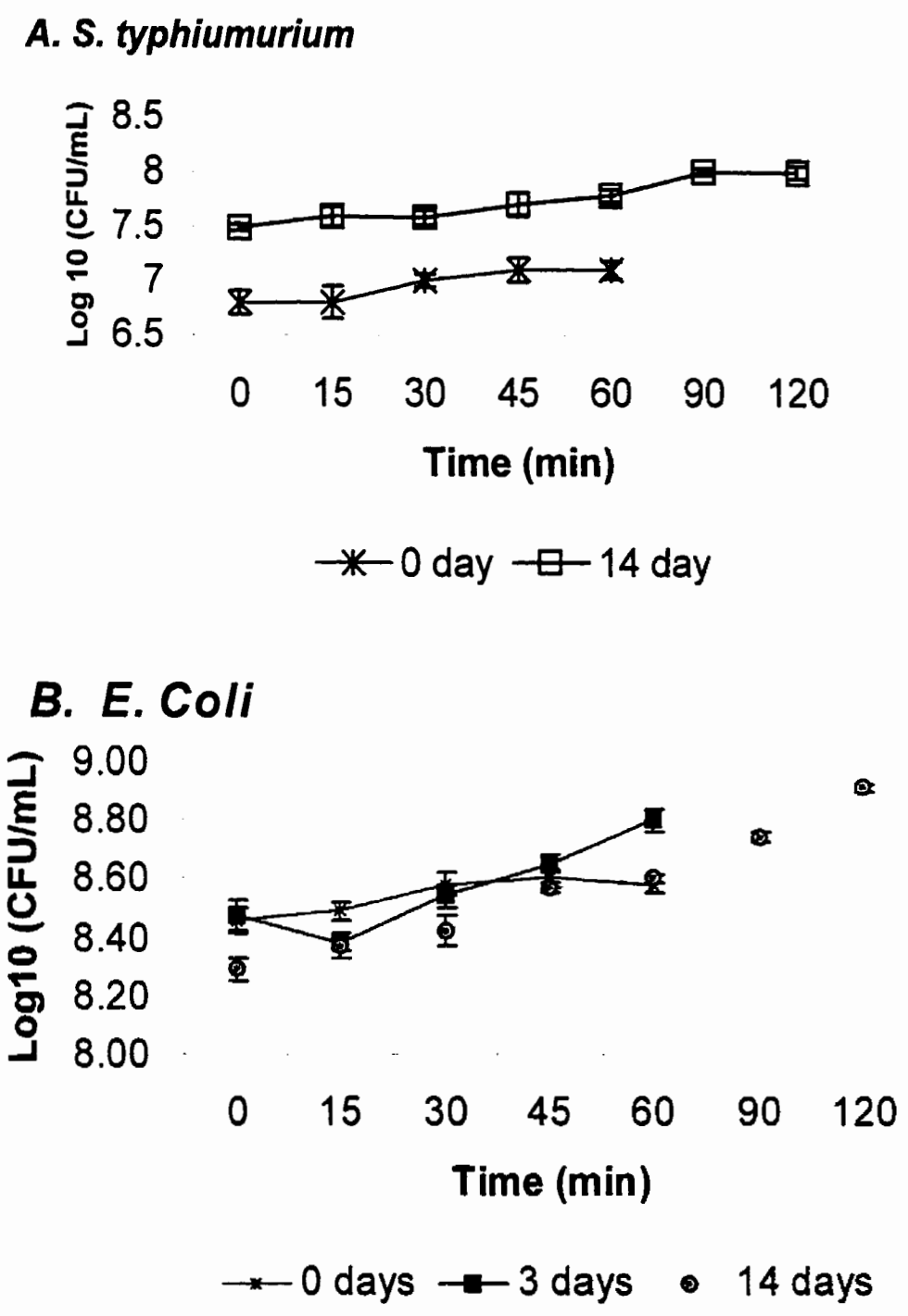


Figure 8. Time Course Study on the effect of hot-room stored spray-dried technical albumen on the growth of *Salmonella typhimurium 266* (A) and *Escherichia coli O157:H7* (B). Bars represent SEM (n=6).

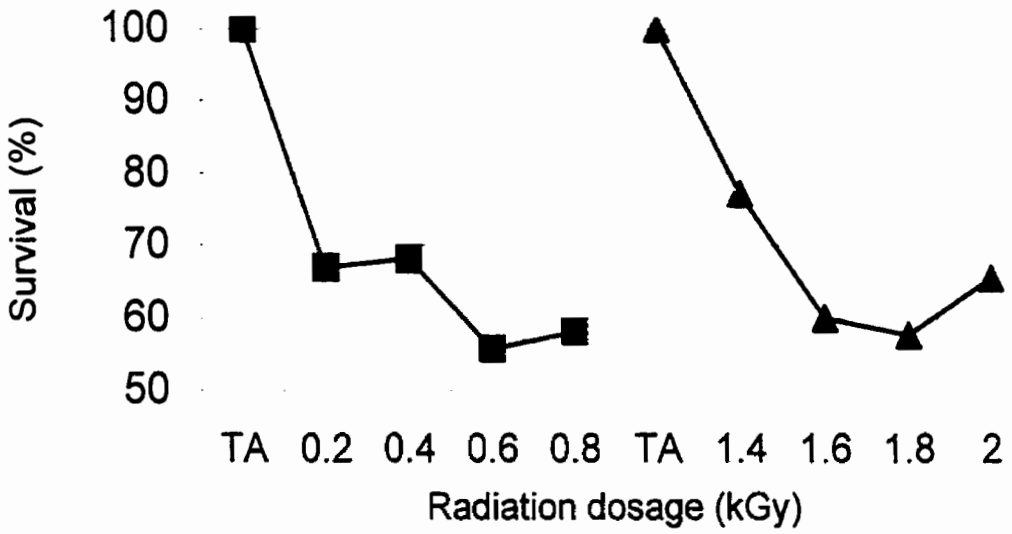


Figure 9. Percent survival of *Escherichia coli O157:H7* following 60 min incubation with technical albumen previously γ–radiated as a solution (■ --■) or powder (▲ ---▲).

performed significantly better than the pelleted SDTA stored in a hot room (70°C) for 3 days ($P<0.05$). The three casein diets of which two contained lysozyme performed similar with respect to DI, BWG and PER. However, the casein diet that contained heated lysozyme performed slightly better than the other two diets (Table 16).

In rats, several AAs showed significant reductions in their digestibility at the distal ileum ($P \geq 0.001$). In most cases the differences were a result of the source of protein, often with AAs threonine, serine, proline, alanine, leucine, phenylalanine, histidine as well as lysine digestibility being lowest for the whole egg diets. In addition, the pelleted diet containing SDTA stored in a hot room (70°C) for 3 days, showed a reduction in the digestibility of several AAs. The remaining diets showed similar AA digestibilities ranging from approximately 80 to 90% (Table 17).

To further examine any potential toxicity of the egg by-products, due to the formation of Maillard products, the liver and kidney weights were determined (Table 18). The kidney weights were similar for all diets with the exception of the pelleted diet composed of SDTA stored in a hot room (70°C) for 3 days. The kidney weights for this diet differed significantly from those of other SDTA diets with the exception of SDTA heated at 80°C for 20 min ($P<0.005$). However, there was no significant difference ($P<0.05$) in the weight of the liver between pelleted SDTA stored in a hot room and the other diets containing SDTA. For the SDWE diets, the liver weight showed a significant reduction over the other diets ($P=0.001$).

TABLE 16. Diet Intake, body weight gain and protein efficiency ration of Sprague-Dawley rats fed diets containing variously treated technical albumen and whole egg by-products (Experiment 1).

Type of Diet/ Protein Source	Diet Intake (g/28 d)	Weight Gain (g/28 d)	PER[2]
Mash Diets			
1. Casein	524[1bc]	220[ab]	3.78[cb]
2. Casein + Lysozyme	528[bc]	225[ab]	3.79[cb]
3. Casein + Heated Lysozyme	525[bc]	224[ab]	3.84[abc]
4. Raw Technical Albumen	499[bcd]	217[ab]	3.92[ab]
5. Spray-dried technical albumen (SDTA)	484[cd]	212[b]	3.94[ab]
6. SDTA heat-treated (20 min; 80°C)	528[bc]	233[ab]	3.96[a]
7. SDTA stored in a hot room (3d; 70°C)	476[de]	184[c]	3.49[d]
8. SDTA radiated (1.8 kGy)	492[bcd]	213[ab]	3.90[ab]
9. Spray-dried Whole Egg (SDWE)	439[e]	162[d]	3.32[e]
10. SDWE heat treated	462[de]	162[d]	3.16[f]
Pelleted Diets			
11. SDTA	536[ab]	231[ab]	3.88[ab]
12. SDTA stored in a hot room (3d; 70°C)	573[a]	236[a]	3.70[c]
Pooled SEM	54.9	31.7	0.30

[1]Mean ± SD; [2]Protein efficiency ratio (rat weight gain divided by protein intake); [abcde]Values within a column with no common superscript differ significantly ($P<0.05$).

TABLE 17. Ileal digestibility of amino acids in Sprague-Dawley fed diets containing variously treated technical albumen and whole egg by-products (Experiment 1).

	Amino Acid[1]												
	Ser	Pro	Ala	Thr	Cys	Val	Met	Ile	Leu	Try	Phe	His	Lys
Mash Diets													
1. Casein	89.87^{a}	94.45^{a}	87.11^{abc}	88.34^{a}	87.34	92.91^{ab}	93.94	92.57^{ab}	94.86^{a}	93.51^{a}	94.48^{a}	92.53^{a}	93.13^{a}
2. Casein + Lysozyme	89.68^{a}	95.01^{a}	87.37^{abc}	88.30^{a}	88.10	93.82^{ab}	95.70	93.26^{ab}	95.23^{a}	92.37^{a}	95.30^{a}	93.49^{a}	94.09^{a}
3. Casein + Heated Lysozyme	89.63^{a}	94.89^{a}	87.67^{abc}	88.1^{a}	87.94	94.46^{a}	95.49	94.36^{a}	95.59^{a}	93.97^{a}	95.24^{a}	92.98^{ab}	93.99^{a}
4. Raw Technical Albumen	89.59^{ab}	87.99^{b}	91.30^{ab}	86.96^{ab}	73.53	91.95^{abc}	94.29	92.12^{abc}	92.70^{b}	88.23^{b}	93.27^{ab}	89.06^{abc}	90.26^{b}
5. Spray-dried technical albumen (SDTA)	86.39^{bcd}	84.37^{bc}	88.92^{ab}	82.02^{bcd}	81.05	89.49^{bc}	94.59	89.98^{abc}	90.90^{b}	84.93^{b}	91.22^{cb}	86.09^{c}	87.98^{bc}
6. SDTA heat treated (20 min; 80°C)	84.07^{de}	82.74^{cd}	87.09^{ab}	79.18^{de}	77.80	88.51^{cd}	92.78	89.19^{bc}	89.82^{b}	82.47^{bc}	89.39^{cd}	83.84^{cd}	84.71^{c}
7. SDTA stored in a hot room (3d; 70°C)	88.37^{abc}	86.42^{bc}	89.97^{bc}	84.65^{bc}	81.88	90.04^{bc}	95.11	90.87^{abc}	91.81^{b}	86.67^{b}	92.59^{abc}	87.62^{bc}	86.97^{c}
8. SDTA radiated (1.8 kGy)	86.07^{cde}	84.66^{bc}	88.92^{abc}	82.49^{cd}	80.74	88.82^{cd}	94.68	89.81^{cb}	90.76^{b}	84.07^{bc}	91.38^{bc}	85.53^{c}	85.88^{c}
9. Spray-dried Whole Egg (SDWE)	71.57^{f}	69.83^{e}	77.12^{e}	67.20^{f}	64.54	78.59^{f}	87.97	80.85^{e}	80.11^{e}	65.70^{e}	79.40^{f}	70.55^{e}	73.85^{e}
10. SDWE heat treated	73.06^{f}	71.34^{f}	80.86^{d}	70.37^{g}	62.52	82.11^{e}	90.08	84.72^{d}	83.82^{d}	71.97^{d}	83.85^{e}	72.82^{e}	74.00^{de}
Pelleted Diets													
11. SDTA	88.05^{abc}	88.29^{b}	91.06^{a}	84.45^{bc}	83.08	91.31^{abc}	96.45	92.72^{ab}	92.65^{b}	86.11^{b}	92.45^{abc}	88.28^{abc}	88.49^{bc}
12. SDTA stored in a hot room (3d; 70°C)	83.13^{e}	80.24^{d}	85.85^{c}	77.18^{e}	72.91	86.16^{d}	91.85	88.09^{cd}	86.76^{c}	80.17^{cd}	88.36^{d}	79.99^{d}	76.86^{d}
Pooled SEM	6.09	8.03	3.93	6.77	5.20	4.66	5.21	3.83	4.57	8.19	4.62	7.26	7.08

[1]Ser= serine, Pro=proline, Ala=alanine, Thr=threonine, Cys=cysteine, Val=valine, Met=methionine, Ile=isoleucine, Leu=leucine, Tyr=tyrosine, Phe=phenylalanine, His=histidine, Lys=lysine; [abcdefg]Values within a column with no common superscript differ significantly ($P<0.05$)

TABLE 18. Liver and kidney weights of Sprague-Dawley rats fed diets containing variously treated technical albumen and whole egg by-products (Experiment 1).

Type of diet/ Protein source	Kidney Weight (g/100g body weight)	Liver Weight (g/100g body weight)
Mash Diets		
1. Casein	0.74^{ab}	5.39^{b}
2. Casein + Lysozyme	0.76^{ab}	5.37^{b}
3. Casein + Heated Lysozyme	0.70^{bc}	5.66^{ab}
4. Raw Technical Albumen	0.75^{ab}	5.60^{ab}
5. Spray-dried technical albumen (SDTA)	0.74^{ab}	5.48^{b}
6. SDTA heat treated (20 min; 80°C)	0.70^{bc}	6.10^{a}
7. SDTA stored in a hot room (3d; 70°C)	0.78^{a}	5.51^{b}
8. SDTA radiated (1.8 kGy)	0.73^{ab}	5.79^{ab}
9. Spray-dried Whole Egg (SDWE)	0.72^{ab}	4.43^{c}
10. SDWE heat treated	0.73^{ab}	4.79^{c}
Pelleted Diets		
11. SDTA	0.71^{cb}	5.54^{b}
12. SDTA stored in a hot room (3d; 70°C)	0.65^{c}	5.32^{b}
Pooled SEM	0.07	0.65

^{abc}Values within a column with no common superscript differ significantly ($P<0.05$).

The effect of heat treatment on the ileal *Enterobacteriaceae* counts in growing rats is shown in Table 19. The majority of the diets contained approximately 10^6 CFU (colony forming units)/g digesta. The exceptions were SDTA heated for 20 min at 80°C (10^5 CFU/g), SDTA radiated at 1.8 kGy (10^5 CFU/g). In contrast, the SDWE diets contained higher levels of *Enterobacteriaceae* (10^7 CFU/g digesta).

3.5. Discussion:

In vitro protein digestibility studies on autoclaved egg albumen indicated that there was potential to optimize protein digestibility by 30-40% over raw egg albumen using moist heat. Kato *et al.*, (1990) showed that it was also possible to destabilize proteins using dry heat over a period of several days. It was postulated that storage in a hot-room (70°C) could improve protein digestibility. Dry-heat only slightly improved the protein digestibility of the egg by-products. Since one of the primary objectives of this study was to concurrently optimize protein digestibility and the novel antimicrobial activity of lysozyme, the hot-room storage that best met both conditions was selected for further analysis *in vivo*. Ibrahim *et al.* (1996a, b) showed that the novel antimicrobial activity of lysozyme was optimal when lysozyme retained 50% of its catalytic ability to cleave NAM and NAG residues in the peptidogiycan. As can be seen from this study (Table 15), the length of hot-room storage that most closely correlates to a 50% reduction in enzymatic activity in lysozyme is 3 days. In addition, no further improvement was noted in the *in vitro* protein digestibility for the egg by-products

TABLE 19. Effect of dietary protein source and heat treatment on ileal counts of *Enterobacteriaceae* (Experiment 1).

Type of diet/ Protein source	Log_{10} CFU[1] /g digesta
Mash Diets	
Casein	6.28 ± 0.04^{e}
Casein + Lysozyme	6.98 ± 0.04^{b}
Casein + Heated Lysozyme	6.20 ± 0.06^{e}
Raw Technical Albumen	6.77 ± 0.06^{c}
Spray-dried technical albumen (SDTA)	6.38 ± 0.04^{d}
SDTA heat treated (20 min; 80°C)	5.72 ± 0.14^{g}
SDTA stored in a hot room (3d; 70°C)	6.96 ± 0.06^{b}
SDTA radiated (1.8 kGy)	5.90 ± 0.07^{f}
Spray-dried Whole Egg (SDWE)	6.99 ± 0.07^{b}
SDWE heat treated	7.29 ± 0.09^{a}
Pelleted Diets	
SDTA	7.05 ± 0.05^{b}
SDTA stored in a hot room (3d; 70°C)	6.70 ± 0.05^{c}

[1]Colony Forming Units; [abcdefg]Values within a column with no common superscript differ significantly ($P<0.05$).

stored in the hot-room for a period longer than 3 days (Fig. 4). A second commercially viable heat source that was considered *in vivo*, was pelleting. Diets containing SDTA and SDTA stored in a hot room were additionally pelleted to further optimize protein digestibility and lysozyme activity. In this context, the *in vivo* PER assay focused primarily on commercially viable heat treatments with the SDTA heated in accordance to the parameters that optimized the novel antimicrobial activity of pure lysozyme in the *in vitro* assays. One exception was SDTA moist heated at 80°C for 20 minutes. In pure lysozyme studies, Ibrahim *et al.* (1996a, b) showed that lysozyme heated in phosphate buffer for 20 min at 80°C reduced the survival of *E. coli* and *Salmonella enteritidis* to 13.0% and 59.2%, respectively. In their study, heat-treated lysozyme was freeze-dried and later reconstituted. Our study used freshly prepared lysozyme solutions. As seen in Fig. 6, the survival rate of *E. coli* and *S. typhimurium* were reduced to 0.08% and 50.95%, respectively, following incubation with thermally denatured lysozyme. Another treatment that was selected for *in vivo* evaluation was SDTA radiated at 1.8 kGy. This preparation showed some ability to reduce the survival of *E. coli in vitro*. In this context, pure lysozyme radiated as a solution at 1.4 kGy produced the greatest repression in growth of the target microoganisms (Fig. 7). Further analysis of technical albumen, incubated as a powder or as a solution demonstrated that *E. coli* survival rate could be reduced by 45-50% (Fig. 9). In addition to the casein control diets, raw TA and SDTA were assessed *in vivo* to determine the benefits of the selected heat treatments.

One primary concern of including technical albumen (TA) in monogastric diets is the presence of avidin. Therefore, the raw technical albumen (RTA) diet was designed to assess the impact of avidin on biotin availability. The literature is riddled with studies documenting the negative impact of avidin on growth due to biotin deficiency (Cunha *et al.*, 1946; Castledine *et al.*, 1978; Kratzer *et al.*, 1988; Burley and Vadehra, 1989; Pastoor *et al.*, 1991). However, in the current study, rats fed RTA showed no reduction in BWG or PER relative to animals fed casein or SDTA, suggesting that there was no effect of biotin deficiency on growth parameters. No other biotin deficiency symptoms, such as hair loss were observed. Studies by Kopinski *et al.* (1989c) separated biotin deficiency syndrome from the effect of egg protease inhibitors. Their data was later substantiated by Van Nevel *et al.* (2000) who found that 1 gram of egg powder can inhibit 40 mg or trypsin. Since trypsin inhibitors are thermally unstable, it was postulated that heating the egg by-products proteins improve overall protein digestibility. In fact, the diets containing SDTA stored in the hot room (70°C) for 3 days and pelleted SDTA stored in the hot room (70°C) for 3 days had poorer growth parameters than the remaining TA and casein containing diets. Although heat treatment (hot-room storage or pelleting) reduces bacterial counts and protease inhibitors in the egg products (Peters, 1967), too much heat can damage proteins and negatively impact PER values (Friedman, 1996b). Eggs, on a dry matter basis, contain 49.2% protein, 43.9% lipids and 3.93% carbohydrates (Stadelman and Cotterill, 1977; Burley and Vadehra, 1989; American Egg Board, 1998; Manitoba Egg Producers, 2000). The degree to

which the Maillard reaction may occur between proteins and reducing sugars (composing a fraction of the total carbohydrate content) is difficult to assess. In addition, the Maillard reaction may occur between proteins and oxidized fatty acids (Friedman, 1996a). Although spray-drying limits heat degeneration at high drying temperatures relative to other drying techniques such as flash drying (Hayashi, 1989; Kats *et al.*, 1994b), sub-optimal parameters during spray-drying may increase fatty acid oxidation making the product more susceptible to subsequent Maillard reactions (Guardiola *et al.*, 1997). If extensive Maillard products are formed, they can impact on digestive physiology, by inhibiting digestion and absorption of proteins (Oste *et al.*, 1987; Pitotti *et al.*, 1994; Friedman, 1996a, b). Although the AA digestibility was not significantly lower for SDTA stored in a hot room, the SDTA stored in a hot room and pelleted diets showed a significant reduction ($P<0.05$) in the digestibility of several key AAs including: threonine, leucine, valine, histidine and lysine.

To assess the benefits of the lysozyme present in the TA products, two additional casein diets were included in the study. One diet contained native lysozyme while the other contained thermally denatured lysozyme (20 min; 80°C). The benefits of added lysozyme in the diets, although minimal, were seen through the elevated PER value in the casein + lysozyme and casein + heated lysozyme diets over the casein control. The benefits of lysozyme present in the diets containing egg by-product was not substantiated by the results of the *in vitro* time course study with both *E. coli* and *S. typhimurium* (Fig. 8). In this

trial, the ability of lysozyme present in SDTA to impede bacterial growth was negligible.

Lysozyme has several possible mechanisms of action *in vitro*. Initial studies showed that thermal denaturation of lysozyme changes the surface hydrophobicity, enabling it to more effectively interact with the cell membrane of gram-negative bacteria and act via an agglutination mechanism to subsequently kill gram-negative bacteria due to membrane disturbances (Ibrahim *et al*, 1996a, b). In addition, heat-treated lysozyme may be able to activate endogenous autolysins (Ibrahim *et al.*, 1997). A third mechanism may involve the removal of divalent cations, Ca^{2+} and Mg^{2+}, from the outer bacterial membrane (Ibrahim *et al.*, 1997). In this regard, removing divalent cations, which are integral components in electrostatic interactions between lipopolysaccharides (LPS) and the carboxyl groups of membrane proteins, may induce membrane disturbances (Ibrahim *et al.*, 1996a). In 1998, Ibrahim modified his theory on lysozyme's ability to scavenge divalent cations from bacterial membranes. He proposed that heat-treated lysozyme operates through a promoted binding affinity, thus competing with Ca^{2+} for binding sites on the outer bacterial membrane. This theory was further substantiated in a study by During *et al.* (1999) in which mutant T4 lysozyme and synthetic peptides that mimic heat-denatured lysozyme were able to insert into and destabilize the bacterial membrane. In the gastrointestinal tract, the ability of lysozyme to compete with Ca^{2+} binding sites may prevent some gram-negative bacteria from attaching to the calcium affiliated with the cells lining the intestinal lumen. The mechanism by which lysozyme is

effective could be similar to the competitive exclusion mechanism proposed for prebiotics (Spring, 1995; Gibson, 1998; Sanders, 1998; Spring and Privulescu, 1998). As lysozyme could compete for binding sites, inhibiting bacteria from attaching to the lumen, it may exert anti-adhesive and attenuative properties. Table 19 shows the benefits of heating TA to induce the novel antimicrobial activity of lysozyme. SDTA heated at 80°C for 20 min gave the lowest counts of *Enterobacteriaceae* in the small intestine of the rat. Based on *in vitro* studies, the level of gram-negative bacteria was not expected to be reduced over the control diets or the SDTA diet. In addition, the casein diet containing heat-treated lysozyme showed a numerical reduction in the level of *Enterobacteriaceae* when compared to either the casein control or the casein diet containing native lysozyme. Therefore, it is believed that lysozyme may act as a competitive inhibitor for luminal binding sites. Animals fed radiated SDTA (1.8 kGy) also showed a reduction in *Enterobacteriaceae* levels. This was supported by earlier *in vitro* work (Fig. 9). Although the mode of action is uncertain, it is suspected that radiation disrupts the disulphide bonds of lysozyme in a manner similar to thermally denatured lysozyme. Further studies are required to better understand the mechanism through which radiated lysozyme exerts its activity against gram-negative bacteria.

Since the casein diet containing heat-treated lysozyme resulted in performance equal to the raw TA diet, only part of the improvement in PER can be attributed to the presence of lysozyme. In addition, rats tend to respond better to diets containing elevated levels of SAAs (Raiten *et al.*, 1998).

Therefore, the AA profile of the TA products contributed in part to the improvement in growth parameters seen over the casein control diet. The diet that performed the best contained heated SDTA (20 min; 80°C). Overall, the WE products performed the poorest of all the diets, possibly due to the higher level of fat in the diets. Jenkins and Mitchell (1989) documented an inverse relationship between the level of dietary fat and the PER value in rats: as the level of dietary fat increases the PER decreases. In addition to a reduced PER, increased lipid peroxidation in the liver may result in a fatty liver, as evidenced by the pale yellow colour of the liver. Further, as the PER decreases, the kidney weights tend to increase (Jenkins and Mitchell, 1989). In the current study, this was not always the case. For SDTA stored in a hot room, there was an increase in the kidney weights. However, in the SDTA stored in a hot room and pelleted, there was a significant reduction in the kidney weights over the SDTA dry-heated diet, suggesting that the extent of protein damage may lead to reduced protein digestion (Table 18). Boyd *et al.* (1966) showed that the liver weights of animals fed whole eggs were greater than animals fed egg whites. Childs and Ostrander (1976) showed similar results suggesting that the increased liver weights were conducive to lipid and cholesterol accumulation. In contrast, liver weights were lowest for animals fed the WE diets and greatest for rats fed SDTA heat-treated at 80°C for 20 min (Table 18). Although all organ weights were corrected to 100 g body weight, the reduced DI (Table 16) and AA digestibility (Table 17) of whole egg diets could affect the extent of deamination in the liver, leading to a lower liver weight. In addition, feeding the SDWE diets resulted in the highest levels of

Enterobacteriaceae bacteria (Table 19). In this context, the fatty acids and monoglycerides present in the SDWE diets may inhibit the growth of gram-positive bacteria (Sprong *et al.*, 1999), allowing the gram-negative *Enterobacteriaceae* population to flourish.

It was hypothesized that thermal denaturation of egg by-products could optimize protein digestibility and lysozyme activity. Thermal-denaturation induced novel antimicrobial activity of lysozyme, as seen in reduced survival rate of *E. coli* and *S. typhimurium in vitro* and reduced numbers of *Enterobacteriaceae* bacterial counts *in vivo*. However, hot room storage of SDTA and SDWE products resulted in reduced growth performance *in vivo*, despite the beneficial effect of heat treatment on protein digestibility observed in the *in vitro* experiment. The reason for this discrepency may be that rats are sensitive to reduced cystine or lysine levels (Sarwar, 1997), lower PER values may indicate the presence of lanthionine or other Maillard products in the diets containing hot-room stored egg by-products. Overall, the TA diets performed better than the control diets or the diets containing SDWE. The SDTA heat-treated at 80°C for 20 min as well as the radiated SDTA (1.8 kGy) had some of the highest PER and BWG values, in addition to the lowest *Enterobacteriaceae* counts ($P<0.05$). Further research is needed to elucidate the mode of action of the radiated lysozyme products. Since the rat assay is an acceptable model for weanling-pigs (Thomson *et al.*, 1994, 1995), it would appear that the TA products have potential as protein and antimicrobial supplements in early-weaned pig diets. However, due to the presence of antinutritive factors in eggs (*i.e.* avidin,

trypsin inhibitors, Maillard products) caution needs to be exercised when extrapolating the results to pigs (Yu *et al.*, 1996). Therefore, to assess SDTA products for use in the agricultural industry as a novel protein and antimicrobial supplement further *in vivo* trials are required with weanling-pigs.

3.6. Implications

Data from the current study demonstrates that it is possible to combine nutrition with microbial regulation. The induction of novel bactericidal activity of pure lysozyme against gram-negative bacteria via thermal treatment or γ-radiation, the reduction of pathogenic bacteria *in vitro* via γ-radiation of technical albumen and the reduction of *Enterobacteriaceae* in rats fed heat-treated or γ-radiated SDTA provides an exciting opportunity to reduce the use of antibiotics as growth promotants in animal agriculture. In addition, the ability of SDTA to meet or exceed the PER value of casein, demonstrates the excellent AA profile of this product.

4. Manuscript 2

THE APPLICATION OF EGG BY-PRODUCTS AS VALUABLE PROTEIN SUPPLEMENTS IN BROILER CHICKEN DIETS.

4.1. Abstract

Egg by-products have received little attention as a poultry feedstuff despite their excellent AA profile, energy content and the presence of anti-bacterial proteins. The nutritive value of the egg by-products was evaluated in two poultry experiments. The first experiment was a completely randomized design in which birds were fed corn-soy diets containing 8% of either fish meal (control), spray-dried technical albumen (SDTA), heat treated (hot room storage at 70°C for 3 days) SDTA or heat treated spray-dried whole egg (SDWE). Similar body weight gains (529, 520, 480, 514 g/bird/14 days) and feed conversion ratios (1.33, 1.34, 1.38, 1.32) were observed although a negative effect of heat treatment on the nutritive value of SDTA was evident. This was substantiated by reduced TMEn content of SDTA following hot room storage (5.32 vs 4.54 Mcal/kg). Although AA digestibility, as determined at the terminal ileum in Experiment 2, averaged 80-90% for lysine, methionine, cystine and threonine; the AA digestibility, as determined in the TME assay showed a reduction in leucine, threonine, glutamic acid and glycine ($P<0.05$) between SDTA and heat-treated SDTA. Based on the results of the first trial, a long-term production trial was conducted. Five replicates of 60 birds were fed one of four wheat-soy diets: a positive control containing fish meal and antibiotic (PC), a negative control with no antibiotic added (NC), NC+SDTA and NC+SDWE. The test proteins were included at 6% in the starter phase and 5% in the grower phase. The body weight gain and feed conversion averaged 2.14, 2.12, 2.18, 2.25 kg/bird and 1.68, 1.66, 1.61, and 1.60 for PC, NC, NC+SDTA and NC+SDWE, respectively. The high performance

observed for the SDWE diet was substantiated by an increased AME over the PC diet (3212 vs 2956 kcal/kg). In comparison to the PC diet, a reduction in the population of gram-negative *Enterobacteriaceae* was observed for NC+SDTA (7.78 vs 6.25 Log_{10} CFU/g feces). It is evident from this study that the substitution of fish meal with egg by-products could further improve broiler chicken performance.

4.2. Introduction

Egg-breaking facilities produce substantial quantities of by-products each year that are unsuitable for human consumption. Egg by-products such as technical albumen (TA) or whole egg (WE) have received little attention as a poultry feedstuff despite their excellent AA profile, energy content and the presence of anti-bacterial proteins (*i.e.* lysozyme).

Egg by-products are an excellent source of AAs and are rich in methionine and cystine, which could complement feedstuffs that are low in these two essential AAs. In addition, egg by-products have an excellent fatty acid profile, closely resembling the profile of poultry (abdominal) fat (Sim, 1970; Peebles *et al.*, 2000). Despite the excellent AA and fatty acid profiles, few studies have evaluated the incorporation of egg by-products in broiler chicken diets (Johnson and Parsons, 1997; Junqueira *et al.*, 2000).

The presence of lysozyme offers a unique opportunity to combine nutrition and modification of the gastrointestinal microflora. In its native conformation, lysozyme catalytically cleaves the N-acetylmuramic acid (NAM) and N-acetylglucosamine residues (NAG) located in the peptidoglycan of bacterial cell walls. The activity of native lysozyme against gram-negative bacteria is limited as the NAM and NAG residues are protected by a lipopolysaccharide outer-membrane. Several research groups have tried to chemically modify the structure of lysozyme to improve its ability to access the NAM and NAG residues in gram-negative bacteria (Proctor and Cunningham, 1988, 1993; Yang and Cunningham, 1993 and Ibrahim *et al.*, 1993, 1994 a, b, 1996a, b). However,

Ibrahim *et al.* (1996a, b; 1997) showed that thermal denaturation of lysozyme induces a novel antimicrobial activity that is independent of its catalytic activity (native lysozyme). It is theorized that the novel antimicrobial activity of lysozyme results via increased affinity for bacterial (gram-negative and gram-positive) cell walls (Ibrahim *et al.*, 1996 a, b; Ibrahim, 1998; During *et al.*, 1999).

The objectives of this study were: (1) to assess the ability to incorporate the egg products into broiler chicken diets, (2) to optimize the nutrient availability and (3) to evaluate the potential for lysozyme to modify the gastrointestinal microflora.

4.3. Materials and Methods:

4.3.1. Materials

Canadian Inovatech Inc., Winnipeg, MB, CANADA provided spray-dried technical albumen (SDTA), SDTA stored in a hot room (70°C) for 1 or 3 days, spray-dried whole egg (SDWE), and SDWE stored in a hot room (70°C) for 1 day.

4.3.2. TME Assay

Two groups of twelve single-comb white adult leghorn cockerels were randomly assigned to one of three egg by-products to assess the true metabolizable energy (TME_n) content as described by Sibbald (1986) with some modifications (Zhang *et al.*, 1994). The birds were housed in individual wire metabolic cages with a 14-hour light cycle. All animals had access to fresh water throughout the experimental period. Following a 28-hour fasting period, each group of birds was precision-fed either 6.25 or 12.5 g of the test product, blended

with a non-nitrogen diet (90% glucose, 10% canola oil) to a total of 25 g. During the next 48 hours, the excreta from each bird was collected. The excreta was then freeze-dried, ground to pass through a 1 mm sieve and pooled for each group for analysis of gross energy, nitrogen (Kjeldahl) and AA content.

4.3.3. Broiler Chicken Experiment 2

Two hundred ten Cobb crossed male broiler chickens obtained from Carleton Hatcheries, Grunthal, MB, were used to assess feed intake, body weight gain and feed to gain ratio of diets containing heat treated technical-grade egg products. After a 5-day acclimation period, the chicks were randomly assigned to one of six dietary treatments. Chickens were housed in groups of five per pen (5 birds/pen, 7 pens per treatment), such that the initial weight of all pens was similar. All birds had a 24 hour light cycle and *ad libitum* access to feed and water. Experimental diets were formulated to meet NRC (1994) requirements and contained 22% protein, 1.0% calcium, 0.45% available phosphorous, 1.15 % lysine (min.), 0.50 % methionine (min.), 0.90% cystine plus methionine (min.), 0.87% threonine (min.) and 0.97% linoleic acid (min.) (Table 20). Chromic oxide was added to the diets as an internal marker. The control diet contained 8% fish meal. All remaining diets replaced fish meal on an equal basis with either spray-dried technical albumen (SDTA), SDTA stored in a hot room (70°C) for 1 and 3 days, spray-dried whole egg (SDWE), and SDWE stored in a hot room for 1 day. Birds were weighed on days 0, 7 and 14. Feed intake was determined at the end of each 7-day period.

TABLE 20. Composition and calculated analyses of egg by-product diets used in the broiler chicken growth trial (5-19 days of age), Experiment 2.

	Diet					
	1 (control)	2	3	4	5	6
Ingredients (%)						
Corn	59.06	58.01	58.01	58.01	53.31	53.31
Soybean meal	26.05	26.05	26.05	26.05	30.85	30.85
Fish meal	8					
Spray-dried technical albumen (SDTA)		8				
SDTA stored in a hot room (1d; 70°C)			8			
SDTA stored in a hot room (3d; 70°C)				8		
Spray-dried whole egg (SDWE)					8	
SDWE stored in a hot room (1d; 70°C)						8
Mineral premix	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix	1	1	1	1	1	1
$CaCO_3$	1.35	1.75	1.75	1.75	1.80	1.80
Biophosphate	0.60	1.35	1.35	1.35	1.2	1.2
DL-Methionine	0.14	0.04	0.04	0.04	0.04	0.04
Vegetable Fat	3	3	3	3	3	3
Chromic Oxide	0.3	0.3	0.3	0.3	0.3	0.3
Calculated Composition:						
ME (kcal/kg)	3077	3260	3260	3260	3307	3307
Protein (%)	20.93	21.01	21.01	21.01	20.97	20.97
Ca (%)	1.04	0.99	0.99	0.99	1.01	1.01
Available P (%)	0.48	0.45	0.45	0.45	0.45	0.45
Lysine (%)	1.11	1.09	1.09	1.09	1.07	1.07
Methionine + Cystine (%)	0.90	0.99	0.99	0.99	0.83	0.83
Threonine (%)	0.78	1.18	1.18	1.18	1.05	1.05

[1]Mineral premix provided, per kg complete diet Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.36 mg; [2]Vitamin premix provided, per kg complete diet vitamin A, 8255 IU; vitamin D_3, 1000 IU; vitamin E, 10.9 IU; vitamin B_{12}, 0.0115 mg; vitamin K, 1.1 mg; riboflavin, 5.5 mg; niacin, 53.3 mg; choline, 1.02 g; folic acid, 0.75 mg; ethoxyquin, 125 mg.

At the termination of the experiment, excreta samples were collected over a 4-hour period, frozen, freeze-dried, equilibrated at ambient temperature for 24 h and finely ground. In addition, 10 birds per treatment group were sacrificed by cervical dislocation as approved by the Animal Care Committee of the University of Manitoba, to collect ileal digesta. Excreta samples were analysed for chromic oxide (internal marker), gross energy and nitrogen (Kjeldahl) content. Ileal digesta was analysed for chromium and AA content.

4.3.4. Broiler Chicken Experiment 3

One thousand two hundred day-old commercial broiler cockerels were randomly assigned to 20 floor pens (60 birds/pen) to assess the potential of SDTA and SDWE to replace fishmeal and virginiamycin in broiler diets. Birds were exposed to decreasing/increasing phase lighting (Dunn-Rite program). The initial temperature was held at 32°C and was decreased by 2.5°C per week until the air temperature reached 24°C. Each pen was additionally equipped with an electric brooder for 3-5 days to ensure adequate heating. All birds had *ad libitum* access to feed and water. Birds were assigned to one of four dietary treatments: (1) a positive control containing antibiotics (PC), (2) a negative control without antibiotics (NC), (3) NC substituting SDTA for fishmeal and (4) NC substituting SDWE for fishmeal. All diets were formulated as a starter and grower ration (Table 21). Feed consumption, body weight gain and feed efficiency, corrected for mortalities, were determined on day 21 and 37. Mortality was recorded daily as it occurred.

TABLE 21. Composition and calculated analyses of egg by-product diets used in the broiler chicken performance trial (1-37 days of age), Experiment 3.

	Starter				Grower			
	1	2	3	4	1	2	3	4
Ingredient (%)								
Wheat	64.88	64.88	65.11	60.76	74.98	74.98	75.42	71.81
Soybean meal	21.4	21.4	21.3	25.8	12.5	23.5	12.3	16
Fish meal	6	6			5	5		
Spray-dried technical albumen			6				5	
Spray-dried whole egg				6				5
Mineral premix[1]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix[2]	1	1	1	1	1	1	1	1
$CaCO_3$	1.43	1.43	1.75	1.77	1.48	1.48	1.74	1.75
Biophosphate	0.71	0.71	1.27	1.16	0.38	0.38	0.85	0.76
L-Lysine					0.09	0.09	0.19	0.18
DL-Methionine	0.08	0.08	0.07	0.01	0.01	0.01		
Threonine					0.06	0.06		
Tallow	4	4	3	3	4	4	3	3
Virginiamycin	+	-	-	-	+	-	-	-
Coccidiostats	+	+	+	+	+	+	+	+
Calculated composition:								
ME (kcal/kg)	3019	3019	3035	3099	3098	3098	3104	3156
Protein (%)	23.0	23.0	22.99	23.01	20.03	20.03	20.01	20.01
Ca (%)	1	1	1	1	0.9	0.9	0.9	0.9
Available P (%)	0.45	0.45	0.45	0.45	0.35	0.35	0.35	0.35
Lysine (%)	1.19	1.19	1.10	1.10	1.00	1.00	1.00	1.00
Methionine + cystine (%)	0.89	0.89	1.04	0.90	0.73	0.73	0.86	0.79
Threonine (%)	0.83	0.83	1.09	1.00	0.74	0.74	0.9	0.83

[1]Mineral premix provided, per kg complete diet Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.36 mg; [2]Vitamin premix provided, per kg complete diet vitamin A, 8255 IU; vitamin D_3, 1000 IU; vitamin E, 10.9 IU; vitamin B_{12}, 0.0115 mg; vitamin K, 1.1 mg; riboflavin, 5.5 mg; niacin, 53.3 mg; choline, 1.02 g; folic acid, 0.75 mg; ethoxyquin, 125 mg.

At the end of experiment, excreta samples were collected as described in Experiment 2. The samples were then analysed for gross energy and nitrogen (Kjeldahl) content. Apparent metabolizable energy (AME) content was calculated according to Hill *et al.* (1960). In addition, 15 birds per treatment were randomly selected for *Enterobacteriaceae* enumeration in their distal ileum. The birds were killed by cervical dislocation, the abdominal cavity exposed and the contents of ileum collected into sterile containers. *Enterobacteriaceae* was determined by serially diluting 1 g of digesta in saline (0.85%), plating 1 mL on violet red bile agar and incubating at 35°C for 24 hours. *Enterobacteriaceae* levels were represented as Log_{10} CFU/g digesta.

4.3.5. Chemical and Statistical Analyses

Chemical and statistical analyses were performed according to the methodology described in Manuscript 1 of this thesis.

4.4. Results

The true metabolizable energy (TME_n) of spray-dried whole eggs (SDWE) was higher than that of spray-dried technical albumen (SDTA). In addition, the hot room stored SDTA had a significantly reduced TME_n value when compared to SDTA ($P< 0.05$; Table 22). AA analysis of the TME_n samples also showed reduced digestibility of leucine, threonine, glutamic acid and glycine between SDTA and either SDWE or hot-room stored SDTA (Table 23). In Experiment 2, chickens fed SDTA stored in a hot room (70°C) for 1 or 3 days had lower body weight gains than the control (fishmeal), untreated SDTA or SDWE diets ($P<$

TABLE 22. True Metabolizable energy (TME_n) content of spray-dried whole egg (SDWE), spray-dried technical albumen (SDTA) and SDTA store in a hot room for 3 days at 70°C.

Sample	TME (Mcal/kg DM)
SDWE	5.69 ± 0.059[1a]
SDTA	5.42± 0.068[b]
SDTA stored in a hot room (3d; 70°C)	4.54 ± 0.059[c]
Pooled SEM	0.027

[1]Mean ± SD; [abc]Means with no common superscripts differ significantly (P<0.05)

TABLE 23. Amino acid digestibility of spray-dried whole egg (SDWE), spray-dried technical albumen (SDTA) and SDTA stored in a hot room for 3 days at 70°C (TME_n assay).

Amino Acid	SDWE	SDTA	Hot room stored SDTA	Pooled SEM
Asparginine	77.79	79.80	76.84	3.11
Threonine	69.68[b]	78.12[a]	71.89[b]	4.80
Serine	68.83[b]	76.21[a]	74.79[ab]	4.98
Glutamic acid	75.74[b]	82.53[a]	77.28[b]	3.67
Proline	62.20	67.05	60.05	8.31
Glycine	51.97[b]	66.51[a]	56.52[b]	6.52
Alanine	81.69	83.38	79.65	2.49
Cystine	58.83	64.26	62.06	6.17
Valine	81.60	84.60	83.14	2.68
Methionine	88.52	91.47	91.50	2.26
Isoleucine	86.69	88.75	86.64	3.32
Leucine	84.79[ab]	88.09[a]	83.81[b]	2.81
Tyrosine	76.08	80.35	77.51	4.82
Phenylalanine	86.69	85.82	86.58	3.05
Histidine	51.72	58.70	52.48	5.83
Lysine	76.51	74.07	70.93	3.87
Arginine	80.64	84.62	79.70	3.56
Tryptophan	76.45[b]	80.84[a]	74.17[b]	2.93

[ab]Means within rows with no common superscripts differ significantly ($P<0.05$)

0.001). The feed to gain ratio showed that the hot room stored SDTA products performed 4% poorer than the control diet and 6% poorer than the diets containing SDWE ($P<0.001$; Table 24). The apparent metabolizable energy (AME) content of the diets did not differ significantly among the treatments ($P>0.5$; Table 25). With respect to AA digestibilities in the whole egg diets, valine, methionine, isoleucine, leucine, histadine and lysine had lower values than the other diets ($P<0.05$; Table 26). In addition to the SDWE diets having lower lysine digestibilities, the diet containing SDTA stored in a hot room for 3 days also showed a reduction in lysine digestibility.

In the broiler chicken performance trial (Experiment 3), birds fed diets containing SDWE had the largest body weight gain in the starter phase. Although the NC diet containing SDTA had the best FCR (1.382), it was not statistically different from the NC diet containing SDWE ($P>0.05$; Table 27). During the grower phase, diets containing egg by-products continued to perform statistically better than either of the control diets ($P<0.05$). The SDWE diet did perform slightly better during the grower period as well as for the entire trial (Table 27). Reduced levels of *Enterobacteriaceae* were observed in the distal ileum of birds fed the NC diet containing SDTA when compared to either the positive or negative control diets ($P>0.05$; Table 28). The AME content did not differ significantly between the diets ($P>0.05$; Table 29). Mortality (6.4%) was independent of treatment.

TABLE 24. Growth performance of broiler chickens (5-19 days of age) fed diets containing spray-dried technical albumen (SDTA), SDTA stored in a hot room (70°C) for 1 or 3 days, spray-dried whole egg (SDWE) and SDWE stored in a hot room (70°C) for 1 day (Experiment 2).

Diet	Feed Intake (g/bird/14 days)	Body Weight Gain (g/bird/14 days)	FCR
Control	704[1a]	529[a]	1.33[b]
Spray-dried technical albumen (SDTA)	694[a]	520[a]	1.34[b]
SDTA stored in a hot room (1d; 70°C)	654[b]	477[c]	1.37[a]
SDTA stored in a hot room (3d; 70°C)	664[b]	480[bc]	1.38[a]
Spray-dried whole egg (SDWE)	661[b]	506[ab]	1.31[b]
SDWE stored in a hot room (1d; 70°C)	677[ab]	524[a]	1.32[b]
Pooled SEM	24.7	23.7	0.031

[1]Mean ± SD; [abc]Means within columns with no common superscripts differ significantly (P<0.05)

TABLE 25. Apparent metabolizable energy (AME_n) content of diets fed to broiler chickens. (Experiment 2)

Diet	AME_n (kcal/kg)
Control	3184[1]
Spray-dried technical albumen (SDTA)	3339
SDTA stored in a hot room (1d; 70°C)	3221
SDTA stored in a hot room (3d; 70°C)	3262
Spray-dried whole egg (SDWE)	3255
SDWE stored in a hot room (1d; 70°C)	3266
Pooled SEM	40.5

TABLE 26. Amino Acid digestibility of diets containing spray-dried technical albumen (SDTA), SDTA stored in a hot room (70°C) for 1 or 3 days, spray-dried whole egg (SDWE) and SDWE stored in a hot room (70°C) for 1 day fed to broiler chickens (5-19 days of age), Experiment 2.

Diet	Control	SDTA	SDTA-1d	SDTA-3d	SDWE-1d	SDWE-3d
Alanine	89.10	88.64	87.39	88.03	85.98	83.97
Arginine	90.52	92.03	90.50	89.53	88.78	86.35
Aspartic acid	83.23	85.61	84.75	84.03	83.40	81.73
Cystine	85.06	87.04	85.33	82.94	80.18	81.02
Glutamic acid	91.09	91.90	91.22	90.94	90.14	88.48
Glycine	83.57	83.14	81.96	82.44	80.85	78.92
Histidine	93.08^{a}	91.89^{a}	91.44^{a}	90.99^{ab}	90.63^{ab}	88.68^{b}
Isoleucine	88.55^{a}	88.35^{a}	87.44^{a}	87.13^{a}	85.42^{ab}	82.46^{b}
Leucine	90.76^{a}	90.95^{a}	90.35^{a}	89.98^{a}	88.25^{ab}	86.31^{b}
Lysine	88.19^{a}	87.82^{a}	86.37^{ab}	84.59^{ab}	84.42^{ab}	81.26^{b}
Methionine	91.41^{a}	90.87^{ab}	88.28^{abc}	89.45^{ab}	86.64^{bc}	84.40^{c}
Phenylalanine	86.20	89.59	89.05	88.67	87.02	83.46
Proline	86.64	88.47	88.45	88.61	87.75	86.87
Serine	85.19	84.16	83.44	84.56	81.44	80.16
Threonine	82.95	82.31	81.79	81.59	79.75	77.87
Tyrosine	85.85	87.37	86.10	85.95	83.86	79.73
Valine	86.29^{a}	85.92^{a}	84.41^{a}	84.56^{a}	82.65^{ab}	79.70^{b}

^{abc}Means with no common superscripts within a row differ significantly ($P<0.05$)

TABLE 27. Growth performance of broiler chickens (1-37 days of age) fed diets containing spray-dried technical albumen (SDTA) and spray-dried whole eggs (SDWE) as a replacement for fishmeal (Experiment 3).

Diet	Feed intake (kg)	Body weight gain (kg)	FCR
A. Starter period			
Positive control	1.03[a]	0.71[b]	1.445[ab]
Negative control (NC)	1.04[a]	0.71[b]	1.49[a]
NC + SDTA	0.99[b]	0.72 [b]	1.38[c]
NC + SDWE	1.05[a]	0.75[a]	1.40[bc]
Pooled SEM	0.011	0.010	0.0159
B. Grower period			
Positive control	2.56[a]	1.43[bc]	1.79[a]
Negative control (NC)	2.47[b]	1.42[c]	1.74[a]
NC + SDTA	2.51[ab]	1.46[b]	1.72[b]
NC + SDWE	2.52[ab]	1.49[a]	1.69[b]
Pooled SEM	0.027	0.012	0.020
C. Overall			
Positive control	3.59	2.14[bc]	1.68[a]
Negative control (NC)	3.51	2.12[c]	1.66[a]
NC + SDTA	3.50	2.18[b]	1.61[b]
NC + SDWE	3.58	2.25[a]	1.60[b]
Pooled SEM	0.035	0.015	0.015

[abc]Means with no common superscripts differ significantly ($P<0.05$)

TABLE 28. Effect of dietary protein source and virginiamycin on ileal gram-negative *Enterobacteriaceae* counts in broiler chickens (37 days of age) (Experiment 3).

Diet	Bacterial Count (Log_{10} CFU/g digesta on a dry matter basis)
Positive control	7.8
Negative control (NC)	6.8
NC + SDTA	6.2
NC + SDWE	8.1
Pooled SEM	1.34

TABLE 29. Apparent metabolizable energy (AME) content of diets containing spray-dried technical albumen (SDTA) and spray-dried whole egg (SDWE) products (Experiment 3).

Diet	AME (kcal/kg)
Positive Control	2825 ± 144[1]
Negative control (NC)	2902 ± 205
NC + SDTA	3094 ± 23
NC + SDWE	3073 ± 140
Pooled SEM	152.5

[1]Mean ± SD

4.5. Discussion

Spray-dried technical albumen (SDTA) and spray-dried whole egg (SDWE) can be successfully incorporated into broiler chicken diets. The growth parameters of birds fed egg-products were as good or better than the fishmeal control. This is in contrast to earlier research by Junqueira *et al.* (2000), who showed that birds fed dried whole egg did not perform as well as the control diet. However, a study by Johnson and Parsons (1997) demonstrated that SDWE had a better protein efficiency ratio (PER) than other animal protein sources.

Overall the birds fed diets containing SDWE performed better than those containing SDTA. In Experiment 2, the FCR for birds fed diets containing SDWE or SDTA were 1.335 and 1.308, respectively. Throughout Experiment 3, the birds fed the diet containing SDTA had poorer weight gain than those fed the diet containing SDWE ($P<0.05$). The FCR was not different. The slight improvement in the growth performance of animals fed the diet containing SDWE may be attributed to the higher level of dietary fat. Sulistiyanto *et al.* (1999) demonstrated that the energy from fat sources is better utilized than the energy from either carbohydrates or proteins. While there is no statistical advantage to the source of dietary fat, the weight gains of birds fed poultry fat were numerically greater than for birds fed corn oil at a 3% inclusion rate (Peebles *et al.*, 2000). Although, the birds in the current study were not fed poultry (abdominal) fat, Sim (1970) demonstrated that the saturated fatty acid composition in poultry fat and egg yolks is highly correlated. Although both SDTA and SDWE contain fat, the level of fat in SDWE exceeds the level found

in SDTA (15.4 vs 25.4%). The higher fat content directly translated into higher TME_n content of SDWE relative to SDTA (5.693 vs 5.417 Mcal/kg DM). The higher level of energy, in particular from lipids, and a fat profile that more closely resembles poultry fat resulted in numerically better growth performance of birds fed diets containing SDWE.

It was hypothesized that thermal denaturation of SDWE and SDTA through hot room storage would optimize protein digestibility and eliminate antinutritive properties of some proteins including trypsin inhibitors and avidin. Van Nevel *et al.* (2000) found that whole egg powder can inhibit 40mg of trypsin /g of egg powder. In addition, avidin, found in egg albumen, makes unheated dried whole egg biotin deficient, as the biotin levels found in egg yolk are inadequate to counteract the presence of avidin (Kratzer *et al.*, 1988). Therefore, animals fed diets containing heated egg by-products were anticipated to perform better than the unheated egg products. In Experiment 2, birds were fed SDTA, SDTA stored in a hot room (70°C) for 1 or 3 days, SDWE or SDWE stored in a hot room (70°C) 1 day. The birds fed the hot room stored egg products did not perform as well as animals fed unheated products. In the case of SDTA, there was a significant reduction in body weight gain and FCR ($P<0.05$). Birds fed SDTA (unheated) gained 520.0 g/14d/bird with a FCR of 1.335; however, birds fed heat-treated SDTA gained 476.6 or 480.4g/14d/bird with a FCR of 1.372 and 1.382 after hot room storage for 1 or 3 days respectively. SDWE showed a numerical increase in feed efficiency from 1.31 without hot room storage to 1.32 after 1 day of hot room storage. The difference

between heated and unheated egg products was corroborated by the TME_n values for SDTA and SDTA stored in a hot room for 3 days. The TME_n study showed that the metabolizable energy was reduced by 881 kcal/kg DM following hot room storage for 3 days. In addition, hot room storage reduced the digestibility of several AAs including threonine, serine, glutamic acid, glycine, leucine and tryptophan ($P<0.05$). On average (dry matter basis) eggs contain 49.2% protein, 43.9% lipids and 3.93% carbohydrates (Manitoba Egg Producers, 2000; American Egg Board, 1998; Burley and Vadehra, 1989; Stadelman and Cotterill, 1977). The formation of Maillard products can significantly reduce the AA availability (Friedman, 1996b). In addition to classical reactions between reducing sugars (*i.e.* glucose) and proteins, the Maillard reaction may occur between proteins and oxidized fatty acids (Friedman, 1996a). Guardiola *et al.* (1997) noted that dry heating (*i.e.* hot room storage) augments the risk of fatty acid oxidation and the subsequent formation of Maillard products. Spray drying limits the degeneration of heat labile products (Hayashi, 1989; Kats *et al.*, 1994b). However, sub-optimal parameters during spray drying may increase fat oxidation making the product more susceptible to subsequent Maillard reactions (Guardiola *et al.*, 1997). As is evident from the TME assay, both metabolizable energy and protein digestibility are reduced following hot-room storage for 3 days (Table 22 and 23). The reduced metabolizable energy content could result from reduced lipid availability due to lipid-protein interactions. Low digestibility of AAs further supports the concept of the Maillard reaction occurring between fatty acids and proteins. Although the reduction in histidine, isoleucine, leucine, and

lysine availability between heated and unheated SDTA and SDWE diets for was significant in Experiment 2 ($P<0.05$; Table 26), it was not as pronounced as that seen in the TME assay (Table 23).

In addition to optimizing protein digestibility, it was believed that hot room storage might induce the novel antimicrobial activity of lysozyme against gram-negative bacteria. In Experiment 3, the four diets were designed to evaluate the benefits of lysozyme in the egg products. All diets were subjected to additional heat treatment through pelleting. The NC diet containing SDTA had lower *Enterobacteriaceae* counts compared to the positive control (PC), negative control (NC) and NC containing SDWE diets (6.15 vs. 7.78, 6.75 and 8.06 log units, respectively). The PC diet contained virginiamycin, an antibiotic effective against gram-positive cocci. The presence of virginiamycin in the diet may have caused a shift from gram-positive to gram-negative bacteria, potentially increasing the risk of opportunistic infections (Bovee-Oudenhoven *et al.*, 1999; Sprong *et al.*, 1999). The NC diet containing SDWE tended to have the highest counts, perhaps due to the greater level of fat in the product (25.4% vs. 15.4% for SDWE and SDTA, respectively). Fatty acids and monoglycerides liberated in the gastrointestinal tract inhibit gram-positive bacteria with a lesser effect on gram-negative bacteria. Due to the reduction of the gram-positive bacteria and the opportunistic nature of the gram-negative *Enterobacteriaceae*, increased bacterial counts were expected.

The presence of lysozyme in the NC diet containing SDTA appeared to reduce the level of *Enterobacteriaceae* over the other diets. The additional heat

during pelleting could further induce the novel antimicrobial effects of lysozyme against gram-negative bacteria. Heat-treated lysozyme has several speculated mechanisms of action. Denaturing the catalytic activity of lysozyme alters the hydrophobicity of the enzyme, enabling it to more effectively agglutinate with the cell wall of gram-negative bacteria (Ibrahim *et al.*, 1996a, b). Heat-treated lysozyme may further be able to displace Ca^{2+} or Mg^{2+} from bacterial membranes to stimulate endogenous autolysins or destabilize the lipopolysaccharide and carboxyl groups of membrane proteins (Ibrahim *et al.*, 1996b, 1997). More recently, lysozyme has been shown to act through a promoted binding affinity, competing with calcium for outer membrane binding sites (Ibrahim, 1998; During *et al.*, 1999). In vivo, lysozyme may compete with the calcium binding sites to prevent bacteria from associating with the calcium affiliated with the cells lining the intestinal tract. Thus, the mechanism through which lysozyme acts may be competitive exclusion. Despite a strong trend towards reduced *Enterobacteriaceae* counts in the NC diet containing SDTA, further research is required to optimize the novel antimicrobial properties of lysozyme.

4.6. Implications

The induction of novel bactericidal activity of lysozyme against gram-negative bacteria following heat-treatment showed a trend towards reduced populations of *Enterobacteriaceae*, potentially reducing the levels of anitibiotics required in broiler chicken diets. In addition, SDTA and SDWE can further

improve broiler chicken performance over fish meal. However, a negative effect of hot-room storage (i.e. 3 days at 70°C) on the nutritive value of SDTA and SDWE by-products was documented. Therefore, an alternative to hot room storage needs to be identified to pasteurize the egg products.

5. Manuscript 3

THE POTENTIAL FOR EGG BY-PRODUCTS TO REPLACE SPRAY-DRIED PORCINE PLASMA IN EARLY-WEANED PIGLET DIETS.

5.1. Abstract

Egg-breaking facilities produce substantial quantities of egg by-products each year that are unsuitable for human consumption. Due to the excellent AA profile, the potential for spray-dried egg proteins to replace spray-dried porcine plasma (SDPP) in early-weaned pig diets was investigated in two 3-week performance trials and a 4-week, 2-phase trial. In Experiment 4 and 2, 5 pens containing four piglets (17 ± 1d old) stratified by sex were assigned to the experimental diets in a completely randomized design. Experiment 4 comprised of four corn-soy diets containing 7% of either SDPP, spray-dried technical albumen (SDTA), heat treated SDTA (hot room storage at 70°C for 3 days) or spray-dried whole egg (SDWE). Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratios (FCR) were determined. In addition, five piglets per treatment were euthanized to determine ileal AA and energy digestibilities, as well as *Enterobacteriaceae* levels. Relative to the SDPP diet, ADG, ADFI and FCR were poorer ($P<0.05$) for SDTA, heat treated SDTA and SDWE, with the SDTA performing closest to the SDPP diet. Apparent ileal digestibilities of methionine, lysine and threonine in SDPP and SDTA diets ranged from 80-90% and were generally higher ($P<0.05$) than in the SDWE diet. Ileal digestible energy content was similar ($P<0.05$) in all diets (3.1-3.2 Mcal/kg). *Enterobacteriaceae* levels were lower in the heat-treated SDTA diet than either the SDPP or SDTA diets ($P<0.05$). In the second experiment, the effect of substituting SDPP with varying levels of SDTA was investigated. Pig performance decreased linearly for ADG and ADFI, while it increased for FCR as the level of SDTA was increased in the

diet ($P<0.005$). Diets which substituted 25 or 50% of SDPP with SDTA performed as well as diets containing 7% SDPP. In Experiment 6, 8 pens containing 4 piglets (17 ± 1 d) stratified by sex were assigned to one of three phase I (0-14 d) diets: 7% SDPP, 5.25% SDPP + 1.75% SDTA or 3.5% SDPP + 3.5% SDTA. Animals fed phase I diets performed similar to those in Experiment 5 fed similar diets. In phase II (14-28 d), the experimental products were removed from the diets and animals fed diets containing 1.75% SDTA during phase I had numerically better FCR than the 7% SDPP diet. The results suggest that technical albumen can replace 25-50% of SDPP in early-weaned pig diets without compromising performance.

5.2. Introduction

Efforts to stimulate feed intake and maximize growth performance of weanling pigs often employ phase feeding. However, to stimulate feed intake and maximize performance through increased diet complexity is usually more expensive (Dritz *et al.*, 1996). Spray-dried porcine plasma (SDPP) is one of such ingredients used to improve post-weaning feed intake and weight gain (Hansen *et al.*, 1993; Kats *et al,* 1994a; Coffey and Cromwell, 1995; de Rodas *et al.*, 1995; Bergstrom *et al.*, 1997).

Spray-dried egg products are an interesting alternative to SDPP in early-weaned pig diets. Egg albumen has an excellent AA profile, with high content of methionine. Therefore, it could complement SDPP products known to be deficient in this essential SAA (Kats *et al.*, 1994a; Owen *et al.*, 1995). In addition, the inclusion of egg proteins could reduce the cost of diets containing SDPP by 12 to 13¢ per kilogram (James *et al.*, 1999). Many studies have evaluated the ability to incorporate spray-dried egg products into early-weaned pig diets. To date the trials have produced conflicting results. Owen *et al.* (1993), Nessmith *et al.* (1995) and Van Nevel *et al.* (2000) believe that egg proteins can be successfully incorporated into early-weaned pig diets. Zimmerman (1999), however, demonstrated that there was a linear decrease in the growth performance of weanling pigs as the level of egg product increased from 3 to 9%. Also, no advantage of incorporating egg products into the diets of weanling pigs was reported by De La Llate *et al.* (1998) and James *et al.*, (1999). Such variable responses may be due to different inclusion rates and the content of fat,

avidin or trypsin inhibitors in egg by-products (Owen *et al.*, 1993; Van Nevel *et al.*, 2000).

In addition to having an excellent AA profile, eggs contain several antimicrobial proteins, including lysozyme. Lysozyme, in its native conformation preferentially cleaves the N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) residues in gram-positive bacteria. Although lysozyme can cleave the NAM and NAG residues in gram-negative bacteria, its activity is limited. Several research groups have attempted to modify the structure of lysozyme to improve its ability to reduce the survival of gram-negative bacteria (Proctor and Cunningham, 1988, 1993; Yang and Cunningham, 1993 and Ibrahim *et al.*, 1993, 1994a,b, 1996a,b). Ibrahim *et al.* (1996a) showed that thermally denatured lysozyme has a novel antimicrobial mechanism that is independent of its catalytic activity (native lysozyme). It is believed that heat-treated lysozyme can better interact with the bacterial membrane of gram-negative bacteria, reducing their survival in vitro (Ibrahim *et al.*, 1996a, b; Ibrahim, 1998 and During *et al.*, 1999).

The objectives of this study were: (1) to evaluate the potential for spray-dried technical albumen (SDTA) to replace SDPP in weanling pig diets and (2) to assess the potential antimicrobial activity of lysozyme in SDTA.

5.3. Materials and Methods

5.3.1. Materials

Spray-dried technical albumen (SDTA), SDTA stored in a hot room (70°C) for 1 or 3 days, spray-dried whole egg (SDWE), and SDWE stored in a hot room (70°C) for 1 day were provided by Canadian Inovatech Inc., Winnipeg, MB,

Canada.

5.3.2. Early-weaned Pig Experiment 4

Eighty weanling pigs (Cotswold) averaging 5.8 ± 1.20 kg and weaned at 17 ± 1 days of age were used to asses diets containing technical grade egg products in a 21-day growth trial. Pigs were stratified by sex and assigned by weight in a randomized complete block design to pens containing four pigs per pen, with 5 replicate pens per treatment. Pigs were housed in an environmentally controlled nursery in which the initial temperature was 29.5°C (reduced by 1.5°C per week). Pigs had *ad libitum* access feed and water. At weaning, pigs were assigned randomly to one of four dietary treatments: (1) the control diet containing 7% spray-dried porcine plasma (SDPP), (2) spray-dried technical albumen (SDTA) substituted for SDPP, (3) 7% SDTA stored in a hot room for 3 days at 70°C and (4) 7% spray-dried whole egg (SDWE). The diets were corn/soybean meal/fish meal/lactose-based, were slightly deficient in protein (22%), contained 1.5% lysine and 0.36% methionine and were formulated to meet NRC requirements for calcium and available phosphorus (Table 30).

Upon termination of the experiment, 20 animals were anesthetized with halothan and then euthanized via cardiac puncture with sodium pentobarbital, in a manner approved by the University of Manitoba Animal Care Committee, to collect ileal digesta. Animals were sacrificed 5 hours after the lights came on and ileal digesta was collected from the terminal 30 cm of the small intestine. The digesta was flushed into sterilized containers using 50 mL cold sterile physiological saline. For *Enterobacteriaceae* counts, a portion of the digesta was

TABLE 30. Composition and calculated analysis of experimental rations fed to early-weaned pigs in Experiment 4.

	Ration 1	Ration 2	Ration 3	Ration 4
Ingredient (%)				
Corn	40.00	37.00	37.00	37.00
Lactose	12.66	13.66	13.66	12.66
Soybean meal	20.00	22.00	22.00	23.00
Oat Groats	6.04	6.04	6.04	6.04
Fish Meal	5.00	5.00	5.00	5.00
Spray-dried porcine plasma (SDPP)	7.00	0	0	0
Spray-dried technical albumen (SDTA)	0	7.00	0	0
SDTA stored in a hot room (3 d; 70°C)	0	0	7.00	0
Spray-dried whole egg (SDWE)	0	0	0	7.00
Vegetable oil	4.00	4.00	4.00	4.00
Mineral and vitamin Premix[1]	5.00	5.00	5.00	5.00
Chromic oxide	0.30	0.30	0.30	0.30
Calculated composition				
Digestible energy, (Kcal/kg)	3591	3594	3594	3596
Protein (%)	22.27	22	22	21.98
Lysine (%)	1.5	1.55	1.55	1.58
Methionine (%)	0.36	0.37	0.37	0.37

[1]Premix provided, per kg complete diet: Ca, 0.9%; P, 0.425%; Mg, 0.0125%; Mn, 35 mg; Fe, 152.5 mg; Zn, 137.5; Cu, 125; I, 0.75 mg; vitamin A, 1175 IU; vitamin D_3, 1500; vitamin E, 50 IU; vitamin K, 1.75 mg; choline chloride, 750 mg; niacin, 38 mg; calcium pantothenate, 35.75 mg; riboflavin, 10 mg; thiamin, 20 mg; vitamin B_{12}, 27.5 mg; biotin, 100 μg; folic acid, 0.5 mg.

serially diluted in saline (0.85%), plated in triplicate on violet red bile agar (Becton Dickinson, Cockeysville, MD, USA) using a pour plate-overlay technique and incubated at 35°C for 24 h. Results are expressed as CFU (colony forming units) per gram of digesta (DM basis). The remaining digesta were frozen (-20°C) and freeze-dried for chromic oxide, energy and AA analyses.

5.3.3. Early-weaned Pig Experiment 5

Ninety-six pigs (Cotswold) were used in a 21-day growth assay to determine the effects of replacing 25, 50, 75, or 100% of SDPP with SDTA. Pigs averaging 5.6 ± 1.14 kg and weaned at 17 ± 1 days of age were assigned by weight and stratified by sex to each of five dietary treatments. Each treatment contained five replicates with four pigs per pen, with the exception of the 100% SDTA ration, which, due to limited facility availability, only contained four replicates. Pigs were housed and managed as described in Experiment 4.

Diets were formulated to be slightly deficient in protein (22%) and contained 1.45% lysine and a minimum of 0.36% methionine (Table 31).

5.3.4. Early-weaned Pig Experiment 6

Ninety-six early-weaned pigs (Cotswold) were used in a 28 d, two-phase growth experiment to further asses the effects of substituting SDPP with 25 or 50% SDTA and the impact it may have on phase II growth parameters. Pigs averaging 6.2 ± 1.01 kg and weaned at 17 ± 1 days of age were stratified by sex and assigned by weight to pens (4 pigs per pen, eight pens per treatment). Diets containing SDPP and SDTA were fed during phase I only. Following AA analysis of feed components (Table 32), phase I diets were formulated to contain 1.45%

TABLE 31. Composition and calculated analysis of experimental rations fed to early-weaned pigs in Experiment 5.

	Ration 1	Ration 2	Ration 3	Ration 4	Ration 5
Ingredient (%)					
Corn	39.96	39.45	39.39	39.32	39.26
Lactose	14	14	14	14	14
Soybean meal	19.5	20	20	20	20
Oat groats	6.04	6.04	6.04	6.04	6.04
Fish meal	5	5	5	5	5
Spray-dried porcine plasma	7	5.25	3.5	1.75	0
Spray-dried technical albumen	0	1.75	3.5	5.25	7
Vegetable oil	3.5	3.5	3.5	3.5	3.5
Vitamin and mineral premix[1]	5	5	5	5	5
L-Lysine	0	0.01	0.07	0.14	0.2
Total	100	100	100	100	100
Calculated composition:					
Digestible energy (kcal/kg)	3575	3591	3604	3570	3631
Protein (%)	22.04	21.99	21.74	21.49	21.24
Lysine (%)	1.49	1.45	1.45	1.45	1.45
Methionine (%)	0.36	0.38	0.41	0.43	0.46

[1]Premix provided, per kg complete diet: Ca, 0.9%; P, 0.425%; Mg, 0.0125%; Mn, 35 mg; Fe, 152.5 mg; Zn, 137.5; Cu, 125; I, 0.75 mg; vitamin A, 1175 IU; vitamin D_3, 1500; vitamin E, 50 IU; vitamin K, 1.75 mg; choline chloride, 750 mg; niacin, 38 mg; calcium pantothenate, 35.75 mg; riboflavin, 10 mg; thiamin, 20 mg; vitamin B_{12}, 27.5 mg; biotin, 100 μg; folic acid, 0.5 mg.

TABLE 32. Amino acid composition (%) of feed ingredients used in phase I diets (Experiment 6).

Amino Acid	Corn	SBM[1]	Oat Groats	Fish Meal	SDPP[2]	SDTA[3]
Aspartic acid	0.564	4.744	0.919	5.335	7.214	6.674
Threonine	0.311	1.975	0.431	2.550	4.943	3.398
Serine	0.434	2.526	0.607	0.245	4.524	4.684
Glutamic acid	1.529	8.674	2.536	8.060	10.935	8.621
Proline	0.788	2.525	0.607	3.209	4.667	2.742
Glycine	0.289	1.892	0.590	4.829	2.992	2.353
Alanine	0.508	1.891	0.547	3.669	4.262	3.767
Cystine	0.245	0.738	0.246	0.669	3.150	2.252
Valine	0.310	1.806	0.466	2.388	3.827	3.620
Methionine	0.212	0.594	0.188	1.451	0.704	2.192
Isoleucine	0.216	1.462	0.297	1.800	1.920	2.617
Leucine	0.852	3.151	0.825	3.778	6.862	4.984
Tyrosine	0.230	1.265	0.254	1.636	3.910	2.265
Phenylalanine	0.328	2.069	0.543	1.969	4.046	3.536
Histidine	0.194	1.154	0.232	1.353	2.176	1.386
Lysine	0.263	2.728	0.445	4.396	6.293	4.593
Arginine	0.277	3.184	0.584	3.509	3.572	3.393

[1]Soybean meal; [2]Spray-dried porcine plasma; [3]Spray-dried technical albumen

lysine, and a minimum of 0.36% methionine (Table 33). At the end of phase I (14 days), all animals were switched to a common phase II diet. The phase II diet (Table 33) was formulated to contain 1.25% lysine and 0.34% methionine.

5.3.5. Chemical and Statistical Analyses

Chemical and statistical analyses were performed as described in Manuscript 1 of this thesis. Linear, cubic and quadradic analyses were also utilized as described by Snedecor and Cochran (1980).

5.4. Results

5.4.1. Early-weaned Pig Experiment 4

The pigs fed the control diet containing SDPP gained more weight ($P<0.01$) than the diets containing the spray-dried egg by-products (Table 34). Although the animals on the control diet had a greater ADFI, the differences were not statistically significant ($P>0.1$). In addition, the FCR for the control diet was the lowest, indicating good nutrient utilization. However, there was no statistical difference between the SDPP and the SDTA diet. Furthermore, the pigs fed the SDTA diet performed numerically better than those fed either SDTA stored in a hot room (70°C) for 3 days or the SDWE diets.

Energy and the AA digestibilities are shown in Table 35 and 36, respectively. There were no differences in the digestible energy content among the rations ($P>0.80$). However, there were several AAs including threonine, tyrosine, histadine, leucine, cystine and methionine that were more digestible in the diets containing technical grade egg products than in the control diet containing spray-dried porcine plasma ($P<0.05$). Overall, AA digestibility of egg

TABLE 33. Composition and calculated analysis of experimental rations fed to early-weaned pigs in Experiment 6.

	Phase I			Phase II
	Ration 1	Ration 2	Ration 3	
Ingredient (%)				
Corn	39.8	39.53	39.74	54.29
Lactose	14	14	14	0
Soybean meal	19.5	20	20	24.07
Oat groats	6.04	6.04	6.04	0
Fish meal	5	5	5	4
Spray-dried porcine plasma	7	5.25	3.5	0
Spray-dried technical albumen	0	1.75	3.5	0
Vegetable oil	3.5	3.25	3	2
Mineral and vitamin premix[1]	5	5	5	5
L-Lysine	0.16	0.18	0.22	0.14
Blood meal	0	0	0	1
Dried whey	0	0	0	9.5
Calculated composition				
Digestible energy (kcal/kg)	3569	3572	3573	3477
Protein (%)	22.02	22.00	21.78	20.47
Crude fat (%)	6.25	6.27	6.30	5.31
Arginine (%)	1.19	1.20	1.20	1.12
Cystine (%)	0.51	0.50	0.48	0.37
Phenylalanine (%)	0.95	0.95	0.94	0.85
Histidine (%)	0.54	0.53	0.51	0.51
Isoleucine (%)	0.61	0.63	0.64	0.61
Leucine (%)	1.67	1.65	1.62	1.58
Lysine (%)	1.45	1.45	1.45	1.25
Methionine (%)	0.33	0.36	0.39	0.34
Threonine (%)	1.01	0.99	0.96	0.85
Tyrosine (%)	0.71	0.69	0.66	0.55
Valine (%)	0.89	0.90	0.89	0.83

[1]Premix provided, per kg complete diet: Ca, 0.9%; P, 0.425%; Mg, 0.0125%; Mn, 35 mg; Fe, 152.5 mg; Zn, 137.5; Cu, 125; I, 0.75 mg; vitamin A, 1175 IU; vitamin D_3, 1500; vitamin E, 50 IU; vitamin K, 1.75 mg; choline chloride, 750 mg; niacin, 38 mg; calcium pantothenate, 35.75 mg; riboflavin, 10 mg; thiamin, 20 mg; vitamin B_{12}, 27.5 mg; biotin, 100 μg; folic acid, 0.5 mg.

TABLE 34. Growth parameters of early-weaned pigs fed diets containing spray-dried technical albumen (SDTA), SDTA stored in a hot room (70°C) for 3 days and spray-dried whole egg (SDWE) as substitutes for spray-dried porcine plasma (SDPP) (Experiment 4).

Diet	Average daily feed intake (g)	Average daily gain (g)	Feed conversion ratio
SDPP (control)	323	266[a]	1.22[a]
SDTA	298	212[b]	1.41[ab]
SDTA (70°C; 3d)	268	187[b]	1.44[b]
SDWE	278	194[b]	1.44[b]
Pooled SEM	35.7	40.5	0.13

[ab]Values within columns with no common superscripts differ significantly ($P<0.05$)

TABLE 35. Apparent Digestible Energy (DE) content of diets fed to early-weaned pigs in Experiment 4.

Diet	DE (Mcal/kg)
Spray-dried porcine plasma (control)	3.12 ± 0.27
Spray-dried technical albumen (SDTA)	3.06 ± 0.22
SDTA stored in a hot room (70°C; 3d)	3.22 ± 0.27
Spray-dried whole egg (SDWE)	3.20 ± 0.18
Pooled SEM	0.23

TABLE 36. Apparent Ileal digestibility of essential amino acids of spray-dried porcine plasma (SDPP), spray-dried technical albumen (SDTA), SDTA stored in a hot room (70°C) for 3 days and spray-dried whole egg (SDWE) based diets when fed to early-weaned pigs in Experiment 4.

Amino Acid	SDPP	SDTA	Hot-room stored SDTA	SDWE	SEM
Arginine	84.4	88.3	88.0	88.1	6.11
Cystine	70.5[b]	91.9[a]	92.2[a]	92.0[a]	10.38
Phenylalanine	81.7	86.7	87.9	87.5	6.13
Histidine	78.9[b]	92.3[a]	93.4[a]	93.5[a]	7.90
Isoleucine	78.6	88.8	89.1	89.8	6.97
Leucine	81.4[a]	76.2[b]	79.1[b]	78.5[b]	7.58
Lysine	82.4	83.0	84.2	84.1	5.76
Methionine	85.3[b]	96.3[a]	96.7[a]	96.3[a]	6.26
Threonine	70.8[b]	84.9[a]	85.0[a]	85.6[a]	10.03
Tyrosine	79.9[b]	91.9[a]	93.4[a]	93.2[a]	8.22
Valine	77.8	86.4	86.4	86.7	7.15

[ab]Values within rows with no common superscripts differ significantly ($P<0.05$)

products ranged between 80-90%; whereas, the diet containing SDPP had AA digestibilities that ranged between 70 and 85%.

The ability of SDTA and SDWE products to modify *Enterobacteriaceae* populations in the small intestine was also examined (Table 37). Relative to SDPP, *Enterobacteriaceae* counts in piglets fed the SDTA and SDTA stored in a hot room (70°C, 3 days) were not significantly different. However, the dry-heat employed during the hot-room storage of SDTA reduced the bacterial population below that of the unheated SDTA ($P<0.05$).

5.4.2. Early-weaned Pig Experiment 5

During the 21 d growth trial, ADG and ADFI decreased (linear, $P<0.002$; cubic, $P<0.001$), whereas, FCR increased (linear, $P<0.001$) as SDTA replaced increasing proportions of SDPP in the diet (Table 38). When SDTA replaced 25 or 50% of the SDPP, there was no statistical difference in the ADFI, ADG or the FCR.

5.4.3. Early-weaned Pig Experiment 6

Animals fed phase 1 diets showed similar trends to their counterparts used in Experiment 5 (Table 39). The phase II diet, which did not contain the experimental products, strongly suggest that the animals fed diets containing 25% SDPP substituted with SDTA utilized their feed more efficiently ($P<0.05$) in phase II and had a greater ADG than the animals fed the SDPP diet during phase I. The diet in which 50% of the SDPP was replaced with SDTA performed as well as the SDPP diet. Overall, the animals fed the 75:25 (SDPP: SDTA) diet tended to perform better than the 100:0 or the 50:50 diets.

TABLE 37. Effect of dietary protein supplement on ileal pathogenic Enterobacteriaceae counts in early-weaned pigs (Experiment 4).

Diet	Log_{10} CFU
Spray-dried porcine plasma (control)	6.97 ± 0.12^{ab}
Spray-dried technical albumen (SDTA)	7.05 ± 0.30^{b}
SDTA stored in a hot room (70°C; 3d)	6.60 ± 0.22^{a}
Spray-dried whole egg	7.29 ± 0.10^{c}
Pooled SEM	0.32

[abc]Values within columns with no common superscripts differ significantly ($P<0.05$)

TABLE 38. Growth parameters of early-weaned pigs fed diets containing various levels of spray-dried technical albumen (SDTA) substituted for spray-dried porcine plasma (SDPP) (Experiment 5).

SDPP to SDTA ratio (%)	Average daily feed intake (g)	Average daily gain (g)	Feed conversion ratio
100:0	380[a]	275[a]	1.38[a]
75:25	402[a]	284[a]	1.42[a]
50:50	376[a]	265[a]	1.45[ab]
25:75	333[b]	217[b]	1.54[ab]
0:100	283[c]	175[b]	1.64[b]
Pooled SEM	49.6	47.3	0.18
Linear	P=0.0007	P=0.0015	P=0.0002
Quadratic	P>0.05	P=0.0524	P>0.05
Cubic	P=0.0002	P=0.0001	P>0.05

[abc]Values within columns with no common superscripts differ significantly (P<0.05)

TABLE 39. Growth parameters of early-weaned pigs fed diets containing various levels of spray-dried technical albumen (SDTA) substituted for spray-dried porcine plasma (SDPP) during phase I of Experiment 6.

SDPP to SDTA ratio (%)	ADFI (g)	ADG (g)	FCR
Phase I (0 -14 days)			
100:0	310	259	1.21
75:25	331	269	1.24
50:50	328	256	1.28
Pooled SEM	43.8	44.0	0.11
Phase II (14 –28 days)			
(100:0)	828	568.5	1.46[a]
(75:25)	837	599.1	1.40[b]
(50:50)	800	568.5	1.40[b]
Pooled SEM	48.7	39.2	0.08
Entire trial (0 – 28 days)			
(100:0)	569[ab]	414	1.38
(75:25)	584[a]	434	1.35
(50:50)	557[b]	406	1.37
Pooled SEM	42.0	35.4	0.07

[ab]Values within columns and phase with no common superscripts differ significantly ($P<0.05$)

5.5. Discussion

SDTA and SDWE are excellent sources of SAAs, especially methionine. Results of AA digestibility, in Experiment 4, showed that methionine and cystine are more digestible in diets containing egg by-products than the diet containing SDPP (Table 36). Despite greater methionine, cystine, threonine and histidine digestibilities, the animals fed SDTA or SDWE containing diets had poorer ADG and FCR (Table 34). Since SDPP is known to be low in methionine (Kats *et al.*, 1994a; Owen *et al.*, 1995), the incorporation of egg by-products should complement SDPP's AA profile. In the first experiment, the diet whose performance most closely matched that of the SDPP diet was the SDTA diet. The ADFI and FCR were similar for the two diets ($P>0.05$), although the ADG was better for the animals fed the SDPP diet ($P<0.05$). The second experiment addressed the ability of SDTA to complement the AA profile of SDPP. The SDTA directly replaced SDPP at 0, 25, 50, 75 or 100%. As the level of SDTA increased, there was a linear decrease of ADFI, ADG and FCR, which agreed with earlier work by Zimmerman (1999). However, the performance of animals fed the diets in which 25 and 50% of SDPP was replaced by SDTA performed similar to animals fed the SDPP diet ($P>0.05$). Although egg products contain both avidin and trypsin inhibitors, it is believed that trypsin inhibitors play a larger role in growth repression than avidin. Van Nevel *et al.* (2000) found that whole egg products inhibit 40 mg of trypsin/g of feed. Despite this level, they speculated that inclusion of 5% whole egg products in the diet would not contain significant levels of trypsin inhibiting activity (TIA) to impact the growth of early-

weaned pigs. In SDTA, the TIA level would be expected to exceed that of Van Nevel's product as SDTA contains a greater proportion of egg albumen which contains the trypsin inhibiting protein ovomucoid (Burley and Vadehra, 1989). In Experiment 5, the inclusion of SDTA between 3.5 to 5.25% appears to be the upper inclusion rate of SDTA. To further address lysine concerns in the first two experiments, the AA profile of all proteinacious feed ingredients was determined before formulating the rations for Experiment 6. Furthermore, SDPP and SDTA were removed from the phase II diets to evaluate any residual benefit or detriment in animal performance. In the case of SDPP, it is not uncommon to see a reduction in the feed conversion of animals after the product has been removed, relative to those who did not have SDPP in the phase I diet (Kats *et al.*, 1994a; James *et al.*, 1999). In addition, the nursery environment (Coffey and Cromwell, 1995) and the herd's health status (Bergstrom *et al.*, 1997) may impact the response to SDPP. During the first two weeks of Experiment 6, the diets that contained a mixture of SDPP and SDTA performed similar to the SDPP diet (Table 39). In fact, during phase II, there was a trend for the diet containing 5.25% SDPP and 1.75% SDTA to perform better with respect to ADG and statistically better with respect to FCR ($P<0.05$) relative to the diet containing 7% SDPP. When examining growth over the entire four-week period, animals fed diets containing 1.75% or 3.5% SDTA tended to have an improved FCR. The animals fed diets containing 1.75% SDTA also tended to have greater ADG than animals fed diets containing 7% SDPP. Our data agrees with the findings of Owen *et al.* (1993), who suggested that egg proteins can replace up to 3% SDPP

and Nessmith *et al.* (1995) who documented that egg proteins can be included at a maximum rate of 3.5%. In contrast, James *et al.* (1999) found that although incorporation of egg proteins in the diet was economically beneficial, they had poorer growth response than diets containing SDPP. Further, De La Llata *et al.* (1998) did not find any benefit of replacing SDPP with spray-dried egg albumen. Most trials that have examined the inclusion of egg proteins in early-weaned pig diets have replaced SDPP with the egg products on an equal lysine basis (Owen *et al.*, 1993; Nessmith *et al.*, 1995; De La Llata *et al.*, 1998; James *et al.*, 1999; Zimmerman, 1999; Jaen *et al.*, 2001). However, replacing SDPP with egg proteins on an equal lysine basis does not take advantage of the higher methionine and cystine content of egg by-products and their improved digestibility, as documented in Experiment 4. Often replacing the egg products on an equal lysine basis resulted in the egg products being included in the diets at levels greater than 5%, which was the level cited by Van Nevel *et al.* (2000) to be safe for the inclusion of whole egg products. In addition, the egg products utilized in earlier studies had a high degree of variability in their fat and protein contents, ranging from less than 10% to 40% fat and approximately 45 to 81% protein (Owen *et al.*, 1993; Nessmith *et al.*, 1995; De La Llata *et al.*, 1998; James *et al.*, 1999; Zimmerman, 1999). In the current study the fat content of SDTA was 15.6% and the protein content averaged 64.5%; whereas SDWE contained 25.6% fat and 54.5% protein. Although egg by-products contain an excellent AA profile, they are also of high energy content, due to high fat levels. The additional energy present in egg products was taken into account when

formulating the diets and resulted in no difference in the digestible energy content of rations containing 7% SDTA, 7% SDTA stored in a hot room (70°C) for 3 days or 7% SDWE (Table 35). Despite the isocaloric content of the diets, Owen *et al.* (1993) suggested that the fatty acids of SDTA or SDWE were not as available to the growing pig and resulted in growth repression over diets containing soybean oil.

It was hypothesized that thermal denaturation of SDTA through hot room storage would optimize protein digestibility and reduce the antinutritive effects of trypsin inhibitors or avidin. However, the pigs fed diets containing SDTA stored in a hot room (70°C) for 3 days tended to have poorer growth performance than animals fed the diet containing 7% unheated SDTA (Table 34), despite a trend for improved AA digestibility of lysine, histidine, phenylalanine, tyrosine and isoleucine. It is believed that Maillard products, including carbohydrate- or lipid-protein conjugates, may affect the growth, although it may not be reflected in AA digestibility (Friedman, 1996a). In this context, Maillard reaction products have been shown to act as enzyme inhibitors (including pepsin, trypsin, carboxypeptidase A and aminopeptidase N), mutagens and nephrotoxins (Oste *et al.*, 1987; Pitotti *et al.*, 1994; Friedman, 1996a, b).

In addition to an excellent AA profile, egg products also contain antimicrobial compounds including immunoglobulins and lysozyme. Although other research groups are focusing on the presence of immunoglobulins in the egg product (Jaen, 2001), it is unlikely that the avian immunoglobulins can withstand pelleting temperatures. Lysozyme is a naturally occurring protein that

can cleave NAM and NAG residues in the peptidoglycan of gram-positive bacteria. However, thermally denaturing lysozyme through pelleting to induce a novel antimicrobial activity against gram-negative bacteria (Ibrahim *et al.*, 1996a, b, 1997; Ibrahim, 1998; During *et al.*, 1999) should be investigated. In Experiment 4, a decrease in *Enterobacteriaceae* levels was observed in diet containing SDTA stored in a hot room (70°C) for 3 days ($P<0.05$). The diet containing 7% SDTA had levels similar to SDPP, suggesting that it is as effective at limiting the growth of enterotoxigenic *Escherichia coli* which contribute to the etiology of post-weaning diarrhea (Hampson, 1994; Hampson and Pethick, 1998). Heat-treated lysozyme has an altered hydrophobicity, which allows it to more effectively agglutinate with the cell wall of gram-negative and gram-positive bacteria. This may also allow lysozyme to displace Ca^{2+} or Mg^{2+} from the bacterial membrane to either stimulate autolysins or destabilize the membrane proteins (Ibrahim *et al.*, 1996a, b, 1997). Recent studies by Ibrahim (1998) and During *et al.* (1999) suggest that lysozyme may compete with bacteria for the calcium-affiliated binding sites associated with the cells lining the intestinal tract. In the case of SDWE, the higher fat level of the product may negate the benefits of lysozyme by inhibiting gram-positive bacteria and allowing the proliferation of gram-negative bacteria (Bovee-Oudenhoven *et al.*, 1999; Sprong *et al.*, 1999). The current study indicates a potential for lysozyme to reduce *Enterobacteriaceae* levels; however, further research is needed to elucidate the mode of action and further optimize the novel antimicrobial properties of lysozyme.

5.6. Implications

Data from the current study indicates that it is possible to replace 25-50% SDPP with SDTA in weanling pig diets. The additional methionine in SDTA complements SDPP resulting in improvements in ADG and ADFI. Once the products are removed from the diets, the animals fed the SDPP: SDTA blended diets continue to do as well or better than diets containing SDPP alone. In addition, the presence of lysozyme in the egg by-products may reduce *Enterobacteriaceae* level in the ileum, in a manner similar to that observed for immunoglobulins of SDPP. Further research is needed to optimize the novel antimicrobial effects of lysozyme without jeopardizing protein quality.

6. General Discussion

In vitro studies showed that the protein egg digestibility could be improved through moist heat treatment. However, when commercial forms of heating were employed (hot-room storage), no improvement in protein digestibility was observed over the spray-drying process alone. To further evaluate the nutritive value of variously treated egg by-products, experiments with rats, broiler chickens and early-weaned pigs were conducted. The rat trial contained the most diverse range of products evaluated. In addition to SDTA and SDWE, SDTA and SDWE stored in a hot room (70°C) for 3 days were also evaluated. In broiler chickens, a growth trial, a production trial and a TME assay were used to evaluate the egg by-products. In the growth trial SDTA and two lengths of hot-room storage were used (1 or 3 days) for SDTA and 1 day of hot-room storage for SDWE. The TME assay evaluated SDTA and SDTA stored in a hot-room (70°C) for 3 days. In the early-weaned pig trial, the only heat treatment was hot-room storage of the SDTA. In all three animal models, additional heating (through hot-room storage) was detrimental to the growth of animals. In the rat trial and the first pig experiment, AA digestibility was slightly improved by hot room storage. However, in the growth trial and the TME assay in chickens, there was a trend towards reduced AA digestibility. In all animals, the growth parameters were poorer for the animals fed hot-room stored egg by-products. In the rat trial raw technical albumen was also incorporated into the diets to evaluate the effect of spray drying on nutrient availability in technical albumen.

Raw technical albumen was incorporated into the diets to further evaluate the effect of hot-room storage on antinutritive factors including avidin and trypsin inhibiting proteins. There was significant difference in the growth performance of animals fed raw technical albumen or SDTA. Upon visual inspection of the hot-room stored products, some changes to the colour were noted. While it is difficult to quantitate the extent of the Maillard reaction in the hot-room stored products through AA availability, it is believed that some Maillard products may act as enzyme inhibitors, mutagens or nephrotoxins (Friedman, 1996a, b; Pitotti *et al.*, 1994; Oste *et al.*, 1987). In the chicken and the rat trials, where SDWE was also subjected to hot-room storage, the trend in growth parameters was consistent with that of SDTA.

Both SDWE and SDTA have excellent AA profiles that are high in methionine. It was anticipated that all animals would perform as well on either product. However, in the rat and pig experiments the animals fed diets containing SDWE performed poorer than animals fed the diets containing SDTA. It was speculated by Owen *et al.* (1993) that egg fat is not as available to the pig as other fat sources (i.e. soybean oil). The level of fat was significantly higher in the SDWE product than in SDTA (25.6% vs. 15.4%). In the rat assay, higher dietary fat caused fatty liver syndrome and resulted in a reduced liver weight in animals fed the diets containing SDWE. However, in the broiler chickens, the SDWE product performed equally or better than the diets containing SDTA. Sulistiyanto *et al.* (1999) showed that fat energy is used more efficiently than either protein or carbohydrates in chickens. In addition, Peebles *et al.* (2000) showed that birds

fed poultry fat tended to perform better than animals fed soybean oil. Since the fatty acid composition of poultry fat and egg fat are highly correlated (Sim, 1970) it would be expected that broiler chickens would perform better when fed diets containing SDWE.

In early-weaned pig diets, the ideal ratio of SDPP to SDTA was also evaluated. Although the products have an excellent AA profile, it is believed that the presence of trypsin inhibitors (inhibiting 40mg of trypsin/ g egg product) may impact growth when egg products are incorporated at higher levels in the diet (Van Nevel *et al.*, 2000). Since SDPP is deficient in methionine (Owen *et al.*, 1993, Kats *et al.*, 1994a), it was believed that a blend of SDPP and SDTA would optimize growth, while reducing the cost of the diets (James *et al.*, 1999). It was documented that SDTA can replace 25-50% of SDPP without compromising growth performance. In addition, when SDTA or SDPP were removed from the diet in phase II of the experiment, the animals fed a blend of SDTA and SDPP in phase I had an improved ($P<0.05$) FCR and a trend ($P<0.$) towards lower FCR overall, indicating better nutrient utilization, for the combined phase I and II period.

The presence of lysozyme in egg albumen, offers a unique opportunity to modify the gastrointestinal micro flora through the diet. In its native conformation, lysozyme catalytically cleaves the NAM and NAG residues in the peptidoglycan layer of gram-positive bacteria. Thermal denaturation invokes a novel antibacterial activity in lysozyme (Ibrahim *et al.*, 1996a, b; Ibrahim *et al.*, 1997; Ibrahim, 1998; During *et al.*, 1999). This activity was documented in vitro

with *Escherichia coli O157:H7* and *Salmonella typhimurium 266.* Over native lysozyme, heat-treated lysozyme was able to reduce the survival of *E. coli* and *S. typhimurium* to 0.09 and 13.97%, respectively. In addition, γ-radiation of lysozyme also resulted in similar antimicrobial activity towards gram-negative bacteria. SDTA lysozyme either heat-treated or γ-radiated displayed similar antimicrobial activity. With heat-treated SDTA, there was no reduction in *E. coli* or *S. typhimurium* counts, as noted in a series of time course studies. However, γ-radiated SDTA was able to reduce the survival of *E. coli* by 45-50% over the non-radiated product. *In vivo*, the ability of SDTA and hot-room stored SDTA were examined in rats, broiler chickens and early-weaned pigs. Radiated SDTA was also examined in the rat trial. In the pig and chicken trials, there was a trend towards reduced levels of a family of gram-negative bacteria, *Enterobacteriaceae* over that of SDPP (early-weaned pigs) or virginiamycin (broiler chickens). SDPP is believed to contain immunoglobulins, which are believed to attenuate the level of bacteria. In the rat trial, hot-room stored SDTA did not show reduced levels of *Enterobacteriaceae*. The diets that did show a reduction in *Enterobacteriaceae* contained SDTA moist-heated (80°C for 20 minutes), and radiated SDTA (1.8 kGy). SDWE consistently increased the levels of *Enterobacteriaceae* in the distal ileum. The increased level of fat in SDWE may reduce the survival of gram-positive bacteria and allow for the proliferation of gram-negative bacteria including *Enterobacteriaceae* (Sprong *et al.*, 1999; Bovee-Oudenhoven *et al.*, 1999).

7. Conclusions

1. Induction of a novel bactericidal activity of egg lysozyme against gram-negative *Escherichia coli* and *Salmonella typhimurium* by thermal treatment or gamma-radiation is documented by the results of this study.

2. Gamma radiation of technical albumen reduced the survival rate of pathogenic bacteria *in vitro*.

3. A negative effect of hot room storage (i.e., 3 days at 70°C) on the nutritive value of spray-dried technical albumen and whole egg by products was documented *in vivo* with rats, early-weaned pigs and chickens.

4. It has been demonstrated that spray-dried technical albumen can replace 25% of spray-dried porcine plasma in early-weaned pig diets without compromising performance.

5. It is evident from this study that the substitution of fish meal with the spray-dried technical albumen and whole egg products could further improve broiler chicken performance.

6. Spray-dried technical albumen lysozyme added to animal diets may reduce *Enterobacteriaceae* levels in the distal ileum.

8. References

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