Role of sweet potato fibre on energy utilisation, gut morphology, and gut microbiota in broilers

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(B. Ag. Sc. & M. Animal Sciences)

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Thesis Abstract

Despite considerable research on the feeding value of sweet potato there is a dearth of information on how dietary fibre, in particular total non-starch polysaccharide (NSP) content of sweet potato roots can influence gut health of broiler chickens. It is well established that diet composition and ingredients affect the health of birds by influencing the internal gut environment and microbial status of the gastrointestinal tract of poultry. In developing countries such as Papua New Guinea (PNG), manufactured feed is expensive due to importation of most feed ingredients. Interventions into different practical diets for monogastric livestock using local ingredients for small holder producers are necessary for sustainable and efficient production under local environmental and management conditions.

The impact of several selected local PNG sweet potato varieties with different total NSP contents was studied. Diets based on these varieties were fed to broiler chickens to investigate the effects of the total NSP contents on the apparent metabolisable energy (AME) of diets and growth performance, gut morphology, and detectable levels of *Campylobacter, Salmonella* and *Clostridium perfringens* in the ceca of these birds. Inclusion of an enzyme product in these diets was also assessed. It was found that sweet potatoes from PNG have an average total NSP content of 172 g/kg DM, and generally have higher fractions of insoluble compared to soluble NSPs. AME and digestibility of diets were influenced by the total NSP content. The variety with a low total NSP content had a lower AME value which was improved with enzyme inclusion. Varieties with high total NSP contents had AME values within the required levels for finishing broilers and inclusion of enzymes did not improve or elucidate any effects. Feed intakes were low in broilers fed with sweet potato and this translated into low end weights, weight gains, and poor feed conversion ratios (FCR), despite being high in energy and highly digestible. FCRs did improve with continuous feeding. Digestive capacity of broilers in terms of gut morphology was not influenced despite varying NSP contents. Total NSP contents did however, influence the load of enteric pathogens. Varieties with high total NSP contents had

high levels of *Campylobacter* which were reduced with enzyme inclusion and marginally reduced with continuous feeding of sweet potato but this needs to be further investigated to validate this finding. Levels of *C. perfringens* were high in the variety with a low total NSP content but these levels were comparable to the sorghum diet and may not pose a risk of necrotic enteritis in broilers. Levels of *Salmonella* were low in diets with and without sweet potato. Strategies to reduce campylobacteriosis and salmonellosis in poultry famers and their families as well as product contamination associated with these zoonotic pathogens must be taken into consideration when developing food safety regulations and policies in PNG as broilers raised under these smallholder enterprises supply the informal live broiler chicken industry in PNG. There is currently a lack of surveillance data on the levels of these pathogens under village or smallholder poultry production systems, and information generated here could be used as baseline information for future research in terms of production parameters and managing bird health via nutritional feeding strategies.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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List of Publications

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Chapter 1 The use of sweet potato (*Ipomoea batatas* (L.) Lam) root as feed ingredient for broiler finisher rations in Papua New Guinea

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The use of sweet potato (*Ipomoea batatas* (L.) Lam) root as feed ingredient for broiler finisher rations in Papua New Guinea

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1.1 Abstract

The use of non conventional feedstuffs in poultry rations is now a common practice in developing countries where most of the ingredients used in the production of commercial poultry stockfeed are imported grains. The prices of these grains are dictated by world market prices and this translates into high retail prices for the end users. The purpose of this review is to highlight the potential of sweet potato root as a poultry feed ingredient especially for finishing off broilers in Papua New Guinea where this root crop is in abundance. This review discusses in general the metabolisable energy value of this crop when compared to maize and its impact on the intake of broilers when processed differently and fed at various inclusion rates. Trypsin inhibitors are the major anti-nutritive factor present in sweet potato roots however these are eliminated with heat moisture treatments. The dietary fibre in sweet potato roots has been shown to have anti-microbial and prebiotic attributes which may be beneficial in promoting good gut health in chickens. The use of exogenous enymes in sweet potato diets is briefly mentioned in this review. All in all, sweet potato roots can be included in diets for broiler chickens at 30 percent without adversly affecting intake of birds if processed correctly. However, to date limited information is available on how sweet potato can enhance digestive capacity of broilers in terms of gut morphology and digestive enzyme activities, as well as the shedding of the main zoonotic bacteria such as *Campylobacter* and *Clostridium perfringens*. The shedding of these bacteria in relation to food safety is important if sweet potato is to be used regularly in finishing off broilers in PNG.

Keywords: sweet potato, broiler chickens, Papua New Guinea, feed, dietary fibre, *Campylobacter*, *Clostridium perfringens*

Abbreviations: PNG, Papua New Guinea; NSP, non starch polysaccharides; AME, apparent metabolisable energy; water soluble non starch polysaccharides, WSNSP; water insoluble non starch polysaccharides WINSP; feed conversion ratio (FCR); sweet potato root meal, SPRM; NE, necrotic enteritis

1.2 Introduction

The cost of growing poultry, pigs and fish (inland aquaculture) is very high as feed alone makes up to 80 percent of the total cost of animal production in Papua New Guinea (PNG) (Ayalew, 2011). Village livestock production is constrained by the high cost of grains imported for making commercial livestock feed. Virtually none or very little locally available ingredients in the form of agro-industrial by-products, or traditional root crops such as cassava, plantain or sweet potato is used by feed manufacturers. The cost of commercial feeds has increased from 56 to 110 percent between February 2003 and July 2011 (Ayalew, 2011). This has placed an economic constraint on backyard, small and semi-commercial poultry farmers who raise poultry as a source of income. Sweet potato can be promoted as a major energy source for poultry, especially broilers in the live broiler chicken markets. It is currently being used by the backyard and small-scale broiler producers as the cheaper feeding options to finish off broilers. Greater and regular use of this root crop in broiler diets can be promoted if more in-depth work is done on understanding production parameters, digestive health and food safety issues associated with this crop when utilised in broiler finisher diets. Continual increases in the cost of conventional ingredients further justify rational and efficient use of this crop in broiler diets in developing countries such as PNG.

1.3 Importance of sweet potato in developing countries

Sweet potato is ranked sixth in the world as the most important food crop after rice, wheat, potatoes, maize and cassava (CIP, 2010). It can grow in marginal conditions, requiring little labour and chemical fertilizers. It is a cheap, nutritious solution for developing countries needing to grow more food on less area of land. It is a major traditional staple and feed crop in the eastern and outlying islands of Indonesia and the highlands of West Papua (Campilan, 2009).

Papua New Guinea is regarded as the second most important centre of sweet potato genetic diversity in the world (Liu et al., 2010). There are about 5000 cultivars grown in PNG; no single cultivar dominates production or trade and there is a wide variation in flesh and tuber skin colour. The national average consumption of sweet potato is 260 kg/person/year (Chang and Kewa, 2014). In PNG, sweet potato is becoming a cash crop for smallholder farmers driven by the need to generate income in a modernizing market economy. Thus, greater utilisation of the crop as an ingredient for raising livestock especially poultry would enhance the value of this crop further for both sweet potato farmers and small-scale poultry producers.

1.4 Chemical composition of sweet potato roots

Sweet potato is composed largely of starch, cellulose, hemicellulose, pectin and sugars. The carbohydrate content accounts for 800–900 g/kg of the total dry mater content. The dry matter content ranges from 130 to 480 g/kg and varies depending on the cultivars, cultural practices and local climatic conditions (Bradbury and Holloway, 1988).

1.4.1 *Starch*

Starch accounts for about 600–700 g/kg of dry matter in sweet potato roots and cultivar characteristics dictate the starch content of roots (Padmaja, 2009). Sweet potato starches are composed of 300–400 g/kg amylose and 600–700 g/kg amylopectin (Padmaja, 2009; Waramboi et al., 2011). Physiochemical properties of sweet potato starch depend at least in part on the amylose/amylopectin ratio thus the digestibility of sweet potato starch is negatively correlated with its amylose content (Zhang and Li, 2004). The reduced digestibility is a result of the amylose forming a tightly packed structure which limits access of amylases (Waramboi et al., 2011).

1.4.2 *Sugar*

Total sugar content in sweet potato cultivars is variable. The range reported by Bradbury and Holloway (1988) is between 3.8 and 56.4 g/kg respectively. Sucrose is the most abundant sugar, followed by glucose and fructose in raw sweet potato roots (Padmaja, 2009; Waramboi et al., 2011). Varietal variations have also been reported in the glucose and fructose levels, with some cultivars containing more glucose whilst fructose is higher in others (Bradbury and Holloway, 1988). There is an increase in the content of maltose when sweet potato roots are cooked and this is associated with the hydrolysis of starch to maltose.

1.4.3 *Protein*

Sweet potato roots are low in crude protein (Table 1). The main amino acid profile of sweet potato roots is provided in Table 2. Sweet potato roots are a poor source of protein and are also lower in lysine and sulphur amino acids (Dominguez, 1992). The most limiting amino acids in poultry are methionine and lysine which are required for effective protein utilisation. These two amino acids as well as cysteine are low in sweet potato roots (Dominguez, 1992). This deficiency in these limiting amino acids can be overcome by adding protein concentrates or synthetic amino acids when used as a feed ingredient in poultry diets.

1.4.4 *Micro-nutrients*

The content of major minerals found in sweet potato roots is shown in Table 2. Apart from these minerals, sweet potato roots are rich in carotenoids which are present as beta-carotene and ranges from 0-20,000 ug/100 g (Woolfe, 1992). The mean values of vitamin C, thiamine (B1), riboflavin (B2) and pantothenic acid are 17, 0.09, 0.06 and 0.59 mg/100 g in fresh roots (Woolfe, 1992).

1.4.5 Dietary fibre

The non-starch polysaccharides (NSP) comprising cellulose, hemicellulose and pectin contribute towards the dietary fibre fraction of sweet potato roots (Padmaja, 2009). Studies conducted by Mei et al. (2010) on the proximate dietary fibre components of sweet potato residues showed that the main dietary fibre fractions obtained were cellulose (31.2 percent), lignin (16.9 percent), pectin (15.6 percent) and hemicellulose (11.4 percent) This was similar to those reported by Salvador et al. (2000). The insoluble cellulose and the soluble hemicellulose and pectin seem to support the notion of a well-balanced content of these components of dietary fibre in sweet potato roots (Holloway et al., 1985; Englyst et al., 1988).

1.4.6 *Anti-nutritive factors*

Raw sweet potato roots contain trypsin inhibitors, and the level of these trypsin inhibitors may vary between different cultivars. The values obtained by Ravindran et al. (1995) ranged from 2.6-32.0 TUI/g, whilst Bradbury and Holloway (1988) reported a mean value of 13 TUI/g from 164 sweet potato samples from the South Pacific. Moist heat treatment at temperatures above 80 degree Celsius is recommended by Zhang and Corke (2001) to effectively eliminate trypsin inhibitors present in sweet potato roots.

1.5 Feeding of monogastric livestock with sweet potato root meal

Wherever sweet potato is cultivated, some part of the plant, in some form, is used in animal production (Scott et al., 2000). Sweet potato and its by-product after processing can be fed to all classes of livestock (Woolfe, 1992). Sweet potato has been fed to pigs, fish and poultry to replace grain in developing countries. Sweet potato leaves and roots have been fed to pigs by numerous researchers as highlighted in Padmaja (2009) and FAO (2012). Provided that an adequate level of protein is supplemented, cooked sweet potatoes may completely replace maize for fattening pigs (FAO, 2012). Sweet potato leaves and roots have been fed to fish

(Padmaja, 2009). Inclusion rates of up to 75 percent sweet potato peel in fish diets has been reported by Olukunle (2006). Sweet potato roots have also been used to feed growing pullets and laying hens (Agwunobi, 1995; Aina and Fanimo, 1997; Ladokun et al., 2006; Ladokun et al., 2007). Inclusion of this root in layer diets should be limited to between 10 and 15 percent with adequate supplementation of protein and vitamin A (FAO, 2012). Dehydrated sweet potato roots have an average Metabolisable energy (ME) value of 14.5 MJ/kg and a mean protein content of 46 g/kg (Table 2). This ME value is similar to that of maize which is around 14.44 MJ/kg whilst the protein content is around 90 g/kg making SPRM comparable to maize as an energy source for broiler diets (Ravindran and Blair, 1991). A total of sixteen sweet potato cultivars from Sri Lanka were analysed for their potential as poultry feed ingredient. The mean AME value obtained for these 16 samples was 14.54 MJ/kg which was similar to that determined for maize samples which was 14.45 MJ/kg (Ravindran et al., 1995). Apart from the AME value being similar to that of maize, the in vitro protein digestibility was well utilized at 75.8 percent. The authors concluded that sweet potato has potential as an energy source for animal feeding in the tropics.

1.6 Chemical properties of sweet potato cultivars from PNG

Nutrient profiles of 22 sweet potato cultivars from PNG were reported by Bradbury and Holloway (1988). The moisture content ranged from 715 g/kg in lowland cultivars to 728 g/kg in highland cultivars, whilst protein content was reported to be around 13 to 17 g/kg, respectively (Bradbury and Holloway, 1988). Starch content ranged from 195 to 203 g/kg, with the cultivars from the lowlands of PNG having a higher starch content compared to cultivars from the highlands. Dietary fibre level of 12 g/kg was obtained for samples from the lowlands compared to highland cultivars which had an average dietary fibre content of 15.3 g/kg (Bradbury and Holloway, 1988). Recent work on several cultivars from PNG on starch digestion and potassium release showed that the cultivars with higher starch content were digested less (Liu et al., 2010). These cultivars had starch content of 590, 630 and 790 g/kg DM basis respectively. The authors postulated that this may be related to the different properties of raw and processed starch of sweet potato roots (Liu et al., 2010). A similar trend was observed by Waramboi et al. (2012) in that some cultivars tested were not completely digested and had fractions of resistant starch. These authors concluded that starch digestibility in sweet potato flour maybe affected by interactions and associations between the starch and non-starch components of the sweet potato roots.

The feeding value of a PNG sweet potato cultivar obtained in an Apparent Metabolisable Energy (AME) bioassay using the total collection method with 21 day-old broilers was found to be 15.39 MJ/kg (Glatz, 2007). This value is higher than that of maize (Ravindran and Blair, 1991) and the product evaluated by Ravindran et al. (1995). This difference may be attributed to the different types of cultivars of sweet potato used by the different researchers as well as the different collection method as broilers consistently derive higher AME values than cockerels (Lopez and Leeson, 2007). Despite this difference, the AME value of the sweet potato cultivar from PNG is comparable to maize and can be utilized as an energy source for finishing off broilers and other poultry types in PNG.

In order to further assess the potential of this crop, thirteen PNG sweet potato cultivars were selected based on their commercial market value and potential as feed material. These cultivars were tested for their NSP contents using the gas chromatography method described by Englyst et al. (1988). The range of total NSP content in these 13 cultivars ranged from109 to 211g/kg DM whilst the proportion of the total water soluble non-starch polysaccharides (WSNSP) ranged from 42 to 105 g/kg DM. Interestingly, only two out of the 13 cultivars analysed had higher fractions of the WSNSP. Dietary fibre is present in all plant derived feed ingredients and unlike starch cannot be degraded by the endogenous enzymes of monogastric animals. Non-starch polysaccharides are anti-nutritive to poultry and other monogastric animals due to their negative effect on digestion processes. These NSPs can either be WSNSP or water insoluble (WINSP). The WSNSP fraction is regarded as anti-nutritive in poultry and other mono-gastric animal and its content in a feed ingredient is therefore a partial indicator of the nutritive value of the ingredient (Liu et al., 2013). The WINSPs on the contrary can affect gut functions and modulate nutrient digestion when included at moderate levels in poultry (Hetland et al., 2004).

Sweet potato from PNG has potential to be used as an energy source in poultry diets as its AME value is comparable to that of maize. These roots however must be used in combination with a protein supplement as the protein content of sweet potato roots are low (Table 1). Additionally, some of the cultivars may contain higher fractions of WINSP compared to WSNSP and may prove beneficial to poultry at gut level.

1.7 Feeding of sweet potato to broiler chickens in PNG

In Papua New Guinea, poultry rations made with different root crops supplemented with concentrate mixes are fed to poultry either as a finisher feed for broilers or as a layer feed for maintaining different laying genotypes (Glatz, 2007; 2013). An assessment of the feeding value of sweet potato roots was conducted to evaluate the form of presentation of these roots to broilers. Sweet potato roots were fed to broiler chickens either as a wet mash or as a dried milled product. Processes involved in the preparation of sweet potato tubers included washing, chopping or grating and boiling. After cooling, the boiled tubers are either used directly as mash or dried and milled before being mixed with matching energy concentrates.

The results showed that bird performance was not affected by the form of presentation and that farmers can process these roots either as freshly cooked wet mash or as a dried milled product (Glatz, 2013). Numerous feeding trials were conducted to assess the growth of broilers fed sweet potato with a concentrate mix. The low energy concentrate mix had a ME content of 9.4 MJ/kg and a crude protein content of 418 g/Kg DM. Feeding options tested were the 50 percent sweet potato plus 50 percent low energy concentrate (SP50L) and 70 percent sweet potato plus

30 percent low energy concentrate (SP70L). Average daily feed intakes of birds fed the 50 and the 70 percent sweet potato diets were 126 and 129 g compared to 154 g for the control diet (Table 3). Birds fed the SP50L diet had the second highest weight compared to the control diet. These birds had by week 7 significantly higher ($P < 0.01$) gains compared to birds on the other experimental diets (Glatz, 2013). The average feed conversion ratio (FCR) of broilers on the sweet potato diets were significantly different ($P < 0.01$) throughout the experiment. The FCR ofthese birds improved by week 7 and this improvement may have been due to the delayed adaptation of birds to the experimental diets and supports the findings reported by Panigrahi et al. (1996).

1.7.1 Growth performance, relative organ weights and gut morphology

Nutrient intake of poultry is affected by both the nutrient composition of the diet and the amount of feed eaten. Processing of the sweet potato roots and the type of cultivar used are the major factors affecting its utilisation in poultry (Panigrahi et al., 1996). Numerous studies have been conducted to assess broiler performance when fed sweet potato root meal (SPRM). SPRM has been fed to broiler chickens at varying inclusion rates of between 10 to 100 percent by a few researchers (Table 3). Inclusion of SPRM at 0, 10, 20 and 30 percent did not affect the average daily intake and FCR of birds (Beckford and Bartlett, 2015). Similar results were reported by (Glatz, 2013).

On the contrary, Panigrahi et al. (1996) suggested that tuber utilisation appeared to be affected by the different degrees of feed intake which was restricted by the high and variable water absorbing nature of the tuber carbohydrates. This view was also expressed by Afolayan et al. (2012) and Maphosa et al. (2003) when trying to explain the decline in body weight gain and feed intake with increasing levels of sweet potato root meal in broiler diets. Effect of SPRM on internal organ weights and other carcass components were reported by Beckford and Bartlett (2015), Afolayan et al. (2012) and Agwunobi (1999). These authors did not observe any significant differences on the relative weight of non-commercial carcass components such as gizzard, hearts, liver and shank due to inclusion of sweet potato roots.

Sweet potato root meal when processed appropriately can be included in the diets of poultry by 30 percent with no adverse effect on intake and FCR (Table 3). Organ weights were not significantly affected with the inclusion of SPRM in finishing broilers. However, mucosal changes such as villi heights and villi and crypt depth or absorption area in the small intestine were not investigated, highlighting the need to investigate if such parameters are enhanced by the inclusion of SPRM with high WINSP content in broiler diets.

1.7.2 Dietary fibre levels of sweet potato and implications on gut attributes

Uncooked starch in sweet potato roots is resistant to enzyme hydrolysis; however this is greatly improved by cooking (Dominguez, 1992). Cooking of sweet potato roots have been shown to increase the dietary fibre level in boiled and steamed sweet potatoesfrom 1.4 to 3.46 percent (Bradbury and Holloway, 1988).The NSPs which are often cellulose, hemicellulose and pectin contribute towards the 'dietary fibre' fraction of sweet potato roots (Padmaja, 2009). Dietary fibre fractions are not degraded by endogenous digestive enzymes of chickens and will have an impact on the physiology of the gut due to its physical presence thereby affecting the mucosa along the gastrointestinal tract of birds (Montagne et al., 2003). These effects can either be positive or negative depending on the type of dietary fibre and the level of inclusion in the diets (Montagne et al., 2003) diets. Minute changes to the gut such as slower digesta transit time associated with WSNSP will trigger microscopic changes to the mucosal layer and this will affect nutrient assimilation (Choct, 2009). A high gut viscosity which is triggered by the presence of WSNSP will decrease the rate of diffusion of substrates and digestives enzymes and hinder their effective interaction at the mucosal surface, thereby acting as a physical barrier to the digestion and absorption of nutrients in the gut (Choct, 1997). A high gut viscosity may also trigger villus cell losses leading to villus atrophy (Montagne et al., 2003).

Nutrient assimilation is also affected by villi height, thus, a decrease in villi height means less surface area for absorption and lower nutrient uptake (Choct, 2009). This may then ultimately compromise growth, health and welfare of birds.

Dietary fibre levels in sweet potato roots seem to be well balanced (Holloway et al., 1985; Englyst et al., 1988) and are often cellulose, hemicellulose and pectin (Padmaja, 2009). There is currently limited information available on the effects of sweet potato fibre on gut morphology of broiler chickens.

1.7.3 Digestive enzymes

Digestive enzymes determine the amount of nutrients available for absorption and are thereby closely associated with nutrient assimilation and absorption in the gut (Iji et al., 2001). Regional activity of mucosal enzymes is related to the digestive capacity in the three different regions of the small intestine (Uni, 2006). The activities of these disaccharidases may be affected by the characteristics of the diets and available substrate (Uni and Ferket, 2004). There is currently limited data available to date on how these digestive enzyme (maltase and sucrase) activities are influenced by the inclusion of SPRM in broiler diets.

1.7.4 Use of exogenous feed enzymes in sweet potato based diets

Regular use of alternative feed ingredients in poultry diets is impeded by high fibre fractions. These fibre fractions are structural carbohydrates which are not digested by the endogenous enzymes in chickens and other monogastric livestock. The presence of moderately high levels of WSNSP in the gut will create a viscous environment which slows down the digesta transit time resulting in the proliferation of non-beneficial bacteria.

Use of exogenous enzymes aids the hydrolysis of this component of the feed thereby reducing its viscosity in the gut of chickens. To date, the work conducted by Nunes et al. (2010) is the only published data on the use of exogenous feed enzymes with SPRM.

1.7.5 Diet, gut microflora composition and possible changes due to sweet potato fibre

The microbial status of the gastrointestinal tract of chickens, is influenced by diet and the internal gut (Apajalahti et al., 2004). The commensal microbial community plays a major role in the health and digestion in chickens (Kleyn, 2013). The chemical composition of the digesta determines the composition of the microbial community in the gastrointestinal tract (Apajalahti et al., 2004). This relationship between digesta composition and gut microflora composition is evident when the numbers of Enterococcus, Lactobacillus, Streptococcus and Escherichia and Lactococcus increased when broilers were fed diets based on different grain types such as sorghum, barley, oat and rye respectively (Apajalahti et al., 2004). Other grains, such as millet, did not significantly change gut bacterial counts (Baurhoo et al., 2011). Recent work by (Singh et al., 2014) showed that coarsely ground corn when included at 300-600 g/kg in diets increased numbers of Lactobacillus from 7.2 to 7.8 CFU/g of digesta and Bifidobacteria from 7.1 to 7.6 CFU/g of digesta as the counts of *Clostridium* (7.45 to 7.0 CFU/g of digesta), *Campylobacter* (7.4 to 6.5 CFU/g of digesta), and Bacteroides (7.0 to 6.1 CFU/g of digesta) decreased with increasing inclusion levels of coarse corn.

Currently there is limited information available in the literature on the gut microflora composition of broilers fed sweet potato based diets or sweet potato residue after starch extraction. However, Takamine et al. (2005) reported an increase in Bifidobacteria in the ceca of rats fed with sweet potato dietary fibre. It has also been studied that sweet potato fibre extract could increase Lactobacilli population and prevent diarrhoea caused by *Salmonella typhimurium* in healthy children (Lestari et al., 2013; Nurliyani et al., 2015). Yoshimoto et al. (2011) reported that fibre enzymatically extracted from three sweet potato varieties exhibited bacteriostatic activity against the *E. coli* and *S. Typhimurium* using micro calorimetry. Yoshimoto et al. (2005) suggested that the pectin and hemicellulose content in sweet potato fibre at one percent concentration enhanced the growth of Bifidobacteria.

Based on the above information available in the literature on sweet potato fibre we can hypothesise that this crop may have specific features that may favour the proliferation of beneficial strains of the gut microflora. This beneficial gut microflora can improve gut health. However, further experimental trials are necessary to assess if sweet potato diets are able to exact such effects in the gut of broiler birds.

1.8 Food safety

Zoonotic bacteria are those that can cause infections in humans. *Campylobacter* spp., *Clostridium perfringens* and *Salmonella* are the most prevalent pathogens derived from poultry that infect humans through foodborne illnesses (Donoghue et al., 2006). The relationship of these zoonotic bacteria with the diet being offered to broiler chickens is vital to reduce bacterial contamination in the end products.

In Papua New Guinea, although the importance of food safety is recognised, there is no coherent national strategy or policy in place to protect food from microbiological hazards throughout the production, processing and supply chain. In order to minimise the load of bacterial contamination in food products, especially by smallholder poultry farmers and consumers of poultry sold via the live chicken markets, control strategies have to be implemented at the farm gate and through the food chain and the consumers. Currently, there is limited information available in literature on incidences of necrotic enteritis (NE) or contamination of *Campylobacter* in commercial broiler chicken farms let alone farms of individual small-scale broiler farmers who supply the live chicken markets in PNG. This is a grey area that needs to be addressed since alternative feeding strategies have been promoted with the use of locally available ingredients such as sweet potato for the farming of broiler chickens destined for the live chicken markets.

1.8.1 Campylobacter

Campylobacter is carried asymptomatically in the gastro intestinal tract of many food animals particularly broiler chickens. *Campylobacter jejuni* is a leading cause of diarrhoeal disease and foodborne gastroenteritis in humans globally (Skånseng et al., 2007) and in PNG this bacteria was detected as one of the causative agents in infantile diarrhoea (Miwatani et al., 1990). Preventative strategies for lowering contamination of chicken products from farm to fork in the small- scale poultry industry which deals with live carcasses are of paramount importance since this organism is part of the natural gut microflora of chickens.

A study conducted by Udayamputhoor et al. (2003) on dietary changes and colonisation of *Campylobacter jejuni* in the chicken gut showed that this bacteria thrived in the gut of birds fed diets with animal protein. A significantly lower colonisation of this organism was found in the ceca of broilers fed plant based protein diets which the authors related to the production of organic acids due to the fermentation of soluble NSPs. *Campylobacter* is considered to be a commensal organism in many avian species, including commercial poultry, and the spread of *Campylobacter* spp. among chickens is very rapid (Keener et al., 2004). Adams (2007) stated that feed based treatments may be needed to control *Campylobacter* shedding on farm.

Whether sweet potato based diets can control gut levels of *Campylobacter* in broiler chickens is still unknown. However, several authors reported that lower gut fermentation products of sweet potato resistant starch may have prebiotic properties (Adeleye et al., 2010; Haydersah et al., 2012). Dietary fibre is preferentially utilised by Lactobacillus and Bifidobacteria species during fermentation in the gut, leading to the production of lactic acid and short chain fatty acids. These organic acids will lower the pH and can maintain the normal microorganism population, thus preventing the establishment of enteric pathogens in the gastrointestinal tract of pigs and poultry (Lindberg, 2014).

1.8.2 Clostridium perfringens

Clostridium perfringens is an important pathogen of poultry and if not controlled, can cause necrotic enteritis (NE) in chickens (Adams, 2007). The presence of this organism in poultry poses a risk of transmission to humans if chicken meat is poorly prepared or stored for too long. NE is the most common and financially devastating bacterial disease in modern broiler flocks (Choct, 2005). *Clostridium perfringens* is widely distributed in the environment and forms part of the normal gut microflora in human as well as animals. This organism is considered to be part of the normal gut microflora of poultry, and predisposing factors must be present to produce clinical NE (Yegani and Korver, 2008). The key risk factor for the development of necrotic enteritis is an intestinal environment that favours growth of *C. perfringens* (Timbermont et al., 2011). Intestinal mucosal damage by coccidiosis in chickens is usually considered as one of the most important predisposing factors in chickens apart from nutrition or diets (Timbermont et al., 2011). Diets based on viscous grains such as wheat, oats, rye and barley with animal protein will predispose chickens to NE (Timbermont et al., 2011). Maize on the other hand, has shown to reduce counts of *Clostridium perfringens* (Singh et al., 2014). The NSP contents in sweet potato may not be as viscous as those observed in the above grains, but may have higher levels of resistant starch which may have prebiotic properties (Adeleye et al., 2010; Haydersah et al., 2012). Currently there is limited information available on whether feeding sweet potato based diets to broilers can predispose the birds to NE. The dietary fibre component of sweet potato has anti-microbial and chemo preventive attributes which may be beneficial in promoting good gut health in chickens by promoting the growth of beneficial bacteria such as Bifidobacteria which in turn may reduce counts of *Clostridium perfringens* in the gut of broiler birds.

1.9 Conclusion

Research into alternative local feed resources as potential substitute for cereal grains in PNG is ongoing. Cassava and sweet potato were identified as potential candidates as these resources are grown locally and are underutilised. Sweet potato is an important feed ingredient and this review has highlighted its potential use as an energy source for livestock diets specifically broiler chickens as its metabolisable energy value is comparable to that of maize and is able to replace maize by up to 30 percent without adverse effect on intakes of broilers. The main anti nutritive factor present in these roots are trypsin inhibitors which can be eliminated with heat moisture treatments. The main factor currently influencing its greater use in broiler diets is its utilisation by chickens. Cultivar choice and appropriate processing methods to improve its digestibility are some of the factors impeding its utilisation by chickens. Limited information is available on how inclusion of sweet potato diets can enhance digestive capacity in broilers as studies to date have only reported on its effect on organ weights. An in-depth knowledge into its effect on digestive capacity and intestinal health of chickens using cultivars with high NSP contents warrants investigation to further promote its use in broiler diets. Work done so far on some PNG cultivars which are highlighted in this review has indicated presence of resistance starch and high WINSP content in some of these cultivars. The dietary fibre component of sweet potato has anti-microbial and prebiotic attributes which may be beneficial in promoting good gut health in chickens. The current trend in research in developed countries focuses on managing gut health via nutrition. More research into the effect of sweet potato fibre in cultivars with high WINSP content is warranted. Information on the effect of these fibres on digestive function, gut physiology and profiling of the gut microbial communities specifically zoonotic bacteria such as *Campylobacter* and *Clostridium perfringens* in chickens will promote the utility of this crop as well as address food safety concerns in the live broiler chicken industry in PNG.

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1.10 References

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Table 1. Nutrient profile of fresh, dehydrated and cooked sweet potato roots presented as g/kg DM basis

	Fresh roots[1]			**Dehydrated roots[2]**			**Cooked roots[3]**
Nutrients	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Dry matter (g/kg As fed)	300	233	372	880	861	932	392
Crude protein	55	26	84	46	30	60	59
Crude fibre	38	28	53	28	20	37	156
Neutral Detergent Fibre (NDF)	113	91	154	104	52	157	-
Acid Detergent Fibre (ADF)	52	40	76	48	34	57	-
Lignin	11	5.0	16	7.0	1.0	11	-
Ether Extract	11	3.0	25	13	5.0	21	-
Ash	6	25	50	31	24	71	-
Starch (Polarimetry) (g/kg DM)	693	-	-	735	682	777	-
Total sugars	91	-	-	70	40	99	-
Gross energy (MJ/kg DM)	17.4	-	-	17.4	16.5	18.9	-
Poultry nutritive values							
AMEn cockerel(MJ/kg DM)				14.5	14.3	14.8	-
AMEn broiler(MJ/kg DM)				15.9	15.6	16.3	-
AME (MJ/kg DM)[4]				15.4			

SOURCE:[1]http://www.feedipedia.org/node/12681(FAO, 2012-2015b);

[2]http://www.feedipedia.org/node/12680[2](FAO, 2012-2015a);

[3]http://www.feedipedia.org/node/12679[3](FAO, 2012-2015a);

[4](Glatz, 2007)

Table 2. Amino acid and Digestible amino acid of SPRM

	Fresh roots[1]			Dehydrated roots[2]		
Amino acids (g/kg Protein)	Mean	Min.	Max.	Mean	Min.	Max.
Alanine	-	-	-	52	45	56
Arginine	-	-	-	36	33	40
Aspartic acid	-	-	-	188	174	197
Cystine	27	-	-	15	11	20
Glutamic acid	-	-	-	122	109	134
Glycine	-	-	-	47	44	52
Histidine	-	-	-	34	30	38
Isoleucine	-	-	-	41	38	46
Leucine	53	-	-	54	49	58
Lysine	40	-	-	36	26	55
Methionine	7.0	-	-	12	9.0	15
Phenylalanine	-	-	-	59	53	68
Proline	-	-	-	56	49	66
Serine	-	-	-	58	56	65
Threonine	47	-	-	54	47	59
Mineral contents (g/kg DM)						
Calcium	1.2	0.5	2.3	1.7	1.0	4.0
Phosphorus	1.5	1.0	2.1	1.6	0.9	2.2
Potassium	12.2	6.4	18.1	9.8	4.1	12.8
Sodium	0.2	0.1	0.9	1.9	1.5	2.6
Magnesium	0.9	0.7	1.2	0.9	0.6	1.2
Manganese	0.051	0.051	0.051	0.009	0.003	0.054
Zinc	0.043	0.010	0.077	0.043	0.016	0.054
Copper	0.007	0.005	0.008	0.007	0.005	0.009
Iron	1.342	0.419	2.264	0.036	0.013	0.062

SOURCE:[1]http://www.feedipedia.org/node/12681(FAO, 2012-2015b);

[2]http://www.feedipedia.org/node/12680[2](FAO, 2012-2015a);

Table 3. Feed intake and feed conversion ratio of broilers fed differently treated SPRM

SPRM Treatment	Diet description	Inclusion (g/kg)	Feed intake/bird/day (g)	Effect	FCR	Effect	Recommendation	Reference
Sun dried & milled	Fed as dry mash; CP-232g/kg; 12.2MJ/kg Replaced maize on a w/w basis;		0-21 days			-	Recommended in starter diets at 400g/Kg maize; higher than that needs to be pelleted to reduce dustiness & improve intake	(Ravindran and Sivakanesan, 1996)
		0	45.19*	-	1.80	-		
		200	46.52*	-	1.86	-		
		400	46.24*	-	1.87	-		
		600	41.91*	↓	1.81			
Sun dried & milled	Fed as dry mash; CP-246g/kg; ME-13MJ/kg Replaced maize at 9, 18, 27 & 36%		0-28 days				Include in starter rations at 270g/Kg and 300g/Kg in finisher rations	(Agwunobi, 1999)
		0	63.0	-	3.70	-		
		90	54.0	-	3.80	-		
		180	63.0	-	4.50	-		
		270	62.0	-	4.4	-		
		360	64.0	-	8.8	↑		

	CP-200g/kg; ME-12.5MJ/kg Replaced maize at 15, 30 & 40%		28-70 days					
		0	150.0	-	5.0	-		
		150	150.0	-	5.77	-		
		300	160.0	-	6.40	-		
		450	150.0	-	8.42	↑		
SPM	Fed as dry mash;		0-35 days				Should not exceed 10% in starter rations and 20% in finisher rations	(Afolayan et al., 2012)
	CP- 210-230g/kg; ME-12.1-12.6	0	58.59*	-	2.10	-		
		100	54.90*	-	2.09	-		
		200	52.97*	-	2.14	-		
		300	53.12*	-	2.27	↑		
		400	51.49*	↓	2.37	↑		
		500	51.67*	↓	2.58	↑		
		600	18.11*	↓	2.82	↑		
	CP-185-207g/kg; ME-12-12.5MJ/kg;		42-63 days					
		0	149*	-	2.83	-		

	Replaced maize at 10, 20, 30, 40, 50 and 60%	100	148*	-	2.79	-		
		200	143*	↓	3.14	↑		
		300	139*	↓	3.53	↑		
		400	139*	↓	3.72	↑		
		500	134*	↓	4.31	↑		
		600	137*	↓	4.83	↑		
Sun dried & milled	Fed as dry mash;		0-28 days				Recommended to feed to finishing broilers at maximum 50%	Maphosa et al. (2003)
	CP-220-230g/kg; ME-10-11MJ/kg; Replaced maize on a w/w basis (g/kg);	0	41.10*	-	1.95	-		
		155	37.71*	-	2.38	-		
		310	34.71*	-	2.58	-		
		465	32.75*	↓	3.16	↑		
		620	25.25*	↓	3.00	↑		
	CP-180-190g/kg; ME-10-11MJ/kg							
			28-56 days					
			116.0*	-	2.52	-		
			126.0	-	3.16	-		
			115.0*	-	3.13	-		

			85.0*	↓	3.74	-		
			95.0**	↓	6.62	↑		
Sundried & grounded	Fed as dry mash;		0-49 days				SPRM to be fed to chickens as a cheap energy source and recommended more work to determine inclusion level for best performance	(Beckford and Bartlett, 2015)
	Starter (CP-230g/kg & ME-13.29MJ/kg)	0	135	-	2.13	-		
		100	141	-	1.96	↓		
	Grower (CP-210g/kg & ME-13.38MJ/kg)	200	124	-	1.95	↓		
		300	124	-	1.95	↓		
	Finisher (CP-180g/kg & ME-13.38MJ/kg)							
	Replaced maize at 10, 20 & 30 w/w of maize							
Preheated, oven dried & milled	Fed as dry mash;		28-49 days					Glatz (2013)
	CP-157-231g/kg; ME-14.2-14.6.	0	152.0*	↑	2.36*	-		
		500	126.0*	-	2.25*	-		

	SPRM was included at 50 & 70% with a concentrate mix (CP-418g/Kg & 9.4MJ/kg)	700	129.0*	-	2.72*	↑	Recommended inclusion of 50% with a concentrate mix

*Average daily feed intake (ADFI) was calculated based on feed intake data and duration of the experiments ;(-)

No effect; ↑-increase; ↓-decrease

1.11 Thesis objectives

In developing countries such as Papua New Guinea (PNG), where manufactured feed is expensive due to the importation of most ingredients, interventions into different practical diets for monogastric livestock using local ingredients for smallholder producers are necessary for sustainable and efficient production under local environmental and management conditions. One such local ingredient is sweet potato (*Ipomoea batatas* (L) Lam). Different dietary interventions influence gut health and digestive capacity of poultry therefore the use of sweet potato, particularly varieties with high total NSP contents may predispose broiler gut health and growth leading to poor performance, and shedding of enteric pathogens such as *Campylobacter, Salmonella* and *Clostridium perfringens* in the ceca of these birds. Therefore, the overarching aim of this research is to investigate whether digestive capacity and gut health of poultry is compromised when fed high levels of sweet potato tuber meal, and if the fibre fraction in sweet potato has the same effect as observed in non-starch polysaccharides of cereal grains. The specific objectives were;

(a) Determine if apparent metabolisable energy (AME) values of poultry diets are influenced by amount and type of NSP present in selected PNG sweet potato varieties

(b) Evaluate the effect of these NSPs on bird performance, gut morphology, and digestive enzyme activities

(c) Evaluate if the presence of *Campylobacter, Salmonella* and *Clostridium perfringens* in the gut of broilers is influenced by NSPs

Chapter 2 Evaluation of the effects of sweet potato (*Ipomoea batatas* (L.) Lam) in broiler diets

Statement of Authorship

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Evaluation of the effects of sweet potato (*Ipomoea batatas* (L.) Lam) in broiler diets

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2.1 Summary

Cereal grains such as maize and wheat are used extensively in feed formulations for poultry as the primary source of carbohydrates. High cost of these grains in many developing countries necessitates the evaluation of other ingredients that are grown locally. Sweet potato is one such crop. The study was conducted as a proof of concept experiment to test the hypothesis that in the presence and absence of enzymes, sweet potato roots when included in diets of broiler chickens may affect the total metabolisable energy content of the diets, exert certain influences on dry matter digestibility of these diets and impact on the production performance and certain gut parameters. A total of 120 chicks were raised on a commercial starter feed from day 0-19. On day 22, the birds were individually weighed and allocated to 96 single bird metabolism cages to conduct a 7-day classical Apparent Metabolisable Energy (AME) assay. The test diets contained 0 and 25% sweet potato flour (SPF) with and without enzyme supplementation (Rovabio Excel AP T-flex) and replicated 24 times. AME of the control diet with and without enzyme was 14.05 and 13.91 MJ/kg whilst the AME of the SPF diets with and without enzymes were 13.45 and 13.43 MJ/kg respectively. AME of SPF was 12.08 MJ/kg. Birds fed the SPF had reduced end weights (P=0.002) and weight gains (P<0.001) leading to higher intake (P=0.004) and FCRs (P<.001). This highlights the need to balance total dietary protein and amino acids levels when using SPF in broiler diets and not SPF per se as SPF diets were not balanced and AME and dry matter digestibility of the diets were comparable to the control. The level of sucrase activity in the jejunum was lower (P<0.001) due to enzyme inclusion. Use of SPF in the current study did not negatively influence the activities of the brush border enzymes maltase and sucrases, gut morphology in the jejunum of broilers or the load of Enterobacteriaceae in the ceca of birds. This finding is promising in that the gut parameters associated with digestive capacity and gut health were not compromised with the feeding of 25 % SPF to broilers.

Keywords*:* Apparent metabolisable energy, dry matter digestibility, intake, feed conversion ratio, digestive enzymes, gut morphology, gut microflora

2.2 Introduction

Energy represents more than 70 % of the total input cost of producing live broilers and therefore it is paramount to assess the energy value of ingredients. The high cost of grains particularly in developing countries, necessitates the evaluation of locally available alternative energy ingredients. Many of these alternative ingredients are of limited use as feed ingredients due to their dietary fibre content and other anti-nutrient properties (Ravindran 2012). For these ingredients to be considered for inclusion in poultry diets, a thorough understanding of their potential and limitations for use in poultry diets is warranted.

Sweet potato can substitute cereal grains as a carbohydrate source in diets of poultry in tropical countries (Ravindran et al. 1995). The potential of sweet potato in food security and global wellbeing has been well recognised with studies performed on its various properties such as processing, utilisation and health importance in humans (Waramboi et al. 2011). Its roots are rich in carbohydrates and vitamin A and its leaves are rich in proteins. It can produce more edible energy per hectare per day than wheat, rice or cassava (Lebot 2009). Sweet potato has high productive efficiency and is a reliable source of energy due to its high starch content and digestibility, but its use as a feed ingredient is limited. The limited use of this crop in poultry diets is associated with its high moisture content, associated high drying cost during processing and its low protein content (Avigen 2015). The low protein, sulphur amino acids and lysine content can be overcome by inclusion of protein concentrates, while the high cost of drying can be overcome by presenting it to birds as a boiled mash as well as use of low-cost appropriate drying techniques (Glatz et al. 2010). However, for sweet potato to meet its potential in the feed ingredient market place, studies are required to determine the opportunities and limitations of its inclusion in poultry diets in order to maximise productive responses in broiler chickens. Sweet potato has been used in the diets of fish, pigs and poultry (Pandi 2006) to replace grain in developing countries. Recent work by Beckford and Bartlett (2015) with Cornish x Rock broiler chickens used discarded sweet potato roots at inclusion rates of 100, 200 and 300 g/kg in the starter, grower and finisher feeds respectively and showed no significant differences in the total feed intake and final live weights of these birds. Feed conversion ratios (FCR) of birds fed with all these sweet potato diets had better FCRs than birds fed with a maize diet indicating that sweet potato roots can be fed to broilers to improve profit margins for farmers (Beckford and Bartlett 2015). These results were similar to those reported by Glatz (2013).

Use of feed enzymes in the poultry feed industry in the last decade was aimed at increasing the feeding value of raw feed materials and to reduce variation in nutrient quality of ingredients thereby reducing feed manufacturing costs and promoting uniformity within and between flocks respectively (Bedford 2000). The addition of exogenous feed enzymes in diets for poultry is one way of increasing the value of such alternative feed ingredients (Choct 2006). The aim of the present study was to determine the apparent metabolisable energy (AME) of sweet potato meal in the presence and absence of enzyme (Rovabio AP TFlex-Adisseo Asia Pacific). Secondary to that was the need to assess other influences on gut functions and cecal microflora.

2.3 Materials and methods

2.3.1 Sweet potato flour (SPF) and enzyme (Rovabio Excel AP T-flex)

Sweet potato flour (SPF) was purchased locally in South Australia. This product is a refined flour and was selected for use in this proof of concept trial as an alternative to sweet potato root meal. The specific enzyme product called Rovabio Excel AP T-flexcontained Endo-1, 4 beta-xylanase, endo-1, 3 9 (4) beta glucanase and 17 other enzyme activities (Adesso, Antony, France) was selected based on its availability in Papua New Guinea (PNG) and other Pacific Island countries.

2.3.2 Bird, housing and management

A total of one hundred and twenty Cobb male broiler day old chicks were obtained from a local hatchery and reared from 0-19 days on litter in a floor pen under infrared brooders in a temperature controlled room. Chicks were fed the Ridley starter feed until the start of the experiment on day 22 post hatch. The nutrient specification for the major nutrients for the starter diet on as fed basis was 220 g/kg crude protein, 13.3 MJ/kg ME, 25 g/kg crude fat, 50 g/kg ash and crude fibre respectively. Feed and water were provided *ad libitum.* Daily temperature readings and mortality were recorded. The temperature was set at 25 ^{0}C and gradually reduced to 22 ^{0}C. The light program was set at 16 hours of light and 8 hours of darkness. This work was approved by the Animal Ethics committee of the Department of Primary Industries and Resources South Australia (PIRSA) AEC Project No. 06/13, and the University of Adelaide AEC Project No. S-2013-074 respectively. All procedures complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.3.3 Experimental diets

Composition of the four assayed diets is presented in Table 1. The basal diet consisted of the standard broiler starter feed, whilst the experimental diets consisted of the basal diet containing 250 g/kg SPF with and without the enzyme product. All diets (including the basal diet) were pelleted using a 7.5 kW Templewood cold pellet press (Slough, Bucks, England) fitted with a 4.0 mm die. Each assay diet was fed to twenty four male broilers housed individually in single metabolic cages.

2.3.4 Apparent Metabolisable Energy

On day 19, birds were placed in pairs into 48 single metabolic cages measuring 26 x 36 x 40 cm for an initial 3-day acclimatisation period. On day 22, the birds were then weighed individually and placed one at a time into each of the 96 single bird metabolic cages. Diets were allocated randomly across the 96 cages for the measurement of the apparent metabolisable energy. All the cages had individual feed troughs and nipple drinkers and each cage was shielded to prevent cross-contamination of excreta.

The classical total excreta collection method was employed for the determination of AME. Feed intake and excreta output were measured quantitatively per cage from day 25 for four consecutive days. Excreta collections from each cage were oven dried overnight daily and then pooled. The pooled dried excreta were then ground and a representative sample from each were taken and stored in airtight plastic containers for the analysis of gross energy.

2.3.5 Chemical analysis

Gross energy (GE) was determined using a Parr isoperibol bomb calorimeter (Moline, Illinois) standardised with benzoic acid. All the samples were tested in duplicate. Other chemical tests were outsourced to Agrifood Technology (Victoria, Australia). The chemical methods used were based on the Australian Fodder Industry Association (AFIA) methods (AFIA 2003).

Calculations

AME of SPF and diets were calculated as follows:

$$AME_{diet} = \frac{(GE_{diet} \times \text{Feed DM consumed}) - (GE_{excreta} \times \text{dry excreta DM})}{\text{Feed DM consumed}}$$

$$AME_{SPF} = \frac{\text{AME of SPF diet} - (\text{AME of basal diet} \times 0.75)}{0.25}$$

The dry matter digestibility of the diets was calculated as follows:

$$\text{DryMatter digestibility} = \frac{\text{Feed DM consumed} - \text{Excreta DM collected}}{\text{Feed DM consumed}}$$

2.3.6 Tissue sample collection

Birds were euthanized on day 32 by intravenous injection of 0.5 ml pento-barbitone (Lethabarb™, Virbac Australia) via the brachial vein. Body weights of the dead birds were recorded prior to dissection for collection of gut tissues and cecal contents. The small intestine was excised and sections were removed from the end of the gizzard to the Meckel's diverticulum. The segments of the small intestine were flushed with phosphate buffer saline, weighed and samples collected for gut morphology and digestive enzyme assays. Tissues for the gut morphological studies were fixed in 10% buffered formalin and later transferred to 70 % ethanol for further processing. The tissues for digestive enzyme analysis were immediately transferred to liquid nitrogen and later stored at -80 ^{o}C until required. Cecal contents were collected into a sterile tube and immediately placed on ice for bacterial cultivation and enumeration.

2.3.7 Histopathology

Intestinal tissues were processed based on procedures described by Iji et al. (2001). Slides were viewed on an Olympus BH-2 microscope at x4 magnification and digitised using an image analysis software (AnalySIS 5 Soft-Imaging System, Germany). Each individual image was calibrated accordingly and measurements (micrometre) of villus height, crypt depth, basal villus width and apical villus width were carried out on 10 intact villi. Apparent villus surface area was estimated from the trigonometric relationship between villus height, villus basal width and villus apical width (Iji et al. 2001).

2.3.8 Digestive enzyme analysis

Activities of the intestinal sucrase (EC 3.2.1.48) and maltase (EC 3.2.1.20) were determined through methods described by Dahlqvist (1964). Briefly, frozen jejunum tissue (250 mg) was homogenised in 1 ml of 10mM phosphate buffer (pH 6.1). The supernatant from each sample was diluted with 50mM phosphate buffer + Triton-X at different concentrations. Supernatants for sucrase assay were diluted with 50mM phosphate buffer + Triton-X (pH 6.1) at 1:200 while samples for maltase assay were diluted with 50mM phosphate buffer + Triton-X (pH 6.5) at 1:1000. Maltose and sucrose were prepared individually with 1.71 g of sucrose (Mr 342.3) and 1.80 g of maltose (Mr 360.31) dissolved in 25 ml of 50mM phosphate buffer (no Triton-X). Standards for each enzyme assay were prepared according to the glucose assay kit (SIGMA, Australia). Enzymes were assayed in 96 micro-plates incubated at 39 ˚C. The plates were then read in a microplate reader at 490nm with a 630nm reference filter. Enzyme activity was expressed as units per milligram of protein. Tissue protein was determined using the Bradford protein assay kit (Bio-Rad, Australia).

2.3.8 Bacterial isolation and enumeration

One gram of cecal content from each euthanized bird was placed into a sterile tube containing 9 ml of sterile phosphate buffered saline and homogenised by vortexing. Subsequent 10-fold serial dilutions of each sample were made to obtain up to 10^{-5} dilution. Then 0.1 ml from dilutions 10^{-3} to 10^{-5} was spread on appropriate selective agar for enumeration of total Lactic acid bacteria, *Escherichia coli* and Enterobacteria. The media used were MRS agar (Oxoid, Australia) for Lactic acid bacteria, MacConkey agar (Oxoid, Australia) for *E. coli* and Violet Red Bile Glucose agar (Oxoid, Australia) for total Enterobacteria. Plates inoculated for the enumeration of Lactic acid bacteria were incubated at 5 % CO_2 whilst those for the *E. coli* and total Enterobacteria were incubated aerobically. All plates were incubated at 37 ^{0}C for 24 h, after which total numbers of bacterial colonies were counted. The counts were converted into logarithmic equivalents and expressed as number of colony forming units (CFU) per gram of cecal content.

2.3.9 Statistical analysis

Analysis of the data was performed using the two way analysis of variance in GenStat®, 15th Edition (VSN International 2011). The difference between the means was identified by the least significant difference and compared using the Duncan's multiple range test.

2.4 Results and discussion

There were no significant differences observed on the AME of diets due to SPF or enzyme supplementation (Table 2). Diets with SPF had AME values comparable to that of the control diet. There were no significant interactions due to SPF and enzymes on AME values. The AME of SPF was calculated to be 12.08 MJ/kg. This value is lower than that of dehydrated roots which has an average AME of 15.9 MJ/kg (http://www.feedipedia.org/node/126802 (FAO, 2012–2015). The dry matter digestibilities of these diets were not significantly affected by the inclusion of SFP and enzymes. Dry matter digestibilities of the SPF diets were comparable to that of the control and there were no significant interactions between SPF and enzyme on digestibility of the diets. AME of SPF with and without enzyme were 13.45 and 13.43 MJ/kg respectively (Table 2). These values are lower than those reported by Ravindran et al. (1995) and Glatz (2013) in sweet potato root meals in broiler diets. The AME of SPF diets may be due to the type of cultivar used and the method of processing (Panigrahi et al. 1996). Different sweet potato cultivars have been found to have varying AME values for poultry. The SPF used in this study yielded an ME of 12.7 MJ/kg DM (Table 1). Work by Ravindran et al. (1995) showed that AME values for poultry ranged from 14.34 to 14.76 MJ/kg for 16 Sri Lankan sweet potato cultivars whilst Banser et al. (2000) reported values of 12.66 and 12.78 MJ/kg in 2 African cultivars which are similar to that of SPF used here. Variability in sugars among cultivars is very high and has been recorded as low as 5.6 % in a local cultivar of the Philippines and up to 38.3 % of dry matter in American cultivars whilst hundreds of cultivars found in Papua New Guinea (PNG) and island Melanesia are also low in sugar content (Lebot 2009). Despite these variations in the ME values for poultry, the ME for SPF used in this study was within the required ME value for growing and finishing broilers (Aviagen 2014). The supplementation of enzymes in the present study did not improve the AME of the diets and may be associated with the type of product used in this study. The dry matter digestibilities of the diets were not significantly affected by the inclusion of SPF and enzymes.

In terms of production parameters, birds fed with the SPF diets had lower end weights (P=0.002), body weight gains (P<.001), higher intakes (P=0.004) and feed conversion ratios (FCR) (P<.001) compared to birds fed with the control and the control with enzyme diets. There were no significant differences due to enzyme supplementation on the end weights, weight gains, intake or FCR in the current study (Table 2). There were no significant interactions between SPF and enzyme inclusion on these growth parameters. The growth performance of broilers fed SPF diets in the present study (Table 2), had lower end weights, body weight gains, high intakes and FCRs at the end of the assay. This can be attributed to the SPF diets being deficient in total protein and essential amino acid content as the diets were not well balanced in the current study and may have compounded this deficiency further as sweet potato is known to be deficient in protein and sulphur amino acids contents. The reduction in body weight gain and higher FCR observed in birds fed with sweet potato diets are similar to those observed in previous studies when sweet potato was included at the rate of 200-300 g/kg in broiler diets (Agwunobi 1999; Maphosa et al. 2003; Afolayan et al. 2012). The higher feed intakes observed for birds fed on the SPF and SPF+E diets in the present study however are contradictory to those reported previously (Agwunobi 1999; Maphosa et al. 2003; Afolayan et al. 2012), when sweet potato was included in these diets for broilers. The present study showed an increase in intake of around 7.5 % in broilers fed with SPF with or without enzyme diets. The marked increased in feed intake of birds in the present study maybe due to a lower lysine content of the SPF diets. Sweet potato roots are lower in sulphur amino acids and lysine (Dominguez 1992) and lysine and cysteine content in sweet potato decreases with increasing temperatures (Panigrahi et al. 1996). The pelleting of SPF diets in the present study may have further reduced lysine content of the SPF diets. It has been reported that pelleting of diets decreases lysine availability (Jensen et al. 1965; Greenwood et al. 2005). Pelleting was found to increase crude protein and lysine requirements for growing turkeys compared to similar diets presented as mash (Jensen et al. 1965), whilst Greenwood et al. (2005) using a dose-response analysis, illustrated that feed form affected estimated lysine needs of broilers when mash diets were pelleted. The increased intake observed in the present study may be indicative of birds increasing their intake to meet this lysine deficiency. Along with dietary energy, dietary protein and amino acids are important regulators of food consumption (Forbes 2006). Birds will attempt to alleviate a marginal deficiency of an essential nutrient by increasing food intake (Forbes 2006) as may be the case in the present study.

In terms of relative organ weights of birds, there were significant differences due to SPF on the relative weights of the liver (P=0.002), jejunum and the ileum of birds fed SPF diets. Birds fed SPF diets had significantly higher liver, jejunum and ileum weights compared to the birds on the control diets (Table 3). A higher liver weight maybe the result of a lower body weight as reported above or may be a consequence due to deficiency in protein and amino acid contents in these diets. A higher relative weight of the jejunum and ileum may be indicative of the high feed intake reported above. The jejunum is where all the major nutrients are digested and absorbed. The prominent role of the jejunum is reflected in the fact that the empty weight of this segment is usually 20 to 50 % higher than the ileum pertaining to a larger amount of material passing though this segment (Hetland and Svihus 2001). Other authors, (Agwunobi 1999; Maphosa et al. 2003; Afolayan et al. 2012) did not observe an increase in the liver and intestinal weights in birds fed sweet potato diets which may be attributed to reduced intakes reported in those studies. The observed higher relative weights in the liver, jejunum and the ileum maybe due to experimental error in that SPF diets were deficient in total dietary protein and amino acids and may not be a consequence of SPF inclusion as such.

In terms of gut morphology, villi height, villi crypt depth, villi crypt depth ratio and apparent surface area in the jejunum of birds fed SPF diets were not significantly different to those of the birds fed the control diets (Table 4). However, the height of the villi in the ileum of birds fed the SPF diets were lower (p=0.017) compared to those of the birds fed the control diets. Other gut morphological traits in the ileum were not affected. There were no significant differences due to enzyme supplementation nor were there any significant interaction on these morphological structures (Table 4). The reduced villi height in the ileum of birds due to SPF is not accompanied by deeper crypt depths and may just be a negative effect of SPF as crypt depths were not affected. A deeper crypt is indicative of high cell turnover to permit renewal of the villus in response to normal sloughing or inflammation from pathogens or their toxins (Moran 1985; Yason et al. 1987; Awad et al. 2011) which may not be happening here. Inclusion of SPF did not have any profound influence on the apparent surface area in the small intestinal segments (jejunum and ileum) in birds fed with the SPF diets. This is promising as gut morphology in regard to apparent surface area is not negatively affected with inclusion of SPF in the current study. Generally, intestinal epithelial cells originate from the stem cells in the crypt and migrate up the villus and are extruded at the villus tip into the intestinal lumen (Uni et al. 2001). Nutrient assimilation is affected by villi heights in that a decrease in villi heights means a reduced surface area available for absorption and lower nutrient uptake (Choct 2009). This may then ultimately compromise growth, health and welfare of birds. In the present study, SPF did not profoundly influence gut morphology in terms of villus height and crypt depth hence did not exert any negative impact on available surface area for nutrient absorption in the jejunum and ileum of broilers.

Tissue protein content and the digestive enzyme activities of the jejunum are presented in Table 5. The jejunal protein content of birds fed the SPF diet were similar to that of the birds fed the control diet, in that there were no significant differences associated with SPF or enzyme inclusion. There were no significant interactions between SPF and enzymes on these parameters. The activities of maltase and sucrase were not significantly affected by SPF in birds fed the SPF diets but sucrase activity was significantly influenced by enzyme supplementation (Table 5). Birds fed diets supplemented with enzymes had lower ($P<0.001$) sucrase activity in their jejunum. This is contradictory to the findings by Shakouri et al. (2009) as these authors reported that the activities of maltase and sucrase were not affected by supplemental enzymes and related this finding to the higher feed intakes observed from birds fed on maize and sorghum. In the present study, higher intakes and heavier jejunal weights were reported for sweet potato diets. The higher bulk of digesta passing through the jejunum in birds fed sweet potato diets may have stimulated the activities of these disaccharidases. Hydrolysis of disaccharides such as maltose and sucrose to glucose occurs at the brush boarder membrane by the brush border digestive enzymes, maltase and sucrase (Caspary 1992). The activities of disaccharidases are influenced by the characteristics of the diets and available substrate, (Chotinsky et al. 2001; Uni and Ferket 2004).

Results from the bacterial enumeration of the cecal content showed that there were no significant differences ($P>0.05$) in the colony counts of Lactic acid bacteria, Enterobacteria and *E. coli* in the ceca of broilers due to SPF or enzyme. There were no significant interactions between SPF and enzyme (Table 6). Currently there is limited information available on the influence of SPF and SPF with enzyme on the load of cecal gut microbes of broiler chickens. However, inclusion of exogenous enzymes in broiler diets has been associated with modulation of the gut microbiota (Kiarie et al. 2013). Addition of glucanase and xylanase in broiler diets *in vivo* were shown to reduce Enterobacteria counts in broilers fed cereal based diets (Rosin et al. 2007; Józefiak et al. 2010). A similar observation in the present study was the lower colony counts of *E. coli* in diets with enzymes. Yang et al. (2008), observed an increase in the number of Coliforms and a reduction in the counts of Lactobacilli with the inclusion of xylanase in wheat based diets. The chemical composition of the digesta can influence the composition of the gut microbial community because different bacterial species have different substrate requirements for growth (Apajalahti et al. 2004). In the present study, the inclusion of SPF in the diet of broilers did not significantly influence the counts of Lactic acid producing bacteria and Enterobacteria in the ceca of broilers. Further studies are essential to investigate the effects of different sweet potato varieties on gut microbiota.

2.5 Conclusion

AME and dry matter digestibility of the diets were not affected by the inclusion of SPF in the diet. The poor growth of birds fed diets with SPF may have been due to the deficiency of dietary protein and overall amino acid contents of the diets and SPF as AME content and dry matter digestibility were comparable to the diets without SPF. Moreover, the use of SPF in the current study did not negatively influence maltase and sucrase activities along the brush border membrane of the jejunum, apparent surface area for absorption in the small intestinal segments or the load of Enterobacteriaceae in the ceca of birds. This finding is promising in that, the gut parameters associated with digestive capacity and gut health were not compromised with the feeding of SPF to broilers. Further studies using sweet potato roots processed for livestock along with molecular techniques to profile cecal bacteria to assess the shedding of the major food borne pathogens such as *Campylobacter, Clostridium perfringens* and *Salmonella* is warranted. Current findings form the baseline for generating vital information on the opportunities and limitations of sweet potato use in livestock feeds, particularly broiler diets.

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Table 1. Composition (g kg-1 DM basis) of the experimental diets

	Experimental Diets			
Ingredients	Basal	SPF	SPF+ E	Basal + E
Commercial starter crumble*	1000	750	750	1000
Commercial sweet potato flour†	-	250	250	-
Enzyme‡	-	-		
Measured nutrient content, (% DM)				
Dry matter	88.8	87.2	87.7	88.9
CP	23.8	18	18.5	24
Fat	6.9	4.9	5	6.1
Ash	4.9	4	3.7	4.6
ADF	4	3.3	3.4	4.5
NDF	10.8	8.3	8.4	11.3
Total starch	37.9	42.2	43.5	30.8
Chemical composition of SPF (% DM)				
Dry matter	84.9			
CP	0.4			
Fat	0.6			
Ash	0.2			
ADF	<0.2			
NDF	<0.2			
Total starch	69.7			
ME (Poultry) MJ/kg DM	12.7			

*The commercial starter crumble was free of any antibiotics and was supplied by Ridley; †Processed sweetpotato flour sold locally in Adelaide. ‡The enzyme product used was included as a powder at 50g/1000kg based on the manufacturer's instructions;

*The commercial starter crumble was free of any antibiotics and was supplied by Ridley;
†Processed sweetpotato flour sold locally in Adelaide.
‡The enzyme product (Rovabio Excel AP T-flex: Endo-1, 4 beta-xylanase, endo-1, 3 9 (4) beta glucanase) used was included as a powder at 50g/1000kg based on the manufacturer's instructions;

Table 2: Effects of the dietary treatments on the AME and the dry matter digestibility of diets and growth of broilers at 27 days posthatch

Treatment								
SPF	Enzyme	Diet AME (MJ/kg) (DM basis)	DM digestibility (g/g)	Start weight (g)	End weight (g)	Weight gain (g)	Feed intake (g/bird)	FCR (g/g)
Control	(-)	13.91[a]	0.643[a]	972.1[a]	1771[a]	799.0[a]	1209[b]	1.52[b]
	(+)	14.05[a]	0.659[a]	972.2[a]	1778[a]	806.1[a]	1200[b]	1.50[b]
SPF	(-)	13.45[a]	0.626[a]	956.6[a]	1680[b]	723.4[b]	1300[a]	1.81[a]
	(+)	13.43[a]	0.649[a]	952.4[a]	1697[b]	744.8[b]	1301[a]	1.75[a]
SEM		0.320	0.018	12.83	21.90	14.71	25.50	0.038
Source of variation								
SPF		NS	NS	NS	<.001	<.001	<.001	<.001
Enzyme		NS	NS	NS	NS	NS	NS	NS
SPF x Enzyme		NS	NS	NS	NS	NS	NS	NS

*Data represent means from 24 replicates per treatment; SEM=Standard error of means; means with the same superscript within a column are not significantly different (P>0.05); FCR-Feed conversion ratio; AME-Apparent Metabolisable Energy; DDM-Dry matter digestibility; †Basal=Control diet; SPF=Sweet potato flour; SPF+E=Sweet potato flour + Enzyme; Basal+E= Control + Enzyme

*Data represent means from 24 replicates per treatment;

†Basal=Control diet; SPF=Sweet potato flour; SPF+E=Sweet potato flour + Enzyme; Basal+E= Control + Enzyme.

Table 3. Effect of the dietary treatments on the relative organs and intestinal segment weights of broilers at 32 days post hatch

Treatment						
SPF	Enzyme	Liver	Pancreas	Duodenum	Jejunum	Ileum
Control	(-)	27.82[c]	1.926[a]	7.342[a]	12.70[ab]	10.81[ab]
	(+)	28.27[bc]	1.832[a]	7.831[a]	12.17[b]	10.18[b]
SPF	(-)	30.58[ab]	1.722[a]	7.538[a]	13.78[a]	11.14[ab]
	(+)	30.83[a]	1.847[a]	7.502[a]	13.20[ab]	11.59[a]
SEM		0.822	0.059	0.213	0.425	0.441
Source of variation						
SPF		0.002	NS	NS	0.015	0.05
Enzyme		NS	NS	NS	NS	NS
SPF x Enzyme		NS	NS	NS	NS	NS

*Data represent means from 24 replicates per treatment; SEM=Standard error of means; Means with the same superscript within a column are not significantly different (P>0.05);

†Basal=Control diet; Basal + E=Control + Enzyme; SPF + E=Sweet potato flour + Enzyme; SPF=Sweet potato flour

*Data represent means from 24 replicates per treatment;

†Basal=Control diet; Basal + E= Control + Enzyme; SPF + E=Sweet potato flour + Enzyme; SPF=Sweet potato flour

Table 4: Effects of dietary treatments on gut morphological traits of broilers at 32 days post hatch

Treatment		Jejunum				Ileum			
SPF	Enzyme	VH	CD	VH:CD	ASA	VH	CD	VH:CD	ASA
Control	(-)	1074[a]	62.91[a]	17.69[a]	0.097[a]	636.6[ab]	60.25[a]	10.98[a]	0.067[a]
	(+)	1163[a]	65.81[a]	18.30[a]	0.105[a]	655.3[a]	64.42[a]	10.67[a]	0.070[a]
SPF	(-)	1082[a]	63.65[a]	18.14[a]	0.098[a]	591.8[ab]	64.29[a]	9.47[a]	0.064[a]
	(+)	1119[a]	65.19[a]	17.77[a]	0.112[a]	564.4[b]	59.11[a]	10.24[a]	0.058[a]
SEM		41.42	2.68	1.103	0.007	27.85	2.88	0.636	0.004
Source of variation									
SPF		NS	NS	NS	NS	0.017	NS	NS	NS
Enzyme		NS	NS	NS	NS	NS	NS	NS	NS
SPF x Enzyme		NS	NS	NS	NS	NS	NS	NS	NS

*Data represent means from 24 replicates per treatment; SEM= Standard error of means; †Basal=Control diet; Basal + E= Control + Enzyme; SPF + E=Sweet potato flour + Enzyme; SPF=Sweet potato flour;

*Data represent means from 24 replicates per treatment;
†Basal=Control diet; Basal + E= Control + Enzyme; SPF + E=Sweet potato flour + Enzyme; SPF=Sweet potato flour

Table 5: Effects of dietary treatments on the tissue protein content and the activities of digestive enzymes in the jejunum of broilers at 32 days post hatch

Treatment SPF	Enzyme	Protein (mg)	Maltase (U/mg protein)	Sucrase (U/mg protein)
Control	(-)	6.48[a]	0.814[a]	0.164[ab]
	(+)	6.87[a]	0.821[a]	0.076[c]
SPF	(-)	6.48[a]	1.017[a]	0.208[a]
	(+)	7.17[a]	0.789[a]	0.096[bc]
SEM		0.357	0.137	0.024
Source of variation				
SPF		NS	NS	NS
Enzyme		NS	NS	<.001
SPF x Enzyme		NS	NS	NS

*Data represent means from 24 replicates per treatment; SEM=Standard error of means; Means with the same superscript within a row are not significantly different (P>0.05);
†Basal=Control diet; Basal + E=Control + Enzyme; SPF + E=Sweet potato flour + Enzyme; SPF=Sweet potato flour;

*Data represent means from 24 replicates per treatment;
†Basal=Control diet; Basal + E= Control + Enzyme; SPF + E=Sweet potato flour + Enzyme; SPF=Sweet potato flour

Table 6: Effects of dietary treatments on the bacterial counts (log CFU/g) in the ceca of broilers at 32 days post hatch

Treatment SPF	Enzyme	Lactic Acid bacteria	Enterobacteria	Escherichia coli
Control	(-)	8.141[a]	7.758[a]	7.999[a]
	(+)	7.626[a]	7.760[a]	7.945[a]
SPF	(-)	7.943[a]	7.774[a]	8.174[a]
	(+)	7.627[a]	7.563[a]	7.839[a]
SEM		0.260	0.177	0.172
Source of variation				
SPF		NS	NS	NS
Enzyme		NS	NS	NS
SPF x Enzyme		NS	NS	NS

*Data represent means from 24 replicates per treatment; SEM=Standard error of means; †Basal=Control diet; Basal + E=Control + Enzyme; SPF + E=Sweet potato flour + Enzyme; SPF=Sweet potato flour

*Data represent means from 24 replicates per treatment;
†Basal=Control diet; Basal + E= Control + Enzyme; SPF + E=Sweet potato flour + Enzyme; SPF=Sweet potato flour

Chapter 3 Effects of different sweet potato varieties on performance and levels of enteric pathogens in chickens

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Overall percentage (%)	80%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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1

Effects of different Papua New Guinea sweet potato varieties on performance and level of enteric pathogens in chickens.

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3.1 Abstract

In the last decade, research has targeted the evaluation of local feed ingredients for use in monogastric diets to alleviate the high cost of production of livestock at smallholder levels in Papua New Guinea (PNG). Different dietary interventions to reduce feed costs may come at a cost to the health of the birds as microbial status of the gastrointestinal tract of poultry is influenced by the diet and the internal gut environment. This study was conducted to evaluate the levels of enteric pathogens in the ceca of broilers given sweet potato diets containing varying levels of non-starch polysaccharides (NSP) with and without enzyme inclusion. Selection of a sweet potato variety for use in broilers diets should be based on the total NSP content. In particular, varieties with low soluble NSPs are economical to use as Apparent Metabolisable Energy (AME) values are within the desired range for poultry and there is minimal need to include enzymes to improve NSP digestibility. The use of varieties with a low total NSP content is also advantageous as the numbers of *Clostridium perfringens* were lower in broilers fed with these sweet potato varieties. The levels of *Campylobacter* and *Salmonella* spp. were high in the ceca of birds fed with the sweet potato varieties with high total NSP contents. These levels can be reduced with the inclusion of enzymes. The information generated here will assist in the efficient use of local varieties of sweet potato in PNG by smallholder poultry farmers and the poultry commercial industry.

Keywords: Broiler chickens, enteric pathogens, *Salmonella* spp., *Campylobacter, Clostridium* spp., sweet potato, non-starch polysaccharides

3.2 Introduction

The smallholder broiler industry in Papua New Guinea (PNG) has grown rapidly in the last decade and in 2013 was estimated to be worth around AUS$104 million/annum (Poultry Industry Association-personal communication-Dr Keith Galgal). In addition, the smallholder broiler sector is earning about 39.3 % more than the commercial frozen carcass industry in PNG (Poultry Industry Association-PNG 2015-personal communication-Dr Keith Galgal). The cost of growing poultry is high, as feed alone makes up to 80 % of the total cost of production in PNG. Sweet potato is currently used in a finisher ration for broiler chickens as a cheaper alternative option to the more expensive manufactured finisher stockfeed in PNG. Sweet potato has been used in the diets of fish, pigs and poultry (Pandi 2006) to substitute grains, due to its availability and high carbohydrate (starch) content. Use of non-conventional ingredients in diets for monogastric livestock is limited due tothe high levels of dietary fibre contents in these ingredients (Ravindran, 2012). Dietary fibre is the sum of non-starch polysaccharides (NSPs) and lignin and is a significant part of plant material (Choct, 2015). NSPs are either soluble or insoluble depending on their chemical properties. The soluble fraction of NSPs has been known to exhibit anti-nutritive activities in pigs and poultry, leading to negative changes in gut physiology, gut microflora and gut health, while the insoluble proportion imparts beneficial effects on gut development and secretion of endogenous digestive enzymes (Choct, 2015). Sweet potato has been promoted as a cheaper alternative energy source for poultry, especially for broilers destined for the live broiler chicken markets in PNG (Pandi et al., 2016). The PNG smallholder broiler production system involves more than 50,000 families who produce up to 6 batches of broilers yearly, growing birds up to 42 days-of-age. These birds are sold mostly at the farm gate, at local provincial markets or along roadside markets. This enterprise is mainly family owned at village level and owners' understanding of the implementation of biosecurity and food safety is highly variable. Chickens can harbour foodborne pathogens such as *Salmonella* and *Campylobacter*. Chickens closely interact with humans in the same household and thus potentially expose family members of smallholder poultry farmers to enteric pathogens due to their close contact with poultry. There has been no study which directly links chicken meat consumption with foodborne outbreaks in PNG but consumption of contaminated chicken meat has been identified as one of the most important food vehicles for these organisms worldwide (WHO, 2009). Enteric pathogens such as *Salmonella* and *Campylobacter* species are the most commonly isolated enteropathogens from human gastroenteritis cases (Donoghue, Farnell, Cole, & Donoghue, 2006). A recent report suggested that the NSP from edible plant source was able to inhibit the invasion of *Salmonella typhimurium* across the chicken intestine (Parsons et al., 2014). Moreover, in PNG, the most common and financially devastating disease in modern broiler flocks is necrotic enteritis (NE) which is caused by *Clostridium perfringens*. This organism is considered to be part of the normal gut microbiota of poultry, and predisposing factors must be present to produce clinical necrotic enteritis (Yegani & Korver, 2008). Intestinal mucosal damage by coccidiosis in chickens is usually considered one of the most important predisposing factors in poultry, other than nutrition or diet.

Our hypothesis was that the inclusion of sweet potato (SP) in broiler diets with and without enzyme inclusion can influence the shedding of pathogens such as *Campylobacter, Salmonella* and *Clostridium perfringens,* without affecting broiler performance. In addition to that there is currently limited published information on the presence of enteropathogens in poultry flocks in PNG. This experiment was conducted in PNG to study the effects of the inclusion of different sweet potato varieties with varying levels of NSP contents in broiler diet on the overall performance and levels of *Campylobacter, Salmonella* and *Clostridium* species (spp.).

3.3 Materials and Methods

The current study was undertaken in Lae, Morobe Province, in PNG at the Feed Testing Facility of the National Agricultural Research Institute. The experiment was conducted between April and May of 2014. The average daily temperature for that period was 28 ^{o}C with a relative humidity of around 80 %. Minimum and maximum temperatures recorded for that period were 26 °C and 30 °C respectively. The study was approved by the Animal Ethics Committee of the University of Adelaide, AEC Project No. S-2014-026. All procedures complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

3.3.1 Sweet potato diets

Five commercial sweet potato varieties were purchased from various provincial markets in the Highlands and Morobe Provinces of PNG. These varieties were identified by their local names; Waghi besta (Variety 1), Gimane (Variety 2), Suga (Variety 3), Marasonda (Variety 4) and Rachel (Variety 5). Briefly, fresh sweet potato roots were washed, sliced and oven (Vision Scientific Company, Korea) dried at 60 °C for 16 hours prior to 8 hours of sun drying. The dried chips were milled, packed in bags and stored in storage bins until use. Representative samples (500 g) of these varieties were packed into labelled plastic bags and sent to the Institute of Subtropical Agriculture, The Chinese Academy of Sciences, P.R. China for the analysis of total NSP and compositional sugars as described by Englyst and Cummings (1988) and Englyst, Bingham, Runswick, Collinson, and Cummings (1989). Moisture content, crude protein, fat, ash content, acid detergent and neutral detergent fibre and total starch were analysed by Agrifood Technology Pty Ltd (Victoria, Australia). The chemical methods used were based on the Australian Fodder Industry Association (AFIA) methods (AFIA, 2003). Gross energy values of the assay diets were determined using the PARR 6420 Automatic Isoperibol Calorimeter (Moline, Illinois). Sorghum, soybean, sunflower oil, limestone, di-calcium phosphate, salt and a mineral and vitamin premix were sourced from within PNG from commercial suppliers in ground form. A sorghum-soybean meal basal diet was formulated and five assay diets were developed to replace 250 g/kg of the basal diet with each of the five varieties of sweet potato with and without enzyme inclusion. The specific enzyme product used in this study was Rovabio Excel AP T-flex (Endo-1, 4 beta-xylanase, endo-1, 3 9 (4) beta glucanase with 17 other enzymes (Adesso, Antony, France). The enzyme product used was included as a powder at 50g/1000kg as per the manufacturer's instructions. The composition of the basal diets is shown in Table 1 while the chemical composition of the five tested sweet potato varieties is presented in Table 2. The total NSP content of the tested sweet potato varieties ranged from 162-210 g/kg DM (Table 2). Varieties with total NSP contents below 180 g/kg DM were considered to be low NSP varieties while varieties with total NSP contents above 200 g/kg DM were considered to be high NSP varieties. Varieties with total NSP content between 180-200 g/kg were considered as varieties with medium levels of total NSPs.

3.3.2 Experimental chicks, diets and design

A total of 260 ROSS 308 broiler day olds were bought from a local hatchery (Nuigini Table Birds) and reared on deep litter in two separate floor pens in an open, naturally ventilated shed from day-old to 20 days of age. These chicks were fed with a standard commercial broiler starter crumble (26.2% protein, 5.8% fat, 3.2 % fibre and 7.1 % ash content) up until the start of the experiment at day 21. Data loggers were placed inside the pens for automated recordings of daily room temperatures and relative humidity during the experimental period. Mortality and temperatures were also recorded at regular intervals (0600h, 0100h, 1200h, 1500h, and 1800h). At the start of the assay on day 21, the average weight of the flock was determined so that the individual weight of the experimental birds used in the trial was within ± 200 g of the average flock weight. After calculating the average body weight, 4 birds at a time were weighed and allocated to each of the 48 metabolic cages, measuring 600 x 450 x 380 mm. The experimental design was a completely randomised design with sweet potato and enzymes as the two main treatments factors. The experimental treatments were replicated four times. A total of 12 experimental treatments (including the control) were tested and these were Basal –(Control), basal + enzyme –(Control (+E)) , sweet potato variety 1 (SPV1), sweet potato variety 1 + enzyme (SPV1 (+E)), sweet potato variety 2 (SPV2), sweet potato variety 2 + enzyme (SPV2 (+E)), sweet potato variety 3 (SPV3), sweet potato variety 3 + enzyme (SPV3 (+E)), sweet potato variety 4 (SPV4), sweet potato variety 4 + enzyme (SPV4 (+E), sweet potato variety 5 (SPV5), sweet potato variety 5 + enzyme (SPV5 (+E). These experimental diets were pelleted and fed to 4 experimental birds housed together in a group metabolic cage. All the cages had individual feed troughs and nipple drinkers and each cage was shielded to prevent the cross-contamination of excreta from the neighbouring cages. The Apparent Metabolisable Energy (AME) bioassay was conducted as described by Hughes, (2008), using 21 day old broiler chickens. The assay was conducted from day 21 to day 28 posthatch. Feed intake and excreta output were measured quantitatively per cage from day 25 for four consecutive days. Excreta collections from each cage were oven dried overnight daily and then pooled. Pooled dried excreta from individual cages were ground and a representative sample taken for the determination of gross energy for the calculation of AME values of these diets.

3.3.2.1 Chemical analysis

Gross energy (GE) was determined using a Parr isoperibol bomb calorimeter (Moline, Illinois) standardised with benzoic acid. All the samples were tested in duplicate. Other chemical tests were outsourced.

Calculations

AME of SPF and diets were calculated as follows:

$$\text{AMEdiet} = \frac{(\text{GEdiet x Feed DM consumed}) - (\text{GEexcreta x dry excreta DM})}{\text{Feed DM consumed}}$$

$$\text{AMESPF} = \frac{\text{AME of SPF diet} - (\text{AME of basal diet} \times 0.75)}{0.25}$$

The dry matter digestibility of the diets was calculated as follows:

$$\text{Dry Matter digestibility} = \frac{\text{Feed DM consumed} - \text{Excreta DM collected}}{\text{Feed DM consumed}}$$

3.3.3 Tissue sample collection

At the end of the experiment, for each cage at a time, individual birds were weighed and one bird per cage based on the average group weight was selected for the gut morphology and cecal bacterial enumeration. The selected birds were euthanized by cervical dislocation. Final body weights of the birds were recorded prior to dissection. Gut tissues and cecal contents were aseptically collected. The segments of the small intestine with contents were weighed and then flushed with phosphate buffer saline and 5 cm of each segment were collected for the gut morphological studies. These tissue samples were fixed in 10 % buffered formalin and later transferred to 70 % ethanol for further processing. Cecal contents were collected into a sterile tube and immediately placed on ice for bacterial cultivation and enumeration.

3.3.4 Bacterial isolation, enumeration and detection and quantification by quantitative Real Time PCR

One gram of the cecal content from each euthanized bird was placed into a sterile tube containing 9 ml of sterile phosphate buffered saline and homogenised for the enumeration of *Escherichia coli (E. coli)* and total Enterobacteria. Subsequent 10-fold serial dilutions for each sample were prepared to 10-^{7} dilutions. Then 0.1-mL of samples from $10^{-3,}$ 10^{-4} and 10^{-5} dilutions were inoculated onto the MacConkey and Violet Red Bile Glucose media (Oxoid, Australia) for the enumeration of *Escherichia coli* and total Enterobacteria respectively. The inoculated plates were incubated at 37 ^{0}C for 24 hours, after which the total numbers of bacterial colonies were manually counted. The counts were converted into logarithmic equivalents and expressed as the number of colony forming units (CFU) per gram of cecal contents. Due to the lack of appropriate facilities and frequent power outages in-country (PNG), efforts to culture *Campylobacter* and *Clostridium* failed.

3.3.4.1 DNA extraction from cecal contents

Extraction of DNA from cecal contents was performed using a QIAamp DNA fast DNA stool kit (Qiagen, Hilden, Germany) as per the manufacturer's instruction. The eluted DNA concentrations and quality were determined using a Nano drop (Biolab, Australia). The DNA was then diluted with PCR water to 5 µg/µl and stored at –20°C until required. DNA extraction was performed in PNG. DNA samples were imported into Australia (DAFF Import permit number IP14001999).

3.3.4.2 Quantitative Real Time PCR assay

The details of the primers used in the quantitative real time PCR reactions, are described in Table 2. In order to investigate the detection limit of *Salmonell*a, *Campylobacter* and *Clostridium* species, 200 mg of fecal samples were spiked with each of the specific bacteria at doses ranging from 10^0 to 10^8 CFU/ml. DNA was then extracted from these spiked samples using QIAamp DNA Stool Mini Kit (Qiagen, Australia). The quantitative PCR reaction for the detection of the specific bacteria (*Salmonella*, *Campylobacter* and *Clostridium* spp.) was performed in a total reaction volume of 10 µl containing 5 µl of 1x SYBR Green Master mix (Qiagen, Australia), 0.5 µl each of the forward and reverse primers of the specific target gene, 2 µl of PCR water and 2 µl of sample DNA diluted to 5 µl/µg. Each reaction was performed in duplicate and the reaction volume was dispensed using an automation workstation (Corbett Robotics, Australia). The QPCR assays were conducted in a Rotor-Gene 6000 Series real-time PCR machine (Corbett, Sydney, Australia) under the following conditions; 95 °C for 10 seconds, followed by 40 cycles of 95 °C for 10 seconds and 55 °C for 30 seconds.

To determine the detection limit of the QPCR reaction for each specific bacterium, the reaction was run against a set of standards generated with the spiked samples of known concentrations.

The detection limit of *C. perfringens* was 100 CFUs while detection limits of *Campylobacter* and *Salmonella* were 1000 CFUs respectively.

3.3.5 Histopathology

Intestinal tissues were processed based on procedures described by Iji, Saki, and Tivey (2001). Slides were viewed on an Olympus BH-2 microscope at X4 magnification and digitised using an image analysis software (AnalySIS 5 Soft-Imaging System, Germany). Each individual image was calibrated accordingly and measurements (micrometre) of villus height, crypt depth, basal villus width and apical villus width were carried out on 10 intact villi. Apparent villus surface area was estimated from the trigonometric relationship between villus height, villus basal width and villus apical width (Iji et al., 2001).

3.3.6 Statistical analysis

Analysis of the data was performed using the two way analysis of variance in GenStat®, 15th Edition (VSN International, 2011). The difference between the means was identified by the least significant difference and compared using the Duncan's multiple range test.

3.4 Results

3.4.1 Total Enterobacteria counts and real time PCR counts of Campylobacter, Clostridium and Salmonella species

There were no significant differences ($p<0.05$) in the colony counts of total Enterobacteria and *E. coli* in the ceca of broilers due to the different dietary treatments (Table 4). The counts of total Enterobacteria ranged from 2.301 to 5.602 log CFU/g whilst the counts of *E. coli*, ranged from 4.998 to 5.936 log CFU/g of cecal contents. Birds fed with the SPV5 diet had the lowest count of total Enterobacteria whilst the highest count was observed in birds fed with the SPV1 (+E) diet. The lowest count of *E. coli* was observed in birds fed with the SPV3 diet (4.998 log CFU/g) whilst the highest count was observed in birds fed with the SPV1 diet. There were no significant interactions between the different SP varieties and enzyme inclusion on the numbers of these bacterial species in the ceca of birds fed the different diets (Table 4).

Results from the real time PCR assays on the levels of *Campylobacter*, *C. perfringens and Salmonella* are presented in Table 5. The load of *Campylobacter* (log CFU/g) in the ceca of birds fed the different dietary treatments was significantly (p=0.004) influenced by the SP varieties. Inclusion of enzymes did not significantly (p=0.181) influence the load of this enteric pathogen in the ceca of birds. There was however, a significant interaction (p=0.020) between the different SP varieties and enzyme supplementation on the loads of *Campylobacter* (log CFU/g). The load of *Campylobacter* (log CFU/g) in birds fed with the SPV1, SPV4 and SPV5 diets was reduced with enzyme supplementation while birds fed with the Control, SPV2 and SPV3 diets, increased with enzyme supplementation (Table 5). The lowest level of *Campylobacter* was observed in birds fed with the SPV3 diet (1.271 log CFU/g) whilst the highest was observed in birds fed with the SPV5 diet (5.831 log CFU/g). The levels of *Campylobacter* in birds fed with the SPV5 diets were reduced by 76.4% with enzyme supplementation.

As shown in Table 5, the loads of *C. perfringens* in the ceca of birds were significantly (p=<0.001) influenced by the SP varieties but not by enzyme supplementation (p=0.903). There was no interaction between the enzyme and the different SP varieties. Birds fed with the SPV3 diet had a significantly lower *C. perfringens* load compared to those observed in the ceca of birds fed with the other treatment diets. The load of this enteric pathogen ranged from 2.050 to 3.526 log CFU/g of cecal content. The highest level of this pathogen was observed in the ceca of birds fed with the SPV2 diet. The load of *C. perfringens* in birds fed with this diet (SPV2) was reduced by 3.86 % with enzyme supplementation. The loads of *C. perfringens* in birds fed with the SPV3 diet were significantly lower compared to those in birds fed with the Control and the other SP diets. The levels of this pathogen were however elevated with enzyme inclusion.

The numbers of *Salmonella* spp were not significantly influenced by the SP varieties (p=0.758) or with the enzyme supplementation (p=0.685) (Table 5). However, there was a significant (p=0.047) interaction between the enzyme supplementation and the SP varieties. The load of *Salmonella* ranged from 0.00 to 1.753 log CFU/g of cecal content. The lowest level was observed in birds fed with the control diet whilst the highest was observed in the ceca of birds fed with the SPV5 diet. Inclusion of the enzyme reduced the load of *Salmonella* in the ceca of birds fed with the different SP varieties. Inclusion of the enzyme in the control diet significantly elevated the levels of *Salmonella* whilst the inclusion of enzymes in the SPV5 diet reduced the load of this pathogen by 57.2 %.

3.4.2 NSP levels of the different sweet potato varieties and AME values of the diets

The total NSP content of the tested sweet potato varieties from PNG ranged between 162 and 210 g/kg DM (Table 2). Varieties 3 and 5 had the highest total NSP content of 202 and 210 g/kg DM respectively whilst variety 2 had the lowest total NSP content of 162 g/kg DM. Varieties 1 and 4 had the total NSP content of 197 and 190 g/kg DM respectively. There were considerable differences between the varieties in the relative proportions of the soluble and insoluble fractions of the total NSP in the tested (Table 2).

There were significant differences due to the SP varieties and enzyme supplementation (P<0.001) on the AME values of the different SP diets (Table 6). A significant interaction (P<0.001) between the sweet potato varieties and enzyme supplementation was also observed (Table 6). The SPV2 diet had the lowest AME value while the highest AME was recorded for the Control (+E) diet. In terms of the SP varieties, SPV1 diet had a higher AME value (15.46 MJ/kg) compared to the other SP diets. There was an improvement in the AME of the SPV2 diet with enzyme inclusion (11.22 to 13.97 MJ/kg).

3.4.3 Growth performance of birds fed different SP diets

In terms of bird performance, significant differences were observed on the end weight (p=0.06), weight gain (p<0.001), feed intake (p<0.001) and feed conversion ratio (FCR) (p=0.001) of birds fed the different diets (Table 7). There were significant interactions between the SP varieties and enzyme inclusion on the intake and FCR of birds. Intake of birds fed with the SPV3 diet was improved with enzyme inclusion. Birds fed with the Control (+E) and the SPV1 (+E) diets had significantly higher intakes compared to birds fed with the other diets. In terms of FCRs, birds fed with the SPV1 (+E) diet had significantly higher FCR which is indicative of a high intake and lower weight gain. FCRs of birds fed with the other four SP diets were comparable to that of the birds fed with the Control (+E) diet. As expected, birds fed on the control diet had a significantly lower FCR compared to that of the birds fed on the other test diets (Table 7).

3.4.4 Relative organs weights and jejunal morphology

There were no significant differences (P>0.05) observed on the relative weights of the liver, pancreas and segments of the small intestine of broilers fed with the different SP diets (Table 8). Inclusion of the enzymes in the diets did not significantly influence the above mentioned traits. There was no interaction between the enzymes and the SP varieties on the relative organ weights.

In terms of the jejunal morphology, there were no significant differences (P>0.05) observed in the villus height, villus crypt depth, villus height and crypt depth ratio and the apparent surface area in the birds fed with the different sweet potato varieties (Table 9). The mean villi heights of the jejunum from the birds fed with the different diets ranged from 1105 to 2132 µm, whilst the mean crypt depth measurements ranged from 121 to 246 µm. The highest apparent surface area (0.402 mm^2) was observed in birds fed with the SPV3 (+E) diet. There were no significant (p>0.05) differences observed with enzyme inclusion on the measured traits (Table 9).

3.5 Discussion

Different dietary interventions may affect the health of birds as the microbial status of the gastrointestinal tract of poultry is influenced by the diet composition and the internal gut environment (Apajalahti, Kettunen, & Graham, 2004; Yegani & Korver, 2008). The commensal microbial community of the gut plays a major role in the health and digestion of poultry (Kleyn, 2013), therefore any dietary interventions can either increase or decrease the risk of infection by enteric pathogens.

In the current study, the counts (log CFU/g) of total Enterobacteria and *E. coli* from the ceca of birds fed with the different dietary treatments were not significantly influenced by the different SP varieties nor by enzyme supplementation. This observation is similar to our earlier experiment (unpublished) using sweet potato flour in which the counts of the total Enterobacteria and *E. coli* were not influenced by the inclusion of sweet potato flour or enzyme supplementation.

In the present study, the load (log CFU/g cecal content) of *Campylobacter* was significantly influenced by the different SP varieties but not by the inclusion of enzymes. There was however, a significant interaction between the different SP varieties and enzyme supplementation on the loads of *Campylobacter* (log CFU/g). The load of this pathogen in the ceca of birds was reduced when the enzymes were included in the SPV1, SPV4 and SPV5 diets. These varieties (1, 4 & 5) had medium to high levels of total NSP content (Table 2). In contrast, the load of *Campylobacter* in the birds fed with the SPV2 and SPV3 diets were higher though not significant with enzyme supplementation (Table 5). These two SP varieties (Variety 2 & 3) had the lowest total NSP and lowest soluble NSP components. Earlier studies reported that there was a reduction in the numbers of *Campylobacter* in the ceca of broilers fed with a wheat based diet and NSP degrading enzymes (Fernandez, Sharma, Hinton, & Bedford, 2000; Molnár et al., 2015).

In the current study, the levels of *Salmonella* spp were not significantly influenced by the SP varieties or with enzyme supplementation but a significant interaction between the enzyme supplementation and the SP varieties were observed (Table 5). Teirlynck et al. (2009), suggested that a possible way to control *Salmonella* in broiler chickens was to change the carbohydrate composition, specifically the NSP content in diets for broilers. In addition to that, the supplementation with an enzyme product can decrease the level of *Salmonella* in broilers fed these diets as the hydrolysis of the NSPs by these enzymes will lead to the production of short chain fatty acids (SCFA) (Santos, Ferket, Santos, Nakamura, & Collier, 2008). These SCFAs are known to have bacteriostatic effects (Santos et al., 2008; Van Immerseel et al., 2003).

The levels of *Campylobacter* and *Salmonella* were highest in the birds fed with the SPV5 diet without the enzyme supplementation. This variety had the highest total NSP content with only a marginal difference in the amount of soluble and insoluble NSP fractions (106 g/kg vs. 104 g/kg DM). Similar to *Campylobacter*, the levels of *C. perfringens* in the ceca of birds were significantly influenced by SP variety but not by enzyme supplementation. *C. perfringens* levels were high yet not significantly different in the ceca of birds given the SPV2 diet without enzyme supplementation. The levels of *C. perfringens* were significantly low in the ceca of birds given the SPV3 (Suga variety) diet. This variety had both the lowest absolute and proportional level of soluble NSP. There is limited data available on the levels and the shedding of these enteropathogens in broilers fed with sweet potato diets in PNG. The only available data to date is a study by Tasi (2015) which showed that *Campylobacter* and *Salmonella* are present in poultry farms in PNG but levels were significantly lower than those reported in developed countries.

Soluble NSPs in grains such as wheat, barley and rye are known to create a viscous gut environment in broilers leading to a reduced digesta transit time which creates a conducive environment for the proliferation of pathogens (Annison & Choct, 1991; Choct, 2002; Choct, Annison, & Trimble, 1992; Choct, Hughes, Trimble, Angkanaporn, & Annison, 1995; Choct et al., 1996). In addition to that, enzyme supplementation is associated with the production of SCFAs due to the fermentation of NSPs in the ceca of birds (Rinttilä & Apajalahti, 2013; Van Immerseel et al., 2006). Production of these SCFA would have made the environment more acidic thus reducing the numbers of *Campylobacter* and *Salmonella* whilst *C. perfringens* levels may not have been affected as species belonging to the Clostridiaceae family in the lower intestinal tract are butyrate producers (Teirlynck et al., 2009).

The regular use of alternative feed ingredients in the diets for poultry is impeded by high fibre fractions as these fibre fractions are structural carbohydrates that are not digested by the endogenous enzymes in poultry and other monogastric livestock (Choct, 2006). The use of exogenous enzymes aids the hydrolysis of this feed component, thus reducing the viscosity of the gut in poultry (Pan & Yu, 2014). In the current study, inclusion of the enzymes to the SP varieties with a high total NSP content reduced the levels of *Campylobacter* and *Salmonella* species in the ceca of birds fed on those diets. Apart from the study by Parsons et al. (2014), there is limited information available on how NSPs in crops other than grains, influence the gut microbiota, which may then affect growth performance of broilers raised under less than perfect management conditions. *Campylobacter* spp., *C. perfringens* and *Salmonella* spp are important zoonotic bacteria that infect humans and these pathogens are part of the normal gut microbiota in chickens (Donoghue et al., 2006). The type of diet offered to broiler chickens is vital to reducing bacterial contamination in the end product (chicken meat in this case). In the current study, the numbers of *Campylobacter* and *Salmonella* spp., were higher in the ceca of birds on the sweet potato with the lowest soluble NSP (SPV3-E) whilst *C. perfringens* was highest in birds fed with the SP variety with a low total NSP content. There is an obvious link to the proportion of soluble and insoluble fibre components influencing the load of these enteropathogens in the ceca of broilers. In the present study, the numbers of *C. perfringens* in the sweet potato diets were comparable to that of the birds fed on the control diet and were not influenced by the inclusion of the enzyme. There could be three underlying possibilities for this finding; 1) the NSPs of these sweet potato varieties are not viscous, 2) the levels of insoluble NSPs are higher than the soluble fraction or 3) the diets are highly digestible.

In the present study, the AME values of the different SP diets ranged from 12.38 to 15.46 MJ/kg and can be attributed to the total NSP content of these varieties and place of origin (PNG). The dry matter digestibility (DMD) of the sweet potato diets showed that these dietary treatments were well utilised by the birds (Table 6) and may be due to the diets being offered as pellets (Abdollahi, Ravindran, & Svihus, 2013; Amerah et al., 2013) as pelleting sweet potato diets is necessary due to its dusty texture (Ravindran & Sivakanesan, 1996) however diets must be balanced for protein and amino acid levels.

AME and food utilisation efficiency are often negatively impacted by soluble NSP (Annison & Choct, 1991; Annison, Choct, & Cheetham, 1992; Choct et al., 1995). There is limited information available on the NSPs of roots and tuber crops, and how they may impact on the overall AME of the diet and the FCR of broilers. In the current study, we observed a lower AME value in the SPV2 (Gimane) diet. This variety had a low total NSP content but a higher soluble NSP component (Table 2) and this is similar to that observed in wheat, where varieties with high soluble NSP content had lower AME values (Hughes, 2008; Hughes & Choct, 1999). The other four SP diets had a medium to high total NSP content however their soluble NSP fractions were relatively lower (Table 2). The AME value of SPV2 diet was greatly improved with enzyme inclusion. The use of exogenous enzymes aids the hydrolysis of soluble NSP fractions leading to reduced gut viscosity in poultry, which in turn enables other nutrients to be readily available for absorption (Williams, Geraert, Uzu, & Annison, 1997). Currently, most of the commercial exogenous enzyme products are targeted at the soluble fractions of NSPs in feed ingredients. Insoluble NSPs particularly cellulose are not a practical target for improvement in poultry as no enzyme can effectively release glucose from this component nor does this fraction of NSP create a viscous gut environment in birds. This may be the case here as the impact of enzymes on the AME values from varieties with a high proportion of insoluble NSP were negligible (Table 3).

The inclusion of sweet potato in the present study had a significant influence on the production parameters such as the body weight gains, feed intakes and the FCRs of broilers. Of the sweet potato varieties tested, birds fed with the SPV3 diet had a FCR that was comparable to that of the birds fed on the control diet. This variety had a higher insoluble NSP component and may have enhanced gut digestive parameters (Choct, 2009). Gut morphology in the jejunum of the birds fed with this variety had a higher villi height and a larger apparent surface area available for absorption. The inclusion of sweet potato did not have any significant effect on the gut morphology of birds in the current study (Table 9). The gut morphological traits such as villus height, crypt depth and the apparent surface area in the jejunum of the birds with a high load of *Campylobacter* and *Salmonella* (birds fed with the SPV5 diet) and *C. perfringens* (birds fed with the SPV2 diet) were not affected.

3.6 Conclusion

In conclusion, given that sweet potato will be used extensively in diets for poultry in PNG and other Pacific Island nations in the near future, this study provides useful insights on the effects of feeding local SP varieties on the level of food borne pathogens in chickens. The only significant effect of supplementing enzymes was observed on the levels of *Campylobacter* in birds fed the sweet potato diets. However, birds fed with the sweet potato variety with a high total NSP content had elevated levels of *Campylobacter* and *Salmonella* whilst the level of *C. perfringens* were higher in the birds fed with the sweet potato variety with a low total NSP content. In addition to that, *Salmonella* was not detected in the birds fed on the control diets. The levels of *Campylobacter* were highest in the birds fed four out of the five sweet potato varieties with and without the enzyme supplementation. The lowest level of this enteric pathogen was observed in the birds fed with the sweet potato variety that had the highest insoluble NSP component without the enzyme supplementation. The NSP levels in sweet potato did not affect the gut morphology of birds which highlights the possibility of using different sweet potato varieties for feeding poultry. In terms of the total NSP content, selection of varieties with a low soluble NSP content would be economical as AME values are within the range selected for poultry without the need for enzyme inclusion. Given that the numbers of *C. perfringens* were not significant in broilers fed with these varieties accept for SPV3 (-E), their inclusion in the feed is likely to reduce the risk of necrotic enteritis. On the other hand, *Campylobacter* and *Salmonella* levels in the broilers fed with the sweet potato diets can be reduced with enzyme inclusion. This will ultimately minimize the risk of campylobacteriosis and salmonellosis in the family members of smallholder poultry famers as well as reduce the risks of product contamination for the consumers. To our knowlegde this is the first study conducted in PNG to assess the levels of enteric pathogens such as *Salmonella*, *Campylobacter* and *Clostridium* spp, in the smallholder poultry sector of PNG. This information will be useful for policy makers in addressing food safety issues in the country. In addition to that, these information could also contribute to the implementation of appropriate interventions for reducing the load of enteric pathogens using local resources.

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Table 1. Composition of the basal diet (g/kg DM)

Ingredient	Basal
Sorghum	575
Soybean meal	320
Sunflower oil	60.0
Di-calcium phosphate	20.0
Limestone	11.0
DL-methionine	7.0
Vitamin and mineral premix	2.0
Salt	3.0
Choline chloride (60%)	2.0

Table 2. Chemical composition of 5 local PNG Sweetpotato cultivars

	Variety 1	Variety 2	Variety 3	Variety 4	Variety 5
Dry Matter (g/kg DM)	902	901	891	913	835
Protein (N x 6.25) (g/kg DM)	41	38	48	34	24
Fat (%)	2.5	1.9	1.7	1.8	0.68
Ash (%)	2.7	2.6	2.1	2.3	1.8
Total Starch (g/k gDM)	590	530	580	624	-
Non-starch polysaccharides (g/kg DM)					
Soluble	69	94	64	81	106
Insoluble	128	68	138	109	104
Total	197	162	202	190	210

Variety 1=Waghi besta; Variety 2=Gimane; Variety 3=Suga; Variety 4=Marasonda; Variety 5=Rachel

Table 3. Description of primers selected for Q-PCR reactions for the detection of *Campylobacter*, Clostridium and Salmonella species

Bacteria	Target gene	Primer	Gene Sequence	Size (Bp)	Source
Campylobacter	16S rRNA	F	CAT TGG ATG AGC AAC TTA AAA TC	125	(Cheng & Griffiths, 2003)
		R	TTC TTC CAT TAA ACG GTT GA		
Clostridium perfringens	16SrRNA	CPerf165F	CGCATAACGTTGAAAGATGG	105	(Wise & Siragusa, 2005)
		CPerf269R	CCTTGGTAGGCCGTTACCC		
Salmonella spp	*invA*gene	F	CATTTCTATGTTCGTCATTCCATTACC	132	(Barletta et al., 2013)
		R	AGGAAACGTTGAAAAACTGAGGATTCT		

Table 4. Counts of total Enterobacteriaceae and *E. coli* (Log CFU /g/ml) in chickens fed different sweetpotato diets

Treatment SP variety	Enzyme	Enterobacteria	*Escherichia coli*
Control	(-)	5.079	5.547
	(+)	5.590	5.694
SPV1	(-)	5.201	5.936
	(+)	5.602	5.865
SPV2	(-)	2.540	5.402
	(+)	5.345	5.132
SPV3	(-)	5.429	4.998
	(+)	4.801	5.457
SPV4	(-)	5.318	5.312
	(+)	2.628	5.304
SPV5	(-)	2.301	5.380
	(+)	5.102	5.529
SEM		1.263	0.369
Source of variation			
SP variety		***0.588***	***0.467***
Enzyme		**0.479**	**0.756**
SP variety x Enzyme		***0.298***	***0.945***

Means are not significantly different ($p<0.05$); SEM=Standard error of means

Table 5. Log CFU (g/ml) of cecal content of chickens fed different sweetpotato diets

Treatment SP variety	Enzyme	*Campylobacter*	*C. perfringens*	*Salmonella spp*
Control	(-)	4.095[abc]	3.421[a]	0.000[b]
	(+)	5.734[a]	3.514[a]	1.635[a]
SPV1	(-)	4.840[ab]	3.181[a]	0.970[ab]
	(+)	3.733[abc]	2.940[a]	0.995[ab]
SPV2	(-)	2.461[bc]	3.526[a]	1.003[ab]
	(+)	2.882[abc]	3.390[a]	0.852[ab]
SPV3	(-)	1.271[c]	2.050[b]	0.680[ab]
	(+)	2.077[bc]	2.788[a]	0.440[ab]
SPV4	(-)	2.980[abc]	3.138[a]	1.493[ab]
	(+)	1.375[c]	2.905[a]	1.093[ab]
SPV5	(-)	5.831[a]	3.292[a]	1.753[a]
	(+)	1.376[c]	2.977[a]	0.224[b]
SEM		0.919	0.222	0.469
Source of variation				
SP variety		**0.004**	**<.001**	**0.758**
Enzyme		**0.181**	**0.903**	**0.685**
SP variety x Enzyme		**0.020**	**0.173**	**0.047**

Means within a column with different superscript are significantly different ($p<0.05$); SEM=Standard error of mean.

Table 6. AME values and Dry matter digestibility of treatment diets

Treatment			
SP variety	Enzyme	AME DM (MJ/kg)	DMD (%)
Control	(-)	13.97[b]	65.6[b]
	(+)	15.99[a]	78.5[a]
SPV1	(-)	15.65[a]	79.0[a]
	(+)	15.27[a]	77.8[a]
SPV2	(-)	11.22[c]	66.0[b]
	(+)	13.55[b]	66.2[b]
SPV3	(-)	13.39[b]	66.4[b]
	(+)	13.97[b]	68.3[b]
SPV4	(-)	13.80[b]	68.5[b]
	(+)	13.98[b]	69.0[b]
SPV5	(-)	13.63[b]	66.8[b]
	(+)	13.35[b]	66.6[b]
SEM		0.194	0.029
Source of variation			
(Probability)			
SP variety		**<.001**	**<.001**
Enzyme		**<.001**	**0.008**
SP variety x Enzyme		**<.001**	**<.001**

Means within a column with different superscripts are significantly different (p<0.001; p<0.05); DMD=Dry matter digestibility; SEM=Standard error of means;

Table 7. Summary of main effects of diet and enzyme on performance parameters of broilers

<u>Treatment</u> SP variety	Enzyme	Start weight (g)	End weight (g)	Weight gain (g)	Feed intake (g)	FCR (g/g)
Control	(-)	942[a]	1424[ab]	483[a]	946[b]	1.96[c]
	(+)	930[a]	1416[c]	486[a]	1326[a]	2.75[b]
SPV1	(-)	935[a]	1244[bc]	309[c]	1262[a]	4.09[a]
	(+)	945[a]	1233[c]	288[c]	1192[a]	4.25[a]
SPV2	(-)	949[a]	1267[bc]	318[bc]	919[b]	2.88[b]
	(+)	938[a]	1258[bc]	320[bc]	911[b]	2.88[b]
SPV3	(-)	956[a]	1261[c]	306[c]	869[b]	2.88[b]
	(+)	936[a]	1327[abc]	391[b]	987[b]	2.52[b]
SPV4	(-)	937[a]	1260[bc]	323[bc]	906[b]	2.83[b]
	(+)	946[a]	1246[c]	301[c]	908[b]	3.01[b]
SPV5	(-)	930[a]	1236[c]	306[c]	896[b]	2.93[b]
	(+)	942[a]	1269[c]	328[bc]	894[b]	2.79[b]
SEM		62.5	96.8	46.2	113	0.35
Source of variation						
SP variety		**0.999**	**0.006**	**<.001**	**<.001**	**<.001**
Enzyme		**0.913**	**0.739**	**0.400**	**0.04**	**0.298**
SP variety x Enzyme		**0.992**	**0.948**	**0.215**	**0.004**	**0.045**

Means with the same letter within a main effect are not significantly different ($P > 0.05$); FCR=Feed conversion ratio; SEM=Standard error of means;

Table 8. Dietary effects on the relative organ and intestinal segments (g/kg) of broilers

Treatment SP variety	Enzyme	Pancreas	Liver	Small intestine	Duodenum	Jejunum	Ileum
Control	(-)	2.52	23.54	49.32	12.17	22.06	15.1
	(+)	2.33	22.57	50.34	12.1	23.73	14.51
SPV1	(-)	2.67	24.65	52.45	12.76	23.39	16.3
	(+)	2.9	24.6	49.94	12.62	21.72	15.61
SPV2	(-)	3.07	28.22	50.93	11.69	23.51	15.72
	(+)	3.18	23.83	51.93	12.78	23.16	15.99
SPV3	(-)	3.06	23.67	51.32	12.0	23.89	15.44
	(+)	2.51	24.15	47.75	11.13	21.45	15.17
SPV4	(-)	3.29	26.7	50.3	11.12	23.23	15.94
	(+)	3.1	25.28	54.2	12.68	26.15	15.37
SPV5	(-)	3.11	24.33	51.2	12.35	22.81	16.04
	(+)	3.19	27.01	54.65	13.31	25.37	15.97
SEM		0.564	3.320	3.896	1.796	3.18	2.559
Source of variation							
SP variety		**0.07**	**0.383**	**0.843**	**0.729**	**0.716**	**0.929**
Enzyme		**0.61**	**0.528**	**0.75**	**0.422**	**0.63**	**0.666**
SP variety x Enzyme		**0.776**	**0.436**	**0.749**	**0.753**	**0.427**	**0.999**

Means are not significantly different ($p<0.05$); SEM=Standard error of means

Table 9. Dietary effects on morphological changes in the jejunum of broilers

Treatment SP variety	Enzyme	Villus height (µm)	Crypt depth (µm)	Villus: crypt ratio	Apparent surface area (mm^{2})
Control	(-)	1603	173.0	5.97	0.328
	(+)	1295	185.2	7.37	0.155
SPV1	(-)	1105	118.5	10.85	0.099
	(+)	1703	180.5	9.01	0.288
SPV2	(-)	1517	167.9	10.77	0.207
	(+)	1898	189.1	9.87	0.319
SPV3	(-)	1596	179.8	5.95	0.305
	(+)	2132	245.7	10.23	0.402
SPV4	(-)	1152	121.7	7.47	0.176
	(+)	1625	145.1	12.56	0.284
SPV5	(-)	1319	141.0	9.49	0.131
	(+)	1234	124.5	12.70	0.145
SEM		527.8	61.42	2.759	0.128
Source of variation					
SP variety		**0.871**	**0.756**	**0.621**	**0.675**
Enzyme		**0.390**	**0.435**	**0.252**	**0.439**
SP variety x Enzyme		**0.936**	**0.984**	**0.758**	**0.784**

Means are not significantly different (P > 0.05); SEM=Standard error of means

Chapter 4 Levels of enteric pathogens and growth of broilers fed diets made with three PNG sweet potato varieties with high total non-starch polysaccharide content

Statement of Authorship

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Contribution to the Paper	Performed experiment, data entry, analysis and interpretation, wrote manuscript and responded to editing suggestions by co-authors		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Levels of enteric pathogens and growth performance of broilers fed diets made with three PNG sweet potato varieties with high total non-starch polysaccharide content.

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Short title: Growth performance of broilers fed with sweet potato based feed

4.1 Abstract

In the last decade, research has targeted the evaluation of local feed ingredients for use in monogastric diets to alleviate the high cost of production of livestock on smallholder farms in Papua New Guinea (PNG). This experiment was conducted to study the effects of the inclusion of three PNG sweet potato (SP) varieties with high total non-starch polysaccharide (NSP) contents on the overall performance of broilers and levels of *Salmonella*, *Campylobacter* and *Clostridium species* (spp.) at 42 days post hatch. A total of 200 Ross 308 broiler chicks were obtained from a local hatchery and fed a standard broiler starter ration to day 20 post hatch. At day 21, the birds were weighed and 10 birds were randomly allocated to each of the 20 individual pens in an open, naturally ventilated shed. The sweet potato root meals were processed and included at 30 % in a sorghum basal diet. The experimental design was a completely randomised design with sweet potato as the main treatment factor. There were 4 dietary treatments, Control, sweet potato variety 1 (SPV1), sweet potato variety 2 (SPV2) and sweet potato variety 3 (SPV3). Each dietary treatment was replicated 5 times. The dietary treatments were introduced to the birds on day 21 post hatch and fed over the next 21 days. Birds given the sweet potato diets had significantly lower weekly end weights (24.4 %, 27.97 % and 29.34 %), weight gains (48.8 %, 57.66 % and 64.26 %) and intakes (24.0 %, 26.74 % and 18.83 %) compared to the birds fed the control diet. The feed conversion ratio (FCR) of birds fed with the sweet potato diets improved by day 42. Levels of *Campylobacter, Salmonella* and *Clostridium perfringens* were analysed via real time PCR. The averaged ct values of *Campylobacter* were significantly different ($p<0.001$) and was 28.81 in the control diet, 30.12, 31.84 and 31.93 in the SPV1, SPV2 and SPV3 diets. The averaged ct value of *C. perfringens* was not significant between the different dietary treatments while levels of *Salmonella* were low in the ceca of birds fed different diets.

Keywords: *Campylobacter*, *Clostridium perfringens*, *Salmonella*, broiler chickens, sweet potato

Implications

The current study showed that feeding sweet potato containing high dietary fibre (total NSP) levels over a 3 week period did not negatively impact on the small intestinal morphology of broilers. The levels of *Campylobacter, Salmonella* and *C. perfringens* in the ceca of chickens were variable in that, *Salmonella* levels were low across all dietary treatments; levels of *Clostridium perfringens* were not significantly influenced and there was a marginal decrease in the levels of *Campylobacter* in the ceca of birds on the sweet potato diets. The current study highlights the potential of sweet potato to be used as a prebiotic source in regions where it is grown.

4.2 Introduction

Feeding sweet potato to poultry as an energy source has been reported by numerous researchers (Job *et al.*, 1979, Khan *et al.*, 1994, Panigrahi *et al.*, 1996, Ravindran and Sivakanesan, 1996, Mudoh *et al.*, 1997, Agwunobi, 1999, Maphosa *et al.*, 2003, Ayuk 2004, Ayuk and Essien, 2009, Afolayan *et al.*, 2012). However, there is limited information on whether inclusion of sweet potato in poultry diets influences the levels of enteric pathogens such as *Campylobacter*, *Salmonella* and *Clostridium perfringens*. There have been studies examining the effect of dietary fibre on cecal fermentation products and microflora in rats (Takamine *et al.*, 2005) and the potency of *Lactobacillus* plantarum Dad-13 and sweet potato *(Ipomoea batatas*) fibre as an immunomodulator in rats infected with *Salmonella typhimurium* (Nurliyani *et al.*, 2015). The study by Takamine *et al.* (2005) with rats fed with the sweet potato fibre diet, had heavier cecal content and tissue weights, high moisture content of faeces with butyrate concentration 1.7 times that of the rats fed on the control diet (Takamine *et al.*, 2005). Another study from Indonesia with rats fed with a local sweet potato variety (Bestak) with high insoluble fibre and low soluble fibre investigated immunity in rats infected with *Salmonella typhimurium* (Nurliyani *et al.*, 2015). These researchers found that the soluble fibre content in that sweet potato variety may have had a role in the mucosal immune response through increasing IgA in infected rats, as there was no increase in cecal Lactobacilli (Nurliyani *et al.*, 2015). Lactic acid bacteria in the gut of birds can have immunostimulatory properties therefore, an increase in the Lactic acid bacterial population by means of using prebiotics in the diet of broiler chickens would enhance the natural immune competence ability of the animals by increasing the natural antibody levels, by promoting the immune defence mechanism of birds (Lan *et al.*, 2005).

Currently, there is limited information in the literature on the incidence of necrotic enteritis (NE) caused by *C. perfringens*, as well as *Campylobacter* and *Salmonella* shedding in commercial broiler chickens farmed by smallholders in Papua New Guinea (PNG). This warrents addressing as alternative feeding strategies have been promoted, particularly the use of locally available ingredients, such as sweet potato, for the farming of broiler chickens destined for live chicken markets.

It is hypothesised that inclusion of sweet potato (SP) in broiler diets can influence the shedding of pathogens, such as *Clostridium perfringens*, *Salmonella* and *Campylobacter*, over a prolonged period during the finishing stages of raising broiler chickens under smallholder settings in PNG. This experiment was conducted in PNG to study the effects of the inclusion of three different sweet potato varieties with high total NSP content in broiler diets on levels of *Salmonella*, *Campylobacter* and *Clostridium* species (spp.) and the overall performance of birds from 21-42 days.

4.3 Materials and methods

The study was undertaken in Lae, Morobe Province, PNG at the Feed Testing Facility of the National Agricultural Research Institute (NARI). The experiment was conducted between December 2014 and January 2015. The average daily temperature for that period was 30 ^{o}C with a relative humidity of around 80 %. Minimum and maximum temperatures recorded for that period were 26 ^{o}C and 31 ^{o}C, respectively. The study was approved by the Animal Ethics Committee of the University of Adelaide, AEC Project No. S-2014-026. All procedures complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

4.3.1 Sweet potato diets

Three selected sweet potato varieties were purchased from various provincial markets in the Highlands and Morobe Provinces of PNG. These varieties were identified by local names as Waghi besta (variety 1), Suga (variety 2) and Rachel (variety 3). Briefly, fresh sweet potato roots were washed, sliced and oven dried (Vision Scientific Company, Korea) at 60 ^{o}C for 16 hours. Moisture content, crude protein, fat, ash content, acid detergent and neutral detergent fibre and total starch were analysed by the NARI Chemistry laboratory. The chemical methods used were based on the Association of Official Analytical Chemists (AOAC) methods (AOAC, 1990). Sorghum, soybean, sunflower oil, limestone, di-calcium phosphate, salt and a mineral and vitamin premix were sourced within PNG from commercial suppliers in ground form. A sorghum-soybean meal basal diet was formulated and the three experimental diets were developed by replacing 300 g/kg of the basal diet with each of the three varieties of sweet potato. The diets were offered as mash to the birds. Composition of the basal diets is shown in Table 1.

4.3.2 Experimental chicks, diets and design

A total of 260 Ross 308 broiler day old chicks were purchased from a local hatchery (Nuigini Table Birds) and reared on deep litter in two separate floor pens in an open, naturally ventilated shed from day-old to 20 days of age. The chicks were fed a standard commercial broiler starter crumble (26.2 % protein, 5.8 % fat, 3.2 % fibre and 7.1 % ash content) until the start of the experiment at day 21. Data loggers were placed inside the pens for automated recordings of the daily room temperature and relative humidity during the experimental period. Mortality and temperatures were also recorded at regular intervals (0600h, 0100h, 1200h, 1500h, and 1800h). The experimental design was a completely randomised design with sweet potato as the main treatment factor. The experimental treatments were replicated five times. A total of four experimental treatments (including control) were tested and these were Basal (Control), sweet potato variety 1 (SPV1), sweet potato variety 2 (SPV2) and sweet potato variety 3 (SPV3). These experimental diets were fed to 10 experimental birds housed together in an experimental pen. The experiment was conducted in an open-sided poultry house with a deep litter floor and 20 experimental pens each measuring 4 m × 2.5 m in dimension. Wood chips were provided as bedding material for the birds and fluorescent tubes were used for lighting durig the night throughout the experimental period. Each pen was provided with two feed hoppers and one automated bell drinker. Based on the area and the number of birds, average stocking density was 1 m^2 per bird. Weights of birds were recorded at the start and end of the experiment and at the end of each week. An inventory of birds was taken on a weekly basis. Feed intake per pen was calculated as the difference between feed offered and feed residues in the feed hoppers at the end of each week. Feed conversion ratio (FCR) was calculated weekly for each experimental pen.

4.3.3 Tissue sample collection

At the end of the experiment, individual birds were weighed and three birds per pen based on individual weights were selected for gut morphology and cecal bacterial enumeration. Selected birds were euthanised by cervical dislocation. Final body weights of birds were recorded prior to dissection. Gut tissues and cecal contents were aseptically collected. Segments of the small intestine with contents were weighed and then flushed with phosphate buffer saline and 5 cm of each segment was collected for gut morphological studies. These tissue samples were fixed in 10 % buffered formalin and later transferred to 70 % ethanol for further processing. Cecal contents were collected into a sterile tube and immediately placed on ice for bacterial cultivation and enumeration.

4.3.4 Bacterial isolation, enumeration and detection and quantification by quantitative Real Time PCR

For enumeration of *Escherichia coli* and total Enterobacteriaceae, one gram of cecal content from each euthanised bird was placed into a sterile tube containing 9 ml of sterile phosphate buffered saline and homogenised. Subsequent 10-fold serial dilutions for each sample were prepared to 10-^{7} dilutions. Then, 0.1 mL of samples from $10^{-3,}$ 10^{-4} and 10^{-5} dilutions were inoculated onto the MacConkey and Violet Red Bile Glucose media (Oxoid, Australia) for enumeration of *E. coli* and total Enterobacteriaceae, respectively. Plates were incubated at 37 ^{0}C for 24 h, after which total numbers of bacterial colonies were manually counted. The counts were converted into logarithmic equivalents and expressed as the number of colony forming units (CFU) per gram of cecal content. Due to a lack of appropriate facilities and frequent power outages in-country (PNG), efforts to culture *Campylobacter* and Clostridium failed.

4.3.4.1 DNA extraction from cecal contents

Extraction of DNA from cecal content was performed using a QIAamp DNA fast DNA stool kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. The eluted DNA concentrations and quality were determined using a Nano drop (Biolab, Australia). The DNA was then diluted with PCR water to 5 µg/µl and stored at –20 °C until required. DNA extraction was performed in PNG and DNA samples were imported into Australia (DAFF Import permit number IP14001999).

4.3.4.1 Quantitative Real Time PCR assay

The details of primers used in quantitative real time PCR reactions are described in Table 2. DNA was extracted from spiked samples using QIAamp DNA Stool Mini Kit (Qiagen, Australia). The quantitative PCR (QPCR) reaction for the detection *Salmonella, Campylobacter* and *Clostridium spp*. was performed in a total reaction volume of 10 µl containing 5 µl of 1x SYBR Green Master mix (Qiagen, Australia), 0.5 µl each of forward and reverse primers of the specific target gene, 2 µl of PCR water and 2 µl of sample DNA diluted to 5 µl/µg. Each reaction was performed in duplicate and the reaction volume was dispensed using an automated workstation (Corbett Robotics, Australia). The QPCR assays were conducted in a Rotor-Gene 6000 Series Real time PCR machine (Corbett, Sydney, Australia) under the following conditions; 95 °C for 10 secs, followed by 40 cycles of 95 °C for 10 secs and 55 °C for 30 secs. Detection limit of the QPCR reaction for each specific bacterium was run against a set of standards generated with spiked samples of known concentrations. The detection limit of *C.*

perfringens was 100 CFU, while the detection limit of *Campylobacter* and *Salmonella* was 1000 CFU respectively.

4.3.5 Histological analysis

Intestinal tissues were processed based on procedures described by Iji *et al.* (2001). Histological examination was done on the 5 µm tissue sections cut on a Thermo Scientific Microtome (Microm International GmbH, Walldorf, Hessen, Germany) loaded onto glass slides. These sections were stained with Lillie-mayer's haematoxylin and then counter stained with eosin and mounted in DXP medium. Villus height, crypt depth, villus basal width and villus apical width were measured from 10 randomly selected and intact villi and crypts using a 4X magnification on an Olympus BH-2 microscope (Olympus, Tokyo, Japan) mounted with a Colorview Soft Imaging System CX41 camera (Soft Imaging System, Brook-AncoCorp, Rochester, New York, USA) with the aid of the image analysis program, AnalySIS 5 (Soft-Imaging System, Germany). Villus height was measured from the tip of the villus to the villus crypt junction; crypt depth was defined as the depth of the invagination between adjacent villi; basal villus width was measured across the breath of the base of the villus and the apical villi width was measured across the villus tip. Apparent villus surface area was estimated from the trigonometric relationship between villus height, basal villus width and apical villus width (Iji *et al.*, 2001).

4.3.6 Statistical analysis

Data analysis was performed using one way analysis of variance in GenStat®, 15th Edition (VSN International, 2011). The difference between means was identified by least significant difference and compared using Duncan's multiple range test.

4.5 Results

4.5.1 Total Enterobacteriaceae counts from overnight cultures and Real time PCR analysis of Campylobacter, Clostridium and Salmonella species in the ceca of birds

The counts (log CFU) of the total Enterobacteriaceae and *E. coli* in the ceca of broilers fed with the different dietary treatments are presented in Table 3. There was a significant difference ($p<0.019$) in the viable counts of total Enterobacteriaceae. Birds fed with the SPV1 diet had significantly higher counts of total Enterobacteriaceae compared to birds fed the control, SPV2 and SPV3 diets. Counts of total Enterobacteriaceae ranged from 2.060 to 4.571 log CFU/g of cecal content. There were no significant differences observed in the counts (log CFU/g) of *E. coli* in the ceca of birds fed with the different dietary treatments (Table 3). The counts of *E. coli* ranged from 1.220 to 3.175 log CFU/g of cecal contents. Levels of *Campylobacter* and *C. perfringens* detected via real time PCR are presented as ct values in Table 4. The average ct values of *Campylobacter* was 28.81 which corresponded to 281.36 CFU/g whilst the average ct values of *C. perfringens* in birds fed with different diets were above 30 (Table 4). There was a significant difference ($p<.001$) in ct values of *Campylobacter* in the ceca of birds fed with the different dietary treatments. Birds fed with the different SP diets had significantly higher ct values compared to those fed on the control diet. Feeding the SP diets to birds did not have any significant ($p=0.143$) effect on the ct values of *C. perfringen* (Table 4). The ct values of *C. perfringens* ranged between 30 and 31 (Table 4). The data for *Salmonella* is not presented here as the ct values were high. High ct values are indicative of low levels of bacterial cells which may be the case here. The cut off ct values for *Salmonella* and *Campylobacter* were 33 and the level of detection for these two pathogens were 1000 CFU/g cecal content, while the cut off ct value for *C. perfringens* was 32 with the detection limit of 100 CFU/g cecal content.

4.5.2 Growth performance of birds fed on the different SP diets

Birds fed on the control diet had higher weekly feed intake, than those fed on the SP diets (Table 5). These differences were significant (p<0.001). Body weights of birds fed on the control diet were significantly higher at the end of weeks 4, 5 and 6 compared to birds fed on the SP diets. Birds fed the SPV3 diet had higher weights compared to birds fed with the SPV1 and SPV2 diets. However, at the end of the experiment (week 6), birds fed with the SPV2 had higher end weight compared to those fed with the SPV1 and SPV3 diets. Birds fed on the control diet had a better feed conversion ratio (FCR) (p<0.001) during weeks 4 and 5 (p<0.001; Table5). During week 6, however, birds fed on the SPV1 and SPV2 diets had FCRs comparable to that of birds fed on the control diet. Birds fed with the SPV3 diet had a (p<0.001) higher FCR at the end of week 6, which was reflective of a lower weight gain during the final week (Table 5). Growth performances of birds were similar between the different SP diets in terms of overall end weights, weight gains, intakes and FCRs (Table 5).

4.5.3 Relative organs weights and small intestinal morphology

Significant differences were observed in relative weights of the liver (p=0.001) and pancreas (p=0.003) of birds fed on the different diets (Table 6). The relative liver weight in birds fed on the SPV2 diet was significantly higher compared to birds fed with the control, SPV1 and SPV3 diets. Weights of the duodenum, jejunum and the ileum were not significantly different in birds fed with the different diets (Table 6). However, the relative weights of the ceca were significantly different. The weight of the ceca in birds fed with the SPV1 diet was significantly higher compared to birds fed with the other diets.

In terms of relative length (cm/kg) of small intestinal segments, significant differences were observed in the length of duodenum (p=0.05), jejunum (p=0.027) and the ileum (p=0.05) in birds fed on the different diets (Table 4).

There were no significant differences ($p>0.112$) observed on the villus height in the jejunum of the birds. Birds fed on the SPV3, SPV1, control and the SPV2 diets had villi heights ranging from 1079, 1133, 1353 and 1437 µm respectively. Villus crypt depth, villus: crypt ratio and apparent surface area of birds fed with the control diet were significantly different ($p=0.058$) to those of the birds fed with the SPV3 diet (Table 7). The crypt depth of the birds fed with the control was significantly deeper (304 µm) to those of the birds fed with the SPV3 diet (126 µm). The lowest villus: crypt ratio (4.48) was observed in the birds fed on the control diet, which was significantly different to that of the birds fed on the SPV3 diet. The highest apparent surface area (0.261 mm^2) was observed in birds fed on the SPV2 diet and that was significantly different to those in the birds given the SPV1 and SPV3 diets. There were no significant differences observed on the villus height, villus crypt depth, villus: crypt ratio and apparent surface area from the ileum of birds fed on the different diets (Table 7). Ileal villi heights of the birds fed with the different diets ranged from 467.9 to 814.6 µm, whilst the crypt depth measurements ranged from 84 to 134 µm.

4.6 Discussion

The aim of the current study was to assess growth performance of broilers fed with the three PNG SP varieties with high total NSP contents and to measure the levels of *Campylobacter, C. perfringens* and *Salmonella* spp. at 42 days post hatch.

Overall production performance of monogastric animals are affected by the microbial load and type of microbes present in the gastrointestinal tract due to the type of diet and ingredient composition (Apajalahti and Kettunen, 2006). In the current study, the highest counts (log CFU/g) of total Enterobacteriaceae and *E. coli* were observed in the ceca of birds fed with the SPV1 diet. In the present study, the average detection level of *Campylobacter* was 281.36 CFU/g (ct=28.81) of cecal content and the ct value of *C. perfringens* was above 30 suggesting that this enteric pathogen may be present at a moderate level in the ceca of birds. In our earlier study (unpublished) we assessed the levels of these pathogens in the ceca of birds fed on the same SP varieties tested in the present study, and found that *C. perfringens* level was not significantly influenced by SP diets and that the level of *Campylobacter* and *Salmonella* spp. were high in the ceca of birds fed with the SP varieties with high total NSP content. A similar trend is observed here, however *Salmonella* levels were very low which could be due to the production of short chain fatty acids (SCFAs), specifically butyrate, as work by Takamine *et al.* (2005) shows that rats fed sweet potato dietary fibre had a 1.7 times higher concentration of butyrate. Studies with SCFAs have shown that the level of *Salmonella* was low in broilers fed diets with a number of different NSPs (Babu and Raybourne, 2008). SCFAs are known to have bacteriostatic effects which may have reduced the number of *Salmonella* (Van Immerseel *et al.*, 2003, Santos *et al.*, 2008). Levels of *C. perfringens* in the ceca of birds fed with SP diets seemed low and may relate to the less viscous nature of soluble NSP found in sweet potato and the use of sorghum as the main grain component in the present study. Inclusion of NSPs that are solubilised within the gut environment of birds are known to accentuate digesta viscosity and this is more profound with wheat and rye as these are viscous grains (Pan and Yu, 2014). Chickens can carry a high load of *Campylobacter* without clinical signs as this is part of the commensal microbiota in the gut of broilers, thus the use of SP varieties with high total NSP may have influenced *Campylobacter* levels. The ct values of *Campylobacter* were low in the control diets (281.36 CFU/g), while the treatment groups had high ct values of 30.12, 31.84 and 31.93. Mead (2002) stated that colonisation of *Campylobacter* in chicks could also be reduced by feeding fructo-oligosaccharides. However, whether sweet potatoes used in this study contain fruto-oligosaccharides which may have led to the slight reduction in the levels of *Campylobacter* requires investigation.

Overall performance of broilers observed in the present study would primarily be due to a lower intake of birds fed with the sweet potato diets which translated into low weight gains and end weights and is indicative of an imbalance in the levels of essential amino acids and may not be an impact of sweet potato as such as the diets were not isonitrogenous. The low intake may also be associated with the form of presentation of sweet potato to the birds, as feeding a mash diet could be stressful due to heat stress under humid tropical conditions. This is consistent with past work conducted under similar experimental conditions (Glatz, 2007 and 2013). FCR of birds fed with the SP diets in this study improved at the end of week 6 perhaps due to longer exposure and adaption to the diets and is similar to observations made by the aforementioned author.

The relative liver and pancreas weight observed in birds given the sweet potato diets in the current study is contradictory to those reported by Beckford and Bartlett (2015), Afolayan *et al.* (2012) and Agwunobi (1999). These authors did not observe significant differences in relative weight of the gizzard, hearts, liver and shank due to inclusion of sweet potato in the diet. The contradictory results from the current study, maybe due to the type of SP varieties used in the current study. Sweet potato roots when processed appropriately, can be included in the diets of poultry at 30 percent with no adverse effect on intake and FCR (Pandi *et al.*, 2016).

It is well established that NSPs in cereal grains and legumes directly or indirectly interacts with the gut and the microbiota present in the gut and can either protect against or enhance enteric infections (Montagne *et al.*, 2003). Alterations in the structure and function of the tight junction barrier or inhibition of the inflammatory cascade are some of the effects on the intestinal epithelium by the different enteric bacterial pathogens (Berkes et al., 2003). Shorter or reduced villi heights, deeper crypts and low villus: crypt ratios are some of the modifications observed in the intestinal morphology due to enteric infections which are associated with the presence of stresses such as microbial toxins (Choct, 2009, Ao and Choct, 2013). In the current study, the villi heights in the small intestine of birds fed on the three sweet potato varieties were not significantly different, however the villus: crypt ratio of the birds fed with the control diet were lower compared to the villus: crypt ratio in birds fed with the sweet potato diets. The levels of soluble NSP in the sweet potato varieties or the enteropathogens investigated here did not elucidate such effects on the gut morphology and digestive capacities of broilers indicating that the soluble NSP levels may have been low and that the insoluble fibre could have been well utilised by the cecal gut microbiota. Changes in the intestinal morphology such as shorter villi heights, deeper crypt depth and low villus: crypt ratios are associated with the presences of stresses such as microbial toxins (Choct, 2009, Ao and Choct, 2013). The previously mentioned changes were not observed in the ileum of the birds fed with the different sweet potato diets and may not be pathological as the levels of microbiota is plentiful and more diverse to that of the jejunum. Healthy chickens can harbour *C. perfringens* and counts from 0 to 10^5 CFU/g of intestinal contents are normal however presence of *C. perfringens* even at high numbers do not lead to necrotic enteritis (Timbermont *et al.*, 2011). The levels of *Clostridium perfringens* and the gut environment under the current experimental conditions may not have been favourable to induce necrotic enteritis.

4.7 Conclusion

Feeding sweet potato varieties with high total NSP content over a prolonged period is economically favourable as feed efficiency of birds improved over time. There were no significant effects of NSP contents in sweet potato on the small intestinal morphology of birds, signifying that any variety of sweet potatoe can be included in the diet of broiler chickens without compromising the gut digestive capacity and gut environment. Levels of *Salmonella* spp., and *Clostridium perfringens* were low with prolonged feeding of sweet potato in broiler chickens under the current conditions. Levels of *Campylobacter* spp were maginally reduced with sweet potato feeding over time. This reduction of *Campylobacter* in the ceca of broilers should be further investigated to study the underlying mechanism relating to this observed reduction in broilers fed with sweet potato under PNG conditions.

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Table 1 *Composition of the basal diet (g/kg)*

Ingredient	Basal
Sorghum	575
Soybean meal	320
Sunflower oil	60.0
Di-calcium phosphate	20.0
Limestone	11.0
DL-methionine	7.0
Vitamin and mineral premix	2.0
Salt	3.0
Choline chloride (60%)	2.0
Calculated analysis (% DM)	
Metabolisable energy (MJ/kg)	13.6
Crude protein	19.4
Crude fat	3.9
Crude fibre	2.4
Ash	3.5

Table 2 Description *of primers selected for Q-PCR reactions for the detection of Campylobacter, Clostridium and Salmonella species*

Bacteria	Target gene	Primer	Gene Sequence	Size (Bp)	Source
Campylobacter	16S rRNA	F	CAT TGG ATG AGC AAC TTA AAA TC	125	(Cheng and Griffiths, 2003)
		R	TTC TTC CAT TAA ACG GTT GA		
Clostridium perfringens	16SrRNA	CPerf165F	CGCATAACGTTGAAAGATGG	105	(Wise and Siragusa, 2005)
		CPerf269R	CCTTGGTAGGCCGTTACCC		
Salmonella spp	*invA*gene	F	CATTTCTATGTTCGTCATTCCATTACC	132	(Barletta *et al.*, 2013)
		R	AGGAAACGTTGAAAAACTGAGGATTCT		

Table 3 *Counts of total Enterobacteriaceae and E. coli in chickens fed the different diets*[1]

	Average colony counts					
	Control	SPV1	SPV2	SPV3	SEM	*P*-value
Bacterial spp (Log CFU/ml)						
Total Enterobacteriaceae spp	2.060[b]	4.571[a]	2.182[b]	2.742[b]	0.612	<.0.019
Escherichia coli	1.678[a]	3.175[a]	1.373[a]	1.220[a]	0.630	0.121

SPV1- Variety Waghi best, SPV2-Suga; SP3-Rachel; SEM=Standard error of means

Values within a row with different superscripts differ significantly at $P<0.05$.

Table 4 *Effects of the diets on the ct-values of Campylobacter, clostridium perfringens and Salmonella in broiler chickens at 42 days post hatch*

Tested pathogens	Average ct value				SEM	*P*-value
	Control	SPV1	SPV2	SPV3		
Campylobacter spp	28.81[c]	30.12[b]	31.84[ab]	31.93[a]	2.879	<.001
C.perfringens	30.74[a]	30.74[a]	30.91[a]	31.29[a]	1.256	0.143

SPV1- Variety Waghi best, SPV2-Suga; SP3-Rachel; SEM=Standard error of means

Values within a row with different superscripts differ significantly at $P<0.05$.

Table 5 *Effectsof diets on the weekly and overall performance of broilers at 42 days post hatch*

Performance	Dietary treatments				SEM	*P*-value
	Control	SPV1	SPV2	SPV3		
Weekly weights (g/bird)						
Week 3	886	884[a]	883[a]	898[a]	0.004	0.060
Week 4	1283[a]	1098[b]	1118[b]	1133[b]	0.018	<.001
Week 5	1726[a]	1409[b]	1398[b]	1456[b]	0.025	<.001
Week 6	2182[a]	1754[b]	1705[b]	1687[b]	0.032	<.001
Weekly gain (g/bird)						
Week 4	397[a]	214[b]	235[b]	235[b]	0.018	<.001
Week 5	443[a]	311[bc]	280[c]	323[b]	0.013	<.001
Week 6	455[a]	347[b]	307[b]	231[c]	0.022	<.001
Overall gain	1296[a]	871[b]	822[b]	789[b]	0.030	<.001
Weekly intake (g/bird)						
Week 4	692[a]	602[b]	632[b]	624[b]	0.020	0.024
Week 5	758[a]	633[c]	627[c]	692[b]	0.013	<.001
Week 6	1058[a]	791[b]	723[b]	798[b]	0.026	<.001
Overall intake	2512[a]	2026[b]	1982[b]	2114[b]	0.051	<.001
Weekly FCR (g/g)						
Week 4	1.768[b]	2.903[b]	2.724[b]	2.665[b]	0.156	<.001
Week 5	1.726[c]	2.043[b]	2.245[a]	2.139[ab]	0.064	<.001
Week 6	2.329[b]	2.285[b]	2.436[b]	4.017[a]	0.507	0.079
Overall FCR	1.942[c]	2.334[b]	2.413[b]	2.709[a]	0.098	<.001

SPV1- Variety Waghi best, SPV2-Suga; SP3-Rachel; SEM=Standard error of means

Values within a row with different superscripts differ significantly at $P<0.05$.

Table 6 *Effects of diets on the relative organ and intestinal segments of broiler chickens at 42 days post hatch*

Performance	Dietary treatments[1] Control[2]	SPV1	SPV2	SPV3	SEM	*P*-value
Relative organ weights (g/kg BW)						
Liver	23.81[b]	24.00[b]	26.58[a]	23.30[b]	0.596	0.001
Pancreas	1.70[b]	2.58[a]	2.41[a]	2.22[a]	0.167	0.003
Relative intestinal segment weights (g/kg BW)						
Gizzard	20.04[a]	21.72[a]	22.15[a]	20.81[a]	1.394	0.715
Duodenum	9.94	9.25[a]	9.79[a]	9.59[a]	0.378	0.763
Jejunum	15.95[a]	16.20[a]	17.09[a]	18.00[a]	0.822	0.289
Ileum	13.43[a]	14.74[a]	16.02[a]	14.83[a]	0.734	0.113
Ceca	5.69[b]	8.09[a]	6.95[ab]	6.14[b]	0.537	0.014
Relative lengths (cm/kg BW)						
Duodenum	18.59[ab]	17.53[b]	20.32[a]	18.44[ab]	0.710	0.055
Jejunum	46.24[a]	40.34[b]	45.72[a]	39.88[b]	1.871	0.027
Ileum	43.22[ab]	39.45[b]	45.46[a]	38.43[b]	2.011	0.055

SPV1- Variety Waghi best, SPV2-Suga; SP3-Rachel; SEM= Standard error of means

Values within a row with different superscripts differ significantly at *P*<0.05.

Table 7 *Effects of diets on the jejunal and ileal gut morphological traits of broilers at 42 day post hatch*

Parameters (µl)	Dietary treatments				SEM	*P*-value
	Control	SPV1	SPV2	SPV3		
Jejunum						
Villus height	1353^{a}	1133^{a}	1437^{a}	1078^{a}	178.3	0.112
Crypt depth	304.7^{a}	225.0^{ab}	246.2^{ab}	126.9^{b}	70.99	0.058
VH:CD	4.484^{b}	5.770^{ab}	6.035^{ab}	8.597^{a}	1.585	0.048
APA	0.220^{a}	0.111^{b}	0.261^{a}	0.072^{b}	0.048	0.003
Ileum						
Villus height	654.5^{a}	467.9^{a}	814.6^{a}	587.5^{a}	151.34	0.145
Crypt depth	126.8^{a}	84.00^{a}	134.7^{a}	116.2^{a}	31.52	0.269
VH:CD	5.133^{a}	5.868^{a}	6.110^{a}	5.172^{a}	1.109	0.697
APA	0.054^{a}	0.033^{a}	0.096^{a}	0.046^{a}	0.030	0.187

SPV1- Variety Waghi best, SPV2-Suga; SP3-Rachel; SEM=Standard error of means; APA=Aparent surface area; Values within a row with different superscripts differ significantly at $P<0.05$.

Chapter 5 General discussion and conclusions

Due to the excessive cost of commercially produced stock feed in PNG considerable research has been conducted into the use of non-conventional locally available ingredients to feed poultry and other monogastric livestock. In particular, the research has evaluated the use of sweet potato and cassava in least cost poultry diets produced by feed mills. This research has also recommended the use of sweet potato and cassava supplemented with a protein concentrate to finish broilers (Glatz 2007, Glatz 2013) as well as layer concentrates to feed commercial hybrids, PNG village chickens, and F1 layer crossbreeds (ACIAR 2016). However, the initial research conducted only assessed the growth rate of broilers and egg production of the laying genotypes. The mentioned research efforts did not assess whether the gut health of poultry was affected when birds were fed diets with these local feeds. Maintaining broiler gut health and intestinal integrity to prevent poor growth and mortality is crucial so that profits are maximised for smallholder poultry producers in PNG. Feeding experiments in PNG (Glatz 2007, Glatz 2013) using milled sweet potato included at 50 and 70 % with protein concentrates were associated with low feed intakes which was similar to other published works (Panigrahi et al. 1996, Maphosa et al. 2003, Afolayan et al. 2012). Despite reported decreases in feed intake, poor feed conversion efficiency and low weight gains using local feeds, no attempt has been made to determine why the performance of broilers was affected. Panigrahi et al. (1996) suggested that tuber utilization was affected by variable feed intake as a result of the high and variable water absorption capacity of tuber carbohydrates. Despite considerable research on the feeding value of sweet potato there is a lack of information on how dietary fibre, in particular total non-starch polysaccharide content of the different sweet potato cultivars and varieties can influence gut health and growth of broiler chickens. It is well known that diet composition and ingredients may affect the health of birds by influencing the internal gut environment and hence microbial status of the gastrointestinal tract of poultry (Apajalahti et al. 2004, Apajalahti and Kettunen 2006, Yegani and Korver 2008). The impact of selected local PNG sweet potato varieties with different total NSP content on the gut environment was examined in this thesis. These sweet potato varieties were used in diets for broiler chickens to investigate the effects of sweet potato NSPs on the AME values of the diets, growth performance, gut morphology, and detectable levels of enteropathogens such as *Campylobacter, Salmonella* and *C. perfringens*, in the ceca of broiler chickens. Inclusion of an exogenous enzyme product in sweet potato diets was also assessed to evaluate impact on growth and gut health of broilers.

5.1 Apparent Metabolisable Energy (AME) values and dry matter diet digestibility of diets as influenced by total NSP content.

Currently, there is limited information on the AME values of different PNG sweet potato varieties for use in poultry diets. Work by Glatz (2007) evaluated AME values of sweet potato and other local ingredients from PNG. However as mentioned earlier, this work was limited in that dietary fibre/non-starch polysaccharide contents were not evaluated. The AME value of feeds can be calculated by measuring the gross energy content of the feed and excreta. The feed industry over the last two decades has relied on AME as an estimate of available energy in feed ingredients (Sibbald 1980). In Chapter two of this thesis, a proof of concept study was conducted to evaluate the use of a commercial sweet potato flour (SPF) with a total dietary fibre content of less than 0.2 %. This product was mixed with other ingredients and fed to broilers. The AME of the SPF diet was 13.45 MJ/kg and the dry matter digestibility was 62.6 %. Inclusion of the Rovio T-flex enzyme did not improve the AME or the dry matter digestibility of the SPF diets. In Chapter three, thirteen sweet potato varieties from PNG were analysed for their total NSP content (Appendix 1). Five of the sweet potatoes which are currently considered as commercial varieties were fed to broiler chickens. These varieties had varying total NSP contents and considerable differences in their relative proportion of soluble and insoluble NSPs. These selected varieties were processed into sweet potato root meal and included at 25 % in a sorghum basal diet. These varieties identified with local names were Waghi besta, Gimane, Suga, Marasonda and Rachel and had total NSP content of 197, 162, 202, 190 and 210 g/kg DM respectively. In terms of their relative proportions of soluble and insoluble NSP contents, the lowest soluble NSP and highest insoluble NSP fractions were measured in Suga, while Gimane had the highest soluble NSP fraction and lowest insoluble NSP while Rachel had relatively minute differences between its soluble and insoluble fractions. A brief morphological description of the five varieties that were used in the present studies are provided in Appendix 2. The AME value of the sweet potato diets were significantly influenced by sweet potato variety and the inclusion of the Rovio T-flex enzyme. Significant interactions between the sweet potato varieties and the enzyme on both the AME and the dry matter digestibility of the diets were observed. The variety called Waghi besta had the highest AME value of 15.65 MJ/kg DM and was significantly higher by 10.73 % compared to the control diet and by 28.31, 14.4, 11.82 and 12.91% compared to Gimane, Suga, Marasonda and Rachel respectively. The variety called Gimane had the lowest AME value of 11.22 MJ/kg DM. which was improved with enzyme supplementation (13.55 MJ/kg DM). Varieties with a high total NSP content with a high or well-balanced insoluble fraction relative to the soluble fraction had high AME values that were well within the recommendations to finish broiler chickens (Aviagen 2014). However, the inclusion of an enzyme product (except for the Gimane variety) to these diets did not result in the improvement of both the AME content and the diet digestibility.

The variation in AME observed for the PNG varieties could be due to the type of varieties used and place of origin, as previously reported by Banser et al. (2000) for African varieties and by Ravindran et al. (1995) for Sri Lankan cultivars. The dry matter digestibility of these diets (reported in Chapters three) were above 50 % and ranged from 65 to 79 %. Despite the type of sweet potato product (SPF and sweet potato meal), the dry matter digestibility of the sweet potato diets was high and comparable to the sorghum diet. This is in agreement with Dominguez (1992) who found sweet potato roots are more digestible when processed compared to uncooked or raw starch which is resistant to hydrolysis by amylase. Another factor contributing to the high dry matter digestibility of these sweet potato diets would be low levels of trypsin inhibitors as these are destroyed by processing and or due to the type of cultivar/variety used in this study (Bradbury and Holloway 1988, Ravindran, Ravindran et al. 1995, Zhang and Corke 2001).

5.2 Growth performance, intake and FCR of birds as influenced by NSP content in sweet potato diets

An evaluation of the growth performance of broilers fed with the sweet potato diets was one of the objectives of the research conducted in this thesis. The production performance of broiler chickens can be measured by several parameters. Feed intake, feed conversion ratio (FCR), body weight gains and time to reach market weight are such measurements used to assess the productivity of the flock. FCR is calculated as the ratio of feed intake to body weight gain and reflects how efficient a bird is able to utilise the diets for growth. Digestibility of diets, growing and management conditions and health of birds are some of the major factors that can influence FCR of birds under PNG climatic and environmental conditions.

In Chapter two of this thesis, the intake of birds fed with the SPF diets were higher, however, weight gains and FCRs were lower compared to the control diet. In Chapter three, a similar finding was observed; birds fed with the different sweet potato diets had lower weight gains compared to the control diet. However, intakes of birds fed the different sweet potato diets were variable. Diets using different varieties of sweet potato with high totoal NSP contents (Waghi besta, Suga and Rachel) and the effect of these varieties on growth over a prolonged period were reported in Chapter four of this thesis. These diets were fed to birds from 21-42 days of age. Birds fed with the sweet potato diets also had lower weekly end weights, weight gains, intakes and high FCRs. Overall FCR of birds fed with the sweet potato diets improved over the experimental period and ranged from 2.33 to 2.71. Other researchers suggested that diet palatability, poor diet digestibility, laxative effects due to high soluble sugar content and unidentified nutritive factors as issues affecting intake in broilers fed with varying levels of sweet potato (Banser, Fomunyam et al. 2000, Maphosa, Gunduza et al. 2003, Ayuk 2004, Afolayan, Dafwang et al. 2012) which affected FCRs. Contrary, to those studies, digestibility of diets was measured in this thesis and shows that the digestibility of sweet potato diets is high though intake was low in birds fed with sweet potato diets presented both as pellets (Chapter three) and as mash (Chapter 4). One interesting factor associated with feeding sweet potato in broilers during the finishing stages is that there is low or no mortalities. Low or no mortalities were observed in birds fed with varying levels of sweet potato (Agwunobi 1999, Banser, Fomunyam et al. 2000, Ayuk 2004, Afolayan, Dafwang et al. 2012). This trend was also observed in this thesis where there were no mortalities in Chapters two and three whilst there was a 0.5 % mortality observed in Chapter four. Maphosa, Gunduza et al. (2003) observed increased mortalities with increasing sweet potato and associated that to gastrointestinal disorders caused by soluble sugar contents in sweet potato diets. This is an important aspect of feeding sweet potato to broilers and this thesis show that despite low intakes associated with feeding sweet potato with varying levels of total NSP content, the FCRs of birds improved overtime and this could be associated with a stable gut microbiota with age and may not be an effect of sweet potato per se.

The question that needs to be asked is whether the body weight gains and FCR of birds fed with the sweet potato diets were negatively correlated to the levels of enteropathogens present in the ceca of birds raised under PNG conditions. It is plausible to suggest that the levels of these enteropathogens may have influenced growth of broilers fed with the sweet potato diets. Diet is the greatest determinant of gut microbiota composition which is in turn influenced by the dietary ingredients, nutrient levels, physical structure and processing amongst other factors (Torok et al. 2011). Previous research has reported that birds with poor FCRs contain microbes that negatively influence their nutrient intakes (Torok, Hughes et al. 2011, Stanley et al. 2012, Stanley et al. 2013). The performance of broiler chickens especially the FCR of birds fed with sweet potato diets improved over time which is consistent with findings reported by (Glatz 2013) under PNG conditions. The FCRs values of greater than 2 for birds fed a commercial broiler finisher feed and best bet feeding options using sweet potato mixed with a protein concentrate (Moat 2000, Glatz 2007, Glatz 2012, Glatz 2013) is similar to the results reported in this thesis. There is however limited data enabling comparison of the levels of Enterobacteria, *E. coli*, *Salmonella*, *Campylobacter* or *C. perfringens* observed from the studies reported here with sweet potato diets associated with reduced performance in broiler chickens.

It is also possible that the poor production results reported in Chapter four of this thesis could primarily be due to lower intakes in birds fed with the sweet potato diets which translated into low weight gains and end weights. This lower feed intake could be associated with feeding mash as well as heat stress from high humidity and environmental temperatures. This is consistent with past work conducted under similar experimental conditions (Glatz, 2007, Glatz, 2013).

5.3 Relative weights, gut morphology and digestive enzyme activities as influenced by NSP content in sweet potato diets

5.3.1 Relative organ and intestinal segment weights

Inclusion of sweet potato with varying levels of NSP could influence the levels of enteric pathogens which in turn could affect the digestive capacity of broilers in terms of relative weights of digestive organs, gut morphology and digestive enzyme activities. However, in this study the inclusion of sweet potato in the diet of broilers did not negatively impact on the relative organ weights, gut morphological traits and digestive enzyme activities in the small intestinal segments of broilers. In Chapter two of this thesis, SPF diets did not significantly impact on the relative weights of the pancreas and the duodenum, however, the liver, jejunum and the ileum were heavier in birds fed with these diets. In chapter three, the relative weights of the mentioned traits were not significantly influenced despite the sweet potato varieties having varying level of total NSP content. In Chapter four of this thesis, the relative weights of the small intestinal segment were not influenced by the high total NSP content found in the sweet potato diets, however, relative weights of the liver, pancreas and the ceca were significantly higher in birds fed on the sweet potato diets. The heavier pancreas and liver weights observed in Chapters two and four could be due to the lower body weight of birds fed with sweet potato diets.

5.3.2 Relative intestinal segment weights

Length of the small intestine in broilers is also influenced by dietary ingredients and additives such as antibiotics and enzymes (Engberg et al. 2004, Lázaro et al. 2004, Miles et al. 2006). According to findings in Chapter four, the relative lengths of the individual segments of the small intestines were significantly influenced. The intestinal segments were significantly longer in the birds fed on the SPV3 (Suga) diet compared to those fed on the other sweet potato diets (SPV1-Waghi besta and SPV5-Rachel). Heavier relative weights of the small intestinal segments may be indicative of the considerable differences in the soluble and insoluble fractions in Suga as this variety has the highest insoluble NSP and lowest soluble NSPs leading to a a larger gizzard and liver weights. The larger gizzard and liver weights could be linked to a larger gut capacity due to an increased secretion of digestive amylase and bile salts and prolonged exposure to digestive enzymes in the upper gut and may also be at least in part due to levels of dietary NSP (Hetland and Svihus 2001, Hetland et al. 2005).

5.3.3 Small intestinal morphology

The intestinal mucosal structures such as villi height and crypt depths can reveal useful information on the intestinal function and absorption of available nutrients (Caspary 1992). Nutrient assimilation is affected by villi height as a decrease in villi height means less surface area for absorption and lower nutrient uptake which may ultimately compromise growth, health and welfare of birds (Choct 2009). Villus height, crypt depth and villus: crypt ratio in the jejunum were not significant whilst the villus height in the ileum was lower with enzyme inclusion (Chapter two). The same result was observed for villus height, crypt depth and villus: crypt ratio in the jejunum of birds fed with different sweet potato diets in Chapter three. Generally, there were no significant effects of NSP content in sweet potato on the morphological traits of the small intestine of broilers. Villi height, crypt depths and villus: crypt ratios of birds fed with these two diets were not reduced or deepened. In chapter four, it was found that the villi heights in the jejunum and the ileum of birds fed on the three sweet potato varieties were not significantly different, however the villus: crypt ratios in the jejunum of birds fed with the control diet were lower compared to the villus: crypt ratios in birds fed with the sweet potato diets. It is unclear as to why there was a reduction in these mentioned traits in the jejunum of the birds fed with the control diet. It cannot be linked with a pathogen such as *C. perfringens* because the levels of *C. perfringens* were marginal.

5.3.4 Digestive enzyme activities

Digestive enzymes determine the amount of nutrients available for absorption and are thereby closely associated with nutrient assimilation and absorption in the gut (Iji et al. 2001). Regional activity of mucosal enzymes is related to the digestive capacity in the three different regions of the small intestine (Uni 2006). The activities of these disaccharidases may be affected by the characteristics of the diets and available substrate (Uni and Ferket 2004). There is currently limited data available to date on how these digestive enzyme (maltase and sucrase) activities are influenced by the inclusion of sweet potato in broiler diets. In chapter two of this thesis, tissue protein content and the digestive enzyme activities of the jejunum were studied in birds fed with the SPF diet. The jejunal protein content of birds fed with the SPF diet + enzyme was not significant and there was no significant interaction between SPF and enzymes. The activities of maltase and sucrase were not significant in birds fed with the SPF diets however the inclusion of enzymes significantly influenced the activity of maltase in the jejunum of birds. This observation is contradictory to the findings by Shakouri et al. (2009), as these authors reported that the activities of maltase and sucrase were not affected by supplemental enzymes and could be indicative of the different enzyme product used here. The use of SPF in diets for broilers did not significantly influence brush border enzyme activities in the jejunum of broilers in the present studies.

The levels of soluble NSP in the sweet potato varieties and the levels of enteropathogens did not negatively influence the gut morphology and digestive capacities of broilers indicating that soluble NSP levels contained in sweet potato may have been low compared to the insoluble fractions and that the insoluble fibre could have been well utilised by the ceca gut microbiota. One significant finding is that, birds fed with the variety called Suga had longer villi, deeper crypt depth and lower villi crypt ratio in the jejunum and ileum of birds fed this variety (Chapter three and four). In addition to improved gut capacity, other performance parameters such as weekly weight and feed efficiency, and load of pathogens were better in birds fed this variety (Suga) compared to birds fed with the other sweet potato varieties. This sweet potato variety has the highest insoluble NSP and lowest soluble NSPs. Inclusion of insoluble NSPs at moderate levels in poultry diets have been associated with improved starch digestion, increased intakes associated to a larger gut capacity triggered by increased bulk digesta and faster passage through the gut (Hetland and Svihus 2001; Hetland et al. 2002).

It is well established that NSPs in cereal grains and legumes directly or indirectly interact with the microbiota present in the gut and can either protect against or enhance enteric infections (Montagne et al. 2003). Generally, soluble fibre increases intestinal transit time, delays gastric emptying, delays glucose absorption, increases pancreatic secretion, and slows absorption, where as insoluble fibre decreases transit time, enhances water holding capacity and assists faecal bulking in monogastric animals (Annison and Choct 1991, Choct et al. 1992, Choct et al. 1995, Choct et al. 1996, Choct 2002). The levels of soluble NSP in the sweet potato varieties tested did not elucidate such effects on the gut morphology and digestive capacities of broilers indicating that the soluble NSP levels may have been low and that the insoluble fibre could have been well utilised by the ceca gut microbiota.

5.4 Levels of *Campylobacter, Salmonella* and, *C. perfringens* in the ceca of broilers fed sweet potato diets

Currently, there is limited information in the literature on the incidence of necrotic enteritis (NE) or shedding of *C. perfringens*, *Campylobacter* and *Salmonella* in commercial broiler chickens farmed by smallholders in PNG destined for the live chicken markets. Zoonotic bacteria are those that can cause infections in humans. *Campylobacter, Salmonella* and *C. perfringens* are the most prevalent pathogens derived from poultry that infect humans through foodborne illnesses (Donoghue et al. 2006). *C. perfringens* can negatively affect health of a bird while the other two pathogens can be part of the commensal gut microbiota without causing any clinical signs. The relationship of these zoonotic bacteria with the diet being offered to broiler chickens is vital to reduce bacterial contamination in the end product. Feeding sweet potato to poultry has been reported by numerous researchers; however, research on sweet potato dietary fibre and how it influences the microbiota in chickens are limited.

5.4.1 Viable counts of Total Enterobacteriaceae and Escherichia coli

Salmonella, Escherichia coli, Yersinia pestis, Klebsiella and *Shigella,* are a few of the bacteria belonging to the Enterobacteriaceae family. Overall, food quality and sanitary processing conditions can be judged by levels of coliforms, Enterobacteriaceae and *Escherichia coli* populations (Kornacki and Johnson 2001). Enterobacteriaceae and Enterococci are dominant in the ceca (Mead and Adams 1975) and viable counts of these bacteria decrease in the ceca as broilers grow older, but this decrease seems to be dependent on the type of diet fed to broilers (van der Wielen et al. 2000). In Chapter two, it was found that that the viable counts of the total Enterobacteriaceae and *E. coli* were high in broilers fed on the SPF diets. The same observation was made in Chapter three when birds were fed on the sweet potato root meal diets with varying NSP contents. With prolonged feeding, viable counts of the total Enterobacteriaceae and *E. coli*, were low compared to the levels that were reported in Chapter two in birds fed with the Suga and Rachel varieties. There is limited information on how NSPs in sweet potato affect microbiota *in vivo* in chickens, however it is known that gut microbiota in the ceca of birds, ferment NSPs leading to the production of short chain fatty acids. These short chain fatty acids can inhibit the growth of bacterial species of the Enterobacteriaceae family (van der Wielen, Biesterveld et al. 2000). It can be speculated that the NSP content in sweet potato diets were fermented in the ceca of birds fed with these diets, leading to the production of short chain fatty acids which could have led to the decrease in the viable counts of the Enterobacteriaceae in the ceca of birds (Chapter four).

5.4.2. Salmonella levels in broilers fed with sweet potato diets

In Chapter three it was observed that feeding sweet potato with varying total NSP contents with and without enzymes did not influence the levels of *Salmonella* in broilers. There was however a significant interaction between the enzyme supplementation and the SP varieties. Birds fed on the diet containing the Rachel variety, which has the highest level of soluble NSP had high levels of *Salmonella*. Inclusion of Rovio T-flex to this diet reduced the levels of these enteropathogens in the ceca of broilers fed with this variety. In chapter four, the levels of *Salmonella* were low both in the control and the sweet potato diets with prolonged feeding in broiler chickens. *Salmonella* invasive capacity is regulated by the *Salmonella* pathogenicity island 1, *hil*A and this was down regulated by butyrate in the ceca of birds (Van Immerseel et al. 2003, van Immerseel et al. 2005, Van Immerseel et al. 2006). The majority of the cecal microbiota belong to the Clostridiaceae which are butyrate producers (Teirlynck et al. 2009). Hydrolysis of the NSPs by exogenous enzymes or by other gut microbiota will lead to the production of short chain fatty acids (SCFA) which are known to have bacteriostatic effects on *Salmonella* (Van Immerseel, De Buck et al. 2003, Santos et al. 2008). In chapter three and four of this thesis, the numbers of *C. perfringens* were high compared to levels of *Salmonella* in the ceca of broiler birds fed with sweet potato diets, and is therefore a plausible explanation for the low levels of *Salmonella* observed herein. One study with sweet potato fibre in rats has shown that the butyrate concentration in rats fed with the sweet potato dietary fibre diet was 1.7 times more than that of the rats fed with the control diet (Takamine et al. 2005). Soluble fibre in a local Indonesian sweet potato variety called Bestak was shown to play a role in the mucosal immune response of rats infected with *Salmonella typhimurium* by increasing sIgA (Nurliyani et al. 2015). A similar observation was made by Parsons et al. (2014) with NSP from an edible plant source which were able to inhibit the invasion of *Salmonella typhimurium* across the chicken intestine.

5.4.3 Campylobacter levels in broilers fed with sweet potato diets

Feeding of sweet potato to broilers influenced the load of *Campylobacter* in the ceca of birds (Chapter three and four). The lowest level of *Campylobacter* was observed in birds fed with the Suga variety (1.271 log CFU/g) which has a larger proportion of the insoluble NSPs whilst the highest level was observed in birds fed with the Rachel variety (5.831 log CFU/g) which has the largest proportion of soluble NSPs. This finding is significant in that the proportions of insoluble and soluble NSPs in sweet potatoes can impact on the shedding of *Campylobacter* in broiler chickens. Inclusion of enzymes in the sweet potato diets reduced the levels of this enteric pathogen. Although there is limited data available to compare levels of *Campylobacter* species in monogastric livestock fed with sweet potato diets, earlier studies have shown a reduction in the numbers of *Campylobacter* in the ceca of broilers fed with a wheat based diet and NSP degrading enzymes (Fernandez et al. 2000, Molnár et al. 2015). Colonisation of *Campylobacter* in chicks could also be reduced by feeding fructo-oligosaccharides, which are considered as prebiotics (Mead 2002). Levels of this pathogen were marginally reduced when broilers were fed with sweet potato over a prolonged period (Chapter four). *Campylobacter* is commonly carried in the chicken gut asymptomatically. Some sources of infection for *Campylobacter* in broiler chickens raised on smallholder farms in PNG could be from contaminated water (due to lack of chlorination) and management and husbandry practices.

5.4.4 *Clostridium perfringens in broilers fed sweet potato diets*

The numbers of *Clostridium perfringens* in the sweet potato diets were comparable to that of the birds fed on the control diet and were not influenced by the inclusion of the enzyme (Chapter three). *C. perfringens* was highest in birds fed with the Gimane variety. This variety had a low total NSP content but with higher proportions of soluble NSP content (Chapter three). In Chapter four, the levels of this enteric pathogen were similar across the control and the diets containing Waghi besta, Suga and Rachel varieties (30.74, 30.74, 30.91 and 31.29). Other studies have linked high incidences of necrotic enteritis to the hydrolysis of soluble NSP in the gut of broilers which caused high gut viscosity that led to a slower digesta transit time and proliferation of this pathogen (Annison and Choct 1991, Choct, Annison et al. 1992, Choct, Hughes et al. 1995, Choct, Hughes et al. 1996, Choct 2002). In contrast to those studies, levels of *C. perfringens* in broilers in this study were comparable to the control with slight variation in numbers across the sweet potato diets. Enzyme inclusion did not reduce *C. perfringens* levels. This observation may be indicative of the different composition and type of soluble NSPs present in these sweet potato varieties, higher levels of insoluble NSPs and diets being highly digestible. However, there is limited data available on the potential influence of soluble NSPs in sweet potatoes and their impact on gut viscosity in broilers. In the present studies, given that the numbers of *C. perfringens* were low in the gut of broilers fed with the sweet potato diets the likelihood of the risk of necrotic enteritis is low.

5.5 Potential use of exogenous enzymes with sweet potato meals for broiler diets

Use of feed enzymes in the poultry feed industry in the last decade was aimed at increasing the feeding value of raw feed materials, reducing the variation in nutrient quality of ingredients and thereby lower feed manufacturing costs and promoting the uniformity within and between flocks respectively (Bedford 2000, Choct 2006). The use of exogenous enzymes aids the hydrolysis of soluble NSPs leading to reduced gut viscosity in poultry, which in turn enables other nutrients to be readily available for absorption (Williams et al. 1997). As stated in Chapter one of the thesis, to date, the work conducted by Nunes et al. (2010) is the only published data on the use of exogenous feed enzymes with sweet potato. The objective of including enzymes in this thesis was to assess if enzymes elucidate influences on previously mentioned parameters and gut traits. In chapter two of this thesis I showed that the inclusion of Rovio T-flex in SPF which is a product low in dietary fibre did not influence AME and diet digestibility of sweet potato diets. Bird performance, gut morphological traits and total viable counts of Enterobacteriaceae, Lactic acid bacteria and *E. coli* were not influenced by the inclusion of this enzyme. However, activities of the brush border enzymes were affected. In chapter three, the AME values and diet digestibility were significantly influenced by the addition of Rovio T-flex. Addition of this enzyme, increased intakes of birds fed the control diet but this enzyme did not significantly increase feed intake or other growth parameters and gut morphological traits in birds fed on the diets containing sweet potato. There was however a significant interaction between the sweet potato and enzymes on the levels of *Campylobacter* and *Salmonella* species in the ceca of broilers. Inclusion in this study of the Rovio T-flex which is an enzyme product containing endo-1, 4 beta-xylanase, endo-1, 3 9 (4) beta glucanase did elucidate some influence in the sweet potatoes offered to broilers. Generally, selection of a sweet potato variety for use in broilers diets should be based not only on the total NSP content, but in particular, the proportion and types of insoluble and soluble NSPs in varieties with high total NSP contents as there is a minimal need to include enzymes to improve NSP digestibility. However, levels of *Campylobacter* were high in the ceca of birds fed with Waghi besta, Marasonda and Rachel varieties whilst the level of *Salmonella* was high in the birds offered the diet with Rachel. These sweet potato varieties have a high total NSP contents as well as a fair proportion of soluble NSPs (Chapter three). The levels of these two enteropathogens can be influenced with prolonged feeding without the need to include enzymes (Chapter four).

5.6 Conclusion

Sweet potatoes originating from PNG have an average total NSP content of 172 g/kg DM and range from 109 to 211 g/kg DM whilst the proportion of the total soluble NSP range from 42 to 105 g/kg DM. Only two out of the thirteen varieties analysed had higher proportions of soluble compared to insoluble NSPs. AMEs and diet digestibility are influenced by the total NSP content. The sweet potato variety with a low total NSP had a lower AME value which was improved with enzyme inclusion. AME and dry matter digestibility of the diets are not negatively influenced with sweet potato diets, however diets must be thoroughly balanced for dietary protein contents and amino acid levels. Growth performance of broiler chickens in terms of weight gain and feed intake are low and feed conversion ratios are high in sweet potato diets despite these diets being highly digestible. There is a need to comparatively assess levels of Enterobacteria, *E. coli*, *Salmonella*, *Campylobacter* and *C. perfringens* observed from the studies here to determine if there is a relationship between reduced performance and feeding of sweet potato. Digestive capacity of broilers in terms of the villi height and villi: crypt ratio in the jejunum and the ileum were not negatively influenced with sweet potato feeding, signifying that sweet potato despite varying NSP contents do not impact on broiler gut morphology. Varieties with high total NSP contents had high levels of enteric pathogens such as *Campylobacter* and *Salmonella* while the variety with a low total NSP content had a high level of *C. perfringens.* Birds fed with the diet containing the Rachel variety had high loads of *Campylobacter* and *Salmonella* species while the highest load of *C. perfringens* were observed in birds fed with the diet containing the Gimane variety. One significant finding is that, birds given the sweet potato variety with a high insoluble NSP and low soluble NSP fractions had better growth, enhanced gut capacity, low viable counts of Enterobacteriaceae and low levels of *Campylobacter, Salmonella* and *C. perfringens.* The levels of *Campylobacter* were also influenced with prolonged feeding of sweet potato diets as the levels were marginally reduced. Sweet potato diets could be a least cost option for reducing shedding of *Campylobacter* in broiler chickens by continuous feeding, however this observation needs further validation. Levels of *C. perfringens* were stable with sweet potato feeding thereby reducing the risk of necrotic enteritis in broilers. Despite variable loads of these enteropathogens in broiler chickens fed with sweet potato over time, there is still a high risk of product contamination and campylobacteriosis in family members of smallholder poultry farmers. There is currently a lack of surveillance studies for these pathogens in PNG and as such more needs to be done in that area. Appropriate interventions for reducing the load of enteric pathogens using local resources as poultry feed must be disseminated along with best bet feeding options to smallholder farmers to encourage hygienic and biosafety practices at the farm level. This information could enhance farmer knowledge and upskill farmers with the knowledge on how to protect themselves from enteric infections and reduce contamination of poultry products processed on site and ultimately contribute to promoting food safety issues in the informal live broiler chicken industry of PNG. Strategies to reduce product contamination with enteropathogens in the live broiler chicken industry which involves the 50 000 plus smallholder producers of PNG must be taken into consideration when developing food safety regulations, programs and policies in PNG.

5.7 Recommendations/Future work

Data generated from this study should be used as baseline information for future research in village poultry production systems in Papua New Guinea in terms of production parameters and managing bird health via nutritional feeding strategies. Further work is needed to disseminate and adopt alternative feeding strategies for different poultry species farmed under different farmer production and management systems in PNG. Record keeping and biosafety and biosecurity issues and protocols and procedures are needed to be put in place for smallholder poultry entrepreneurs to prevent poultry product contamination both at farm gate and with end-users of these products supplied via the informal broiler live chicken markets.

Some of the most relevant research works warranted for further investigations with sweet potato in the context of PNG based on economically viable scenarios focusing on small-scale producers are;

- In depth, histological studies on gut secretions and immunity associated with feeding sweet potatoes such as the secretion of mucin and brush border permeability because mucin is the major component of the mucous layer and apart from protecting the gut, it is also involved in filtering nutrients and influences nutrient digestion and absorption
- The best and practical form of presentation of sweet potato to birds under tropical humid environments and profiling of enteropathogens to study if that could improve feed intake and feed efficiency as the current form of milled sweet potato is associated with low intake
- In depth assessment of shedding of *Salmonella* in the different laying genotypes (PNG native chickens, cross bred F1 layer genotypes and ducks) and on egg surfaces from hens fed with sweet potato sold via informal markets. Currently there is a lack of surveillance and prevalence data of this pathogen in backyard and smallholder settings in PNG
- Prevalence of food borne and other relevant pathogens in poultry and other monogastric livestock farmed under smallholder management and farming practices due to a lack of surveillance studies for these pathogens in PNG
- In depth, molecular work to confirm reductions of *Campylobacter* in the gut of broiler chickens fed with sweet potato. The levels of *Campylobacter* may have been high due to other factors such as infections from water source and not sweet potato per se; therefore, further studies must be conducted for broilers on smallholder farms in PNG
- Further studies on the levels of beneficial strains of bacteria such as Lactic acid bacteria in broilers should be undertaken to assess if their levels are influenced with sweet potato as these bacteria have antagonistic activity against pathogenic bacteria. Feeding of

sweet potato has been associated with Bifidobacteria and sweet potato could be a prebiotic source in localities where SP is grown such as PNG

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Appendices

Appendix 1 Contents of non-starch polysaccharides of 13 sweet potato varieties from PNG

Sugar composition (g/kg DM)	Sweet potato varieties												
	Wanmun	Trimun	Marasonda	Gimane	Rachel	Waghi	Ston	Kerot	Suga	Korowes	SI 85	B11	L43
Arabinose	10.45	10.82	8.53	13.21	14.89	16.37	15.44	6.63	17.86	5.73	17.73	7.39	5.92
Xylose	-	-	-	-	-	0.86	1.45	-	-	-	-	-	-
Galactose	16.69	12.81	2.21	10.09	25.23	14.94	26.56	20.33	-	7.70	19.35	24.67	22.27
Glucose	14.98	23.30	68.80	70.77	65.35	34.21	42.69	16.00	46.35	30.41	56.55	26.00	43.22
Mannose	-	2.26	1.75	-	-	2.28	-	-	-	-	-	-	-
Soluble NSP	42.12	49.19	81.29	94.06	105.5	68.66	86.14	42.97	64.21	43.84	93.63	58.06	71.41
Xylose	9.71	8.83	8.66	7.10	5.93	11.43	9.27	6.99	6.58	6.08	7.39	7.08	7.45
Galactose	18.66	13.36	11.26	-	9.66	15.67	10.90	12.68	10.13	-	8.89	-	16.90
Glucose	66.01	59.79	76.18	50.76	77.52	83.45	93.92	34.54	108.3	41.50	101.5	62.51	85.96
Mannose	-	-	-	-	-	-	-	-	-	-	-	-	-
Insoluble NSPs	113.6	98.73	109.2	67.82	104.0	127.9	129.5	65.11	137.5	57.72	126.2	79.00	119.9
Total NSP	155.7	147.9	190.5	161.9	209.4	196.5	215.6	108.1	201.7	101.6	219.8	137.1	191.3

Appendix 2. Brief description of the five sweet potato varieties used in the diets in Chapters 2 and 3 of this thesis

Local name	**Root skin colour**	**Root flesh colour**	**Root shape[1]**
Marasonda	Brownish orange	Cream	Long oblong
Gimane	Cream	Pale yellow	Long oblong
Waghi Besta	Cream	Pale yellow	Elliptic
Rachael	Pink	White	Round elliptic,
Banz Suga	Creamy white	White	Elliptic

1http://keys.lucidcentral.org/keys/sweetpotato/key/Sweetpotato%20Diagnotes/Media/Html/TheCrop/AboutTheCrop/Botany/bot%20st%20rt%20shape.htm

Appendix 3. Images of the five sweet potato varieties used in diets descrised in Chapters three and four

Rachel variety

Waghi besta variety

Gimane variety

Marasonda variety

Suga variety

Appendix 4. Copies of supporting publications

4.1 Evaluation of the performance of broiler chickens fed diets containing different proportions of cassava or sweet potato in an on-station trial in Papua New Guinea

Evaluation of the performance of broiler chickens fed diets containing different proportions of cassava or sweetpotato in an on-station trial in Papua New Guinea

Janet Pandi[1]* *and Phil Glatz*[2]

Abstract

Currently, all commercial stock feeds in Papua New Guinea (PNG) are manufactured using imported grains, and research endeavours are focused on investigating ways of substituting such grains with local resources. The objective of this experiment was to evaluate the performance of broilers fed cassava or sweetpotato blended in different proportions with a high- or a low-energy protein concentrate. Ross 308 chickens were fed either milled cassava or sweetpotato at dietary levels of 0%, 50% and 70%, combined with the corresponding concentrate. The birds were fed a commercial broiler starter diet from hatch to day 20, and experimental diets were introduced at day 21. Body weight gain and feed conversion ratio (FCR) of birds fed the control (0%) diet were significantly superior only in the first 2 weeks. In weeks 6 and 7, birds fed the 50% cassava (CAS50H) and the 50% sweetpotato (SP50L) diets had higher gains and better FCRs compared to the control diet. During the experiment the environmental temperature ranged between 24°C and 31°C and humidity ranged from 75% to 80%; during the latter stages of the trial birds fed the control diets reached a high body weight and were unable to cope with the high temperature and humidity. The FCR of the birds on the test diets improved significantly over time and they reached a market weight of 2 kg by day 49, which is economical for PNG village farmers. It is suggested that 50% cassava and sweetpotato, blended with their respective energy concentrates, can be used to finish off broilers without any deleterious effects on the birds. Greater utilisation of root crops such as cassava and sweetpotato in livestock feed will not only add value to these crops but also improve profit margins for famers and diversify income earning opportunities.

INTRODUCTION

Papua New Guinea (PNG), like other developing nations in the Pacific region, imports large quantities of grains such as sorghum and wheat for the manufacture of commercial livestock feed, especially for the pig and poultry industries. This means that retail prices of feed produced by local commercial feed companies are high, and are considered a constraint by smallholder poultry and pig producers. Based on global trends, in the future there may not be sufficient amounts of feed ingredients such as maize, soybean meal, fishmeal and meat meal to make enough feed to meet the demand from semi-commercial farmers and traditional smallholder farmers (Ravindran 2012).

Demand for poultry products is increasing in PNG, largely due to the many oil and gas development projects in the country. To meet this demand via the village production system, alternative feeding strategies must be found. Farrell (2005) suggested matching poultry production in developing countries with the available feed resources as a way forward to combat rising feed prices. In addition, using poultry production performance indicators (such as feed conversion efficiency and growth rates) from developed countries

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as a benchmark for poultry in developing nations should be avoided as these will be unattainable.

The three major criteria determining the regular use of non-conventional feed resources in poultry diets are: availability of the resources in sufficient quantities; their nutritional value, which must be suitable for poultry; and price, which must be competitive compared to traditional feeds (Ravindran 2012). One of the strategies employed in PNG in the last decade has been to blend a protein concentrate mix with local feed ingredients, such as cassava or sweetpotato, to finish off broiler birds. Different feeding strategies have been trialled, and depending on cost and growth rates, the best options have been recommended for smallholder broiler farmers. The data presented here are from one of these trials. The objective of this experiment was to assess the performance of broiler chickens when fed diets comprising different proportions of cassava and sweetpotato mixed with high- and low-energy protein concentrates, respectively.

MATERIALS AND METHODS

Location

The study was conducted at the National Agricultural Research Institute (NARI)'s Livestock Research Station at Labu (6°40′27″ S, 146°54′33″ E), which is located about 16 km north of the city of Lae. The climate is typically warm and wet with an average daily temperature of about 30°C and 84% relative humidity.

Experimental diets

Four test diets were produced on-site at the same time. The control diet, which was the commercial finisher pellet from New Guinea Table Birds (NGTB), was purchased around the same time from a retail outlet.

The cassava and sweetpotato roots were dried and milled prior to blending with their respective concentrates. The fresh clean roots (peeled in the case of cassava) were processed into flakes using a flake machine. The flakes were then sun dried over 2–3 days until approximately 70% of the moisture had been removed. The dried flakes were then put through a grinding machine or a hammer mill to obtain milled product. The milled meal was stored in storage bins until used.

The milled cassava and sweetpotato were blended with a high-energy (11.8 MJ/kg; 41.9% crude protein (CP)) or a low-energy protein concentrate (9.4 MJ/kg; 41.8% CP), respectively. The four experimental diets were:

- 50% high-energy protein concentrate + 50% cassava (CAS50H);
- 30% high-energy protein concentrate + 70% cassava (CAS70H);
- 50% low-energy protein concentrate + 50% sweetpotato (SP50L);
- 30% low-energy protein concentrate + 70% sweetpotato (SP70L).

The calculated proximate chemical compositions of the diets are summarised in Table 1.

Table 1. Proximate chemical analysis of the experimental diets and the commercial diet used in the trial.

	Diet				
	NGTB finisher (control)	CAS50H	CAS70H	SP50L	SP70L
Energy (MJ/kg)	12.50	15.12	15.11	14.16	14.65
Protein (%)	23.90	22.45	14.63	23.05	15.67
Fat (%)	6.40	5.30	3.46	3.11	2.39
Fibre (%)	4.20	3.13	3.44	3.32	3.11
Lysine (%)	0.80	3.59	3.72	3.45	3.51
Methionine (%)	0.35	1.67	1.64	1.50	1.38
Calcium (%)	1.20	2.23	2.02	1.61	1.05
Avail. phosphorus (%)	0.55	0.47	0.28	0.62	0.41
Sodium (%)	0.05	0.33	0.32	1.14	1.44

NGTB = New Guinea Table Birds; CAS50H = 50% cassava + 50% high-energy concentrate; CAS70H = 70% cassava+ 30% high-energy concentrate; SP50L = 50% sweetpotato + 50% low-energy concentrate; SP70L = 70% sweetpotato + 30% low-energy concentrate.

The trial

The broiler chickens used in the trial were Ross 308 strain purchased from a commercial retail outlet. A total of about 520 day-old chicks were purchased, feather-sexed, weighed and allocated to two separate brooding pens, depending on gender. The chicks were kept in the brooding pens with kerosene lamp heaters during the night and fed on starter feed for 7 days. On day 7, the brooders were removed and the chicks were raised on litter until the experiment began on day 21.

The trial was carried out in a naturally ventilated broiler grow-out shed, with side and partition walls made of chicken wire. The two sides of the shed received either the morning or afternoon sun, and these sides were treated as blocks in the design. Each treatment (diet) was replicated twice on both sides (blocks) of the shed. A total of 20 pens were used, each with dimensions 4 × 2.5 m, and each pen treated as an experimental unit. The experimental design used was a 5 × 4 × 2 randomised complete block design with one treatment factor (i.e. diet).

On day 21, the chickens were weighed and randomly allocated to experimental pens, with 10 males and 10 females in each pen and overall weight of the group in each pen roughly equal. The experimental diets were then randomly allocated to the pens. The birds were introduced to the experimental diets and the feeding continued for 4 weeks. The experiment was terminated on day 49. During the experimental period, all birds had unlimited access to clean and cool water.

At the start of each week during the experiment, body weights of chickens were measured and recorded during the early morning. Feed residues and spillage, if any, were collected, weighed and discarded. All cases of mortalities were recorded and birds were examined for signs of ill health. Ambient room temperature of the broiler shed was recorded at 8 am, 10 am, 12 pm, 2 pm and 4 pm as well as the daily minimum and maximum temperatures.

Data analysis

Data were collected, cross-checked and entered into an Excel® database. Average weekly values for feed intake, weight gain and feed conversion rates were calculated. Statistical analysis was performed using GenStat®, Release 4.2, Discovery Edition (Lawes Agricultural Trust 2005).

RESULTS

Body weight

As expected, the birds fed the control diet had significantly higher weekly weights compared to birds fed the experimental diets ($P < 0.001$; Table 2). In terms of end weights at 49 days, birds on the SP50L diet had the second highest end weight after the control diet, followed by the birds on the CAS50H and SP70L diets. The birds fed the CAS70H diet had significantly lower end weights compared to the other diets ($P < 0.001$). Birds on the experimental diets SP50L, CAS50H and SP70L reached a market weight of 2 kg by the end of week 7.

Body weight gain

The weekly average body weight gains of birds on the control diet were significantly higher in weeks 4 and 5 compared to birds on the experimental diets ($P < 0.001$; Table 2). However in week 6, birds on the CAS50H diet had significantly higher gains compared to birds on the other diets ($P < 0.002$). In week 7 birds on the SP50L diet had significantly higher weight gains compared to birds on the other experimental diets ($P < 0.001$). Birds on the SP70L diet had the second highest weight gain, followed by the birds on the CAS50H ration. The birds on the control and the CAS70H diets had significantly lower ($P < 0.001$) weight gains at the end of week 7.

Feed intake

In terms of average weekly feed intake, in weeks 4, 5, 6 and 7 birds on the CAS70H diet had significantly lower intake compared to those on the other experimental diets ($P < 0.001$). Overall, higher intakes were observed for the sweetpotato diets (SP50L and SP70L) than the cassava diets (Table 2).

Feed conversion ratio

As shown in Table 2, the average feed conversion ratio (FCR) values of broilers on experimental diets were significantly different between the different diets throughout the experiment. In weeks 4 and 5, the birds on the control diet had better FCRs than those on the other diets. In week 7, birds on the SP50L diet had lower feed conversion compared to birds on the other

Table 2. Weekly average body weight, weight gain, feed intake and feed conversion ratio of broilers on the cassava and sweetpotato experimental diets and the control diet.

Age (weeks)	Overall mean	Diet					LSD 5%	*P* value
		NGTB finisher (control)	SP50L	CAS50H	SP70L	CAS70H		
Body weight (kg)								
3	0.906	0.904a	0.906a	0.907a	0.903a	0.909a	0.012	0.838
4	1.177	1.269a	1.219b	1.236ab	1.075c	1.086c	0.041	<0.001
5	1.54	1.855a	1.587b	1.531c	1.488c	1.238d	0.055	<0.001
6	1.892	2.558a	1.810b	1.875b	1.719b	1.500c	0.214	<0.001
7	2.321	2.709a	2.473ab	2.384b	2.231b	1.809c	0.241	<0.001
Weight gain (kg)								
4	0.271	0.365a	0.313b	0.330ab	0.172c	0.177c	0.038	<0.001
5	0.363	0.586a	0.368b	0.295c	0.413d	0.152e	0.03	<0.001
6	0.258	0.269b	0.212bc	0.344a	0.201c	0.263bc	0.062	0.002
7	0.443	0.223c	0.663a	0.509b	0.512b	0.309c	0.115	<0.001
Feed intake (kg)								
4	0.717	0.733a	0.750a	0.704a	0.750a	0.648b	0.048	0.002
5	0.86	1.153a	0.894b	0.763c	0.897b	0.593d	0.041	<0.001
6	0.911	1.445a	0.814b	0.694b	0.991b	0.612b	0.364	0.002
7	0.858	0.924b	1.069a	0.797c	0.969b	0.530d	0.088	<0.001
FCR								
4	2.935	2.017c	2.410c	2.147c	4.404a	3.699b	0.485	<0.001
5	2.644	1.969c	2.433bc	2.587b	2.173bc	4.059a	0.564	<0.001
6	3.74	5.491a	3.854b	2.077c	4.944ab	2.345c	1.45	<0.001
7	2.47	5.483a	1.620b	1.625b	1.907b	1.713b	2.814	0.042

Means followed by the same letter in a row are not significantly different ($P > 0.05$).
NGTB = New Guinea Table Birds; CAS50H = 50% cassava + 50% high-energy concentrate; CAS70H = 70% cassava + 30% high-energy concentrate; SP50L = 50% sweetpotato + 50% low-energy concentrate; SP70L = 70% sweetpotato + 30% low-energy concentrate; LSD = least significant difference.
Source: Glatz (2013).

diets ($P < 0.042$); this figure was significantly different to the FCR of birds on the control diet, but was not significantly different to birds on the CAS50H, SP70L and CAS70H diets. The FCR of birds on the CAS70H diet improved from 3.699 at week 4 to 1.713 at the end of week 7, while FCR on the SP70L diet improved from 4.404 at week 4 to 1.907 at the end of week 7. The birds fed the control diet had significantly higher FCR at the end of week 7 compared to those on the other diets ($P < 0.042$).

DISCUSSION

The results for body weight, weight gain and FCR of birds obtained in this trial are comparable to birds fed commercial stock feed under PNG local conditions. Both cassava mixed with the high-energy concentrate and sweetpotato mixed with the low-energy concentrate showed promising results. Birds fed the experimental diets were able to reach a market weight of 1.8–2.5 kg by 49 days. Birds fed the CAS70H diet had low feed intake throughout the experiment, which may be associated with higher energy values in the feed; the birds had good weight gains, and good FCR at the end of week 7. The results show that these roots can be fed to broilers and can improve profit margins for farmers.

According to the International Society for Tropical Root Crops (http://www.istrc.org/about-the-istrc), the current challenge is for appropriate processing technologies and business enterprise models to be developed for these crops, as unlike grains, root and tuber crops are bulky, have high water content and have a relatively short shelf-life.

Using cassava and sweetpotato as animal feed not only adds value to these crops but also diversifies income earning opportunities for farmers growing these crops. Cassava and sweetpotato are two of the major traditional root crops grown throughout PNG and are important to the livelihoods of many rural farmers. Therefore, any advances in product development for these crops will have a direct impact on improving food security and income generation.

As shown in this paper, these two crops can supply the majority of the carbohydrate in livestock diets provided they are supplemented with other nutrients, such as protein and essential amino acids. The implications of using these local ingredients to feed livestock are huge, especially for smallholder producers who need to maximise farm productivity and increase efficiency.

CONCLUSION

The results in this paper show that it is feasible to feed broiler chickens in PNG on a protein concentrate blended with a local root crop. The two best feeding options based on growth performance appear to be 50% sweetpotato mixed with 50% low-energy concentrate and 50% cassava mixed with 50% high-energy concentrate. More research is needed into appropriate processing technologies for these crops so that their full potential can be realised, for chickens and other livestock. Developing business models for small feed mill operators, incorporating these feeding options, will pave the way for potential investors to fully exploit these crops.

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4.2 Evaluation of the performance of broiler chickens fed diets containing 50% cassava or sweet potato in farmer trials in Papua New Guinea

Evaluation of the performance of broiler chickens fed diets containing 50% cassava or sweetpotato in farmer trials in Papua New Guinea

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Abstract

As part of a project to develop an alternative broiler finisher feeding system using local feed resources, two best-bet diet formulations were trialled. The on-farm trials were conducted at four locations in Papua New Guinea (PNG) and compared growth performance of broilers on the two test diets—50% boiled sweetpotato blended with 50% low-energy concentrate (SP50L) and 50% boiled cassava blended with 50% high-energy concentrate (CAS50H)—against a commercial control. The hypothesis was that growth performance of broilers during the finishing period would not be adversely affected when the commercial broiler finisher was replaced with the alternative diets. The results showed that at all the sites, overall growth performance of broilers on test and control diets was not significantly different, and overall growth performance of broilers fed on the CAS50H and SP50L diets was very good, with birds attaining target market weight of over 2 kg from week 5. It is therefore recommended that the alternative feeding options of boiled sweetpotato and cassava blended with their respective concentrates be promoted as finishing rations for broilers. It is also recommended that further farmer trials, with minimal external input or supervision, be encouraged to consolidate evaluation of this technology and confirm the appropriateness and validity of the technology for extensive use. Further research on the digestibility of sweetpotato cultivars for feeding broilers also needs to be carried out, as well as research into the best way to present cassava roots to broilers.

INTRODUCTION

In Papua New Guinea (PNG) the commercial livestock sector is dominated by broiler chicken production due to the high demand for poultry meat (Gwaiseuk 2001). The chicken industry has grown since the early 1980s under the protection of an import ban and more recently, a high import tariff. Under this scenario, the industry has gained a high level of self-sufficiency. Moat (2001) reported a high demand for live chickens and fresh eggs in both urban and rural areas, and that live broiler chickens in particular command very high prices. The small-scale broiler chicken market operates independently from the vertically integrated commercial frozen broiler industry, and currently produces about six million birds per year for sale in informal local markets. The sale of chickens is the major source of income from the livestock sector of traditional smallholder farming systems, with an estimated 50,000 families currently producing broilers (Glatz 2007).

The carcass weight of live broiler chicken sales is estimated at 7,000 tonnes per year (Kohun et al. 2006). Assuming an average sale price of 25 kina/bird, the subsector has a value of 125 million kina. While this subsector has grown steadily over the years it also faces constraints, the major ones being access to, and the high and rising cost of, day-old chicks and commercial feeds. The high cost is due to the industry using imported hybrid chickens and formulated high-density stock feeds made from imported ingredients. In

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recent years, this problem has become more profound due to major development projects in the country, such as the Liquefied Natural Gas project, which have resulted in a greater demand for chicken meat and more smallholders entering the market.

Recommendations following a survey conducted by the National Agricultural Research Institute (NARI) in 2004 of current feeding practices and attitudes to broiler chicken production include: (1) provision of information and training on use of local feed resources in broiler diets; (2) information and training on on-farm feed mixing; and (3) broiler feeding demonstrations in different rural areas depending on need and cost effectiveness.

The strategy for this trial was to take best-bet diet options that use local feed resources and test them on village farms in a range of locations. A total of 39 farmers were selected from across the country to evaluate the diets and monitor broiler performance, and to provide information on costs, in order to assess the acceptability and profitability of the feeding options. The two experimental diets selected for this evaluation had previously been shown to have promising results based on broiler performance in grow-out trials carried out by NGO partners near to selected farm sites. The NGOs were Christian Leaders' Training College (CLTC) in Banz, Western Highlands Province; Lutheran Development Services (LDS) in Morobe Province; and the OK Tedi Mining Limited (OTML) in Tabubil, Western Province. Grow-out experiments on these partner sites commenced late in 2008 and aimed to establish if selected feeding options were viable for on-farm testing. Based on the results, it was recommended that these two feeding options be tested on-farm.

In the trials conducted at the NGO sites, milled sweetpotato and cassava were used. The sweetpotato or cassava was peeled, chopped, dried and hammer milled, and then mixed with concentrate. The dry form of sweetpotato and cassava was not considered suitable for farmers, as they do not have mills and driers available. To make it more convenient for farmers, it was decided to use boiled and mashed sweetpotato or cassava instead. A grow-out trial on-station at NARI-Labu demonstrated that broiler performance was not affected by the form of presentation of the sweetpotato/cassava component of the diet (Ayalew et al. 2017).

MATERIALS AND METHODS

Sites

The on-farm trials were conducted in the Eastern Highlands, Morobe, Western Highlands and Western provinces of PNG (Figure 1). In Morobe Province, the site was in the Finschaffen District, which is located at the eastern end of the Huon Peninsula and has a population of 36,000 (NSO 2002). Altitude ranges from 0 to 3,000 m above sea level and average annual rainfall is 2,400–3,700 mm. Generally, people in this district are extremely disadvantaged due to relatively high population density on land of limited agricultural potential, poor access to services and low cash income.

In the Eastern Highlands Province, the on-farm trials were conducted in the Obura-Wonenara District. The altitude of the district ranges from 1,400 to 1,800 m above sea level and average annual rainfall is 1,800–4,000 mm. Based on the 2000 census, the population is around 45,000. Generally, the land has low agricultural potential and access to services is moderate. Cash income is also moderate and child malnutrition is a concern.

Trials were also conducted in North Waghi District in the Western Highlands. This district is located in the central east of the province and has a population of 44,000. Altitude ranges from 1,400 to 3,000 m above sea level and average rainfall is 2,300–2,600 mm. Overall, people in this district are not disadvantaged as there is little agriculture pressure and land potential is very high. Access to services is good and cash income is high.

Western Province covers 99,300 km^2 and is the largest province in PNG. The trials here were conducted with 10 famers located along the Kiunga–Tabubil highway, in Sisimakam, Wangbin, Migalsim, Kwiroknai and Tabubil villages. The villages are located in the North Fly district, which has an area of 13,138 km^2 and an average annual rainfall of 8,000 mm. Most of the economy of the district revolves around the Ok Tedi Mine, which is the largest economic entity in the Western Province, accounting for over half of the entire province's economy. There is also a productive rubber industry situated around Kiunga–Tabubil highway.

This paper does not include results from follow-on trials which were conducted at Nawae (Morobe Province), Kainantu Rural (Eastern Highlands Province) and Tambul-Nebliyer (Western Highlands Province).

Housing

The trials were carried out on farms close to where the NGO project partners operated. Housing for the chickens was mostly built from local materials. The houses in the highland sites (Banz and Kainantu) were grass-thatched. The walls were a combination of chicken mesh and a single layer of woven pit-pit mat. The same material was used for flooring. The birds were raised on a slightly elevated position above the floor level. The broiler houses near the coast, particularly in Finschhafen and in Western Province, were made of sago palm thatches and chicken wire mesh was used as walls giving natural ventilation. In sites where sawdust or wood chips were available, these were used as litter.

Where available, drinkers and feeders were purchased; otherwise they were made from bamboo. Most farmers used plastic pan drinkers and feeders. The drinkers were refilled twice daily with clean water, and where boiled mashed feed was used, this was made twice daily as well, usually in the mornings and evenings. In Western Province, where milled cassava was used, feeders were refilled with a certain amount of weighed feed as and when necessary. Farmers were advised to make sure their birds had unrestricted access to feed and clean, cool water throughout the experimental period.

Experimental diets and birds

The birds were distributed to the participating farmers as day-old chicks. Depending on the locality, brooding of these day-old chicks was from day 0 to day 7 in coastal sites, and continued for an additional week in highland sites.

The two experimental diets comprised low- and high-energy concentrates blended with sweetpotato and cassava, respectively. The first diet (SP50L) comprised the low-energy concentrate (metabolisable energy (ME) 15.0 MJ/kg and crude protein (CP) 22.7%) mixed with boiled and mashed sweetpotato at a ratio of about 1:3 on an as-fed basis, which is equivalent to a ratio of about 1:1 on a dry matter basis. The second diet (CAS50H) comprised the high-energy concentrate (ME 16.2 MJ/kg and CP 22.8%)

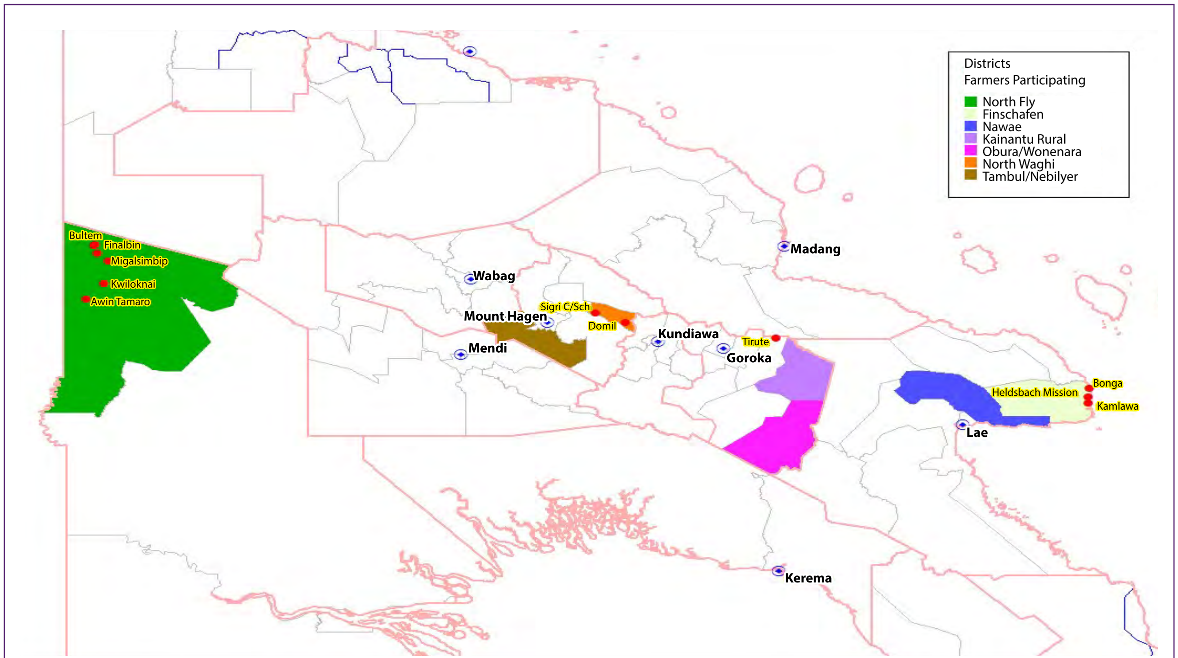

Figure 1. Location of sites where on-farm trials were conducted. Source: NARI-GIS Section.

mixed with boiled and mashed cassava at a ratio of 1:3 on an as-fed basis to give an equivalent ratio of 1:1 on a dry matter basis. Nutrient compositions of the diets, as well as the commercial diets used in the trials, are given in Table 1.

Farmers in the lowlands tested the CAS50H diet and farmers in the highlands tested the SP50L diet, due to the low cost and high availability of these two major staples in the respective locations. Farmers in Western Province tested both the cassava and the sweetpotato diets, however their data for the sweetpotato diet were not included in the analysis due to the fact that sweetpotato is not a staple in the area and is not easily available.

Ross 308 broiler day-old chicks were used in the trials. Participating farmers were selected by the project partner NGOs. These farmers were assisted to buy a box of day-old chicks, two 40 kg bags of commercial broiler starter crumble supplied by New Guinea Table Birds (NGTB), and 60–80 kg of the relevant NARI broiler concentrate bulked up with the respective energy source (sweetpotato or cassava).

From day 1 to day 20, the birds were raised together on commercial broiler starter crumble (Flame). On day 21, birds were weighed and distributed evenly into two separate pens based on their weights. Birds in one pen were fed the NGTB commercial finisher pellets and birds in the other were fed the experimental diet. The experimental phase began on day 21 and ended on day 42. After day 42, the farmers were able to sell their birds.

Data and statistical analysis

Parameters measured were weekly body weights of groups of birds (to calculate average individual weights) and feed intake, which was calculated from the total amount of feed distributed and the feed remaining in bags. There were occasional issues with unreliable recording of leftover feed by the farmers; hence it was not possible to get accurate measurements of feed intake.

A one-way analysis of variance was performed using GenStat® (Lawes Agricultural Trust 2005) to determine whether there were significant differences between the treatment diets in the weekly body weights of birds. Data were managed and analysed separately for the four sites.

RESULTS

Birds fed the cassava diet (CAS50H) in Western Province had lower weekly body weights in weeks 5 and 6 compared to birds fed the commercial finisher feed, as expected, but the weight differences were not statistically significant ($P > 0.05$; Table 2). The birds on the test diet reached market weight of 1.8 kg by week 5 and 2.4 kg by week 6. The coefficient of variation (CV) as shown in Table 2 was above 10% in week 4, 12% in week 5 and 14% in week 6. These large variations may be an indication of variable feed intake of birds due to attributes of the feeds or the feeding practice.

Table 1. Proximate nutrient compositions of the commercial and experimental diets used in the trials.

Nutrient	Commercial diets		Experimental diets	
	Flame	NGTB	SP50L	CAS50H
Protein (%)	24.4	26.2	22.7	22.8
ME (MJ/kg)	*	*	15.0	16.2
Fat (%)	7.7	5.8	2.85	4.95
Fibre (%)	6.8	3.2	1.50	3.95

* Not available.
Composition of the commercial stock feed was obtained from Ayalew and Pandi (unpublished report for Trukai Industries).
NGTB = New Guinea Table Birds; SP50L = boiled and mashed sweetpotato blended with low-energy concentrate (1:1 on a dry matter basis); CAS50H = boiled and mashed cassava blended with high-energy concentrate (1:1 on a dry matter basis); ME = metabolisable energy.

During weeks 5 and 6, birds fed the CAS50H diet in Morobe Province appeared to have lower weekly weights compared to birds fed the control diet, however these differences were not statistically significant ($P > 0.05$; Table 3). In this trial the CV was again quite high, reflecting high variability in the growth of individual birds. We observed farmers who properly mixed the cassava with the high-energy concentrate and had better average broiler performance, but in some cases the cassava was not cooked properly and not mixed well with the concentrate and this may have affected the results.

Results of the trial at Kainantu in Eastern Highlands Province showed no differences between weights of birds fed the sweetpotato test diet and the control diet. Birds fed the SP50L diet had slightly higher body weights during weeks 5 and 6 compared to birds fed the control diet, but these differences were not statistically significant ($P > 0.05$; Table 4). The cooler temperatures and wetter conditions at this site may have contributed to the good end weights; these could also reflect good management of experimental animals, which was supervised by researchers.

In the trials conducted at Banz in the Western Highlands Province, which were supervised by CLTC staff, the birds fed the SP50L diet had lower body weights in weeks 5 and 6 compared with birds fed the control diet, however these differences were not statistically significant ($P > 0.05$; Table 5). Judging by the higher overall end weights, the experimental birds at this site performed better than those at the other sites. This may be due to the farmers' experience—the farmers engaged in the trial had years of experience growing broiler chickens with support from the extension services of CLTC.

Table 2. Weekly end weights of broilers fed the cassava* test diet vs the commercial diet in Western Province ($N = 10$).

Age	Diet	Weekly average bird weight (kg)	Grand mean	LSD	CV (%)	*P* value
Week 4	CAS50H	1.449	1.453	0.145	10.6	0.914
	NGTB control	1.457				
Week 5	CAS50H	1.872	1.961	0.23	12.1	0.112
	NGTB control	2.05				
Week 6	CAS50H	2.411	2.538	0.338	14.2	0.132
	NGTB control	2.665				

* Unlike in the other trials, farmers in this trial were provided with milled cassava meal to mix with the concentrate.
N = number of birds in the group; LSD = least significant difference; CV = coefficient of variance; CAS50H = cassava blended with high-energy concentrate (1:1 on a dry matter basis); NGTB = New Guinea Table Birds.

Table 3. Weekly end weights of broilers fed the cassava test diet vs the commercial diet in Finschhafen, Morobe Province ($N = 8$).

Age	Diet	Weekly average bird weight (kg)	Grand mean	LSD	CV (%)	*P* value
Week 4	CAS50H	1.147	1.148	0.307	24.9	0.995
	NGTB control	1.148				
Week 5	CAS50H	1.511	1.582	0.352	20.8	0.402
	NGTB control	1.652				
Week 6	CAS50H	1.76	1.94	0.488	23.4	0.141
	NGTB control	2.12				

N = number of birds in the group; LSD = least significant difference; CV = coefficient of variance; CAS50H = boiled and mashed cassava blended with high-energy concentrate (1:1 on a dry matter basis); NGTB = New Guinea Table Birds.

Table 4. Weekly end weights of broilers fed the sweetpotato test diet vs the commercial diet in Kainantu, Eastern Highlands Province (*N* = 9).

Age	Diets	Weekly average bird weight (kg)	Grand mean	LSD	CV (%)	*P* value
Week 4	SP50L	1.277	1.29	0.126	9.8	0.665
	NGTB control	1.303				
Week 5	SP50L	1.793	1.727	0.321	18.6	0.398
	NGTB control	1.661				
Week 6	SP50L	2.422	2.408	0.239	9.9	0.814
	NGTB control	2.395				

N = number of birds in the group; LSD = least significant difference; CV = coefficient of variance; SP50L = boiled and mashed sweetpotato blended with low-energy concentrate (1:1 on a dry matter basis); NGTB = New Guinea Table Birds.

Table 5. Weekly end weights of broilers fed the sweetpotato test diet vs the commercial diet in Banz, Western Highlands Province (*N* = 12).

Age	Diet	Weekly average bird weight (kg)	Grand mean	LSD	CV (%)	*P* value
Week 4	SP50L	1.507	1.51	0.117	8.2	0.895
	NGTB control	1.514				
Week 5	SP50L	2.172	2.237	0.133	6.3	0.057
	NGTB control	2.301				
Week 6	SP50L	2.738	2.83	0.203	7.6	0.073
	NGTB control	2.922				

N = number of birds in the group; LSD = least significant difference; CV = coefficient of variance; SP50L = boiled and mashed sweetpotato blended with low-energy concentrate (1:1 on a dry matter basis); NGTB = New Guinea Table Birds.

DISCUSSION

The on-farm trials were conducted with the objective of assessing the acceptability and profitability of the various feeding options. The two feeding options used in this assessment were selected based on previous experiments (Glatz 2013). The results confirmed that these diets based on local ingredients were successful, as the final body weights of birds on the test diets were comparable to those fed the standard broiler finisher feed.

Many studies have been conducted to assess the growth of broilers fed sweetpotato diets at various sweetpotato levels. This study may be the first with 50% sweetpotato to report final body weights that are comparable to birds fed standard commercial diets. Studies by Agwunobi (1999) and Afolayan et al. (2012) showed significantly lower final body weights of broilers fed 50% sweetpotato diets. These differences may be due to diets not being adjusted for protein and energy or the breed of chicken used.

Some studies have evaluated the replacement of cereals with cassava products in poultry diets, and have reported varying results due to differences in the variety used, plant maturity at harvest, ecological conditions of plant growth and processing methods (Chauynarong et al. 2009). The authors of this review on the use of cassava in poultry diets noted that as long as ME, amino acid, mineral and vitamin requirements of poultry are met, a maximum inclusion of cassava at 70% can be achieved, provided the cassava is processed appropriately.

Feed intake is affected by feed form, which in turn affects weight gains and final body weights. The best feed intake occurs on good quality crumble or pelleted feeds, with an even particle size (Aviagen 2014). This could explain the high variation in the weights of birds fed the boiled cassava root. One of the main attributes of boiled cassava roots is friability (Padonou et al. 2005) (friability or mealiness is defined as being easy to crumble or pulverise). We observed that if cassava roots were harvested late, and if they were not boiled long enough, the roots were not friable. Instead the boiled roots were lumpy, which made it difficult to mix thoroughly with the concentrate, leading to uneven particle size of these diets. Beleia et al. (2006) noted that while cooking times of up to 30 minutes are generally accepted, better quality roots require between 10 and 20 minutes cooking. The authors studied the influence of root maturity and cultivar type on the cooking of cassava roots over two planting periods and concluded that age of roots was an important factor, with cooking time being at least double for 25-month-old samples compared to 12-month-old samples. In our trials with the sweetpotato-based diet, the results generally had a lower CV than trials with the cassava-based diet, and this may be because boiled mashed sweetpotato root is easier to prepare as an even mix than boiled mashed cassava roots.

Another factor which may have contributed to the variation in bird weights is inadequate feeding space for birds. High stocking densities and lack of adequate feeders or drinkers may not allow all birds to obtain their equal share of feed. This problem may be overcome by observing the birds feeding and providing more feeders if some birds are missing out.

Another overarching factor that would have affected the performance of broilers at all sites is farmer experience. The greater experience farmers have in raising broilers, the more able they would be in resolving problems with bird management. These include issues such as ensuring adequate feeding space, proper ventilation during warmer temperatures, and litter management. These factors can have a major effect on broiler growth and quality (Aviagen 2014).

CONCLUSION

Overall, performance of broilers on the cassava and sweetpotato diets was very good, and birds attained the target market weight of over 2 kg from week 5. The SP50L diet compared very well with the NGTB commercial diet in terms of body weight. Based on the results of these trials, the alternative feeding options of boiled mashed sweetpotato and cassava, blended with their respective concentrates, can be promoted as a technology for broiler farmers in PNG. However farmer participation to assess broiler performance under their own conditions and management, with minimal input or supervision from NARI, must be encouraged to further evaluate the appropriateness of this technology at the farmer level. The results in this trial on bird performance varied depending on locality and farmer experience, management and attitude. Information on general basic management practices—such as adequate feeding space, litter management, importance of proper ventilation and record keeping—must be made available to farmers in order to realise the full potential of these hybrid birds and improve efficiency and profitability at the farm level. Digestibility of sweetpotato cultivars that are used for feeding broilers also needs to be assessed, as well as the best way to present cassava roots to broilers.

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4.3. Evaluation of the performance of broiler chickens fed mashed or milled formulations of sweet potato-based finisher diets in an on-station trial in Papua New Guinea

Evaluation of the performance of broiler chickens fed mashed or milled formulations of sweetpotato-based finisher diets in an on-station trial in Papua New Guinea

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Abstract

In Papua New Guinea (PNG) it is not known if smallholder broiler farmers should feed their meat birds diets based on dry milled or boiled and mashed sweetpotato. To resolve this question, sweetpotato-based finisher diets were fed to Ross strain broiler chickens as wet mash or dry milled, and growth performance was measured. Birds were fed a standard broiler starter diet until 21 days of age before they were switched to the experimental diets. There were four sweetpotato-based test diets, and a standard commercial broiler finisher diet was used as the control; there were two blocks, two replicates and 12 birds in each replicate. The test diets comprised 50% and 70% boiled and mashed formulations and 50% and 70% dried and milled formulations, each blended with low-energy protein concentrate to make a complete broiler finisher diet with metabolisable energy 15.0 MJ/kg and crude protein 22.7%. Feed intake and weekly live body weight were recorded up to seven weeks of age, and feed conversion ratio (FCR) was calculated on a weekly basis. Dry matter feed intake was variable between diets, as well as weeks, but the variation was not consistent. Live body weights varied significantly between the diets from five to seven weeks of age, with birds fed the control diet performing best followed by birds fed the milled and mashed 50% diet ($P < 0.001$). FCRs were significantly different ($P < 0.001$) for birds fed the diets from five to six weeks of age, with the standard control (FCR = 2.01 and 2.68, respectively) and mashed 50% diet (FCR = 3.00 and 2.25, respectively) performing best, and slight differences between the wet mash and dry milled formulations of the diets. Overall, growth of birds was similar on mashed and milled formulations of the sweetpotato diets. The high moisture content of mashed diets did not affect average feed intake and FCR. It was therefore concluded that both milled and mashed formulations can be promoted, milled formulations through the mini-mills and the mashed formulations for preparation by individual smallholder farmers. Importantly, farmers will not lose out in terms of growth and FCR when they use mashed formulations.

INTRODUCTION

Besides a small number of smallholder contract (out-grower) broiler farmers who supply directly to the country's two big broiler companies for processing, there are an estimated 50,000–60,000 smallholder broiler growers in PNG who are directly involved in market-oriented broiler farming using commercial broiler chickens and stock feed (Kohun et al. 2006; Ayalew 2013). The viability of this smallholder broiler production has been threatened by rising costs of commercial feeds. These feeds, which use imported ingredients, are vulnerable to price fluctuations in major grains on the global market. This is compounded by high transport and other associated costs, and has led to a more than doubling of retail prices for commercial stock feed in the country in recent years (Ayalew 2013). As a result, not only have

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the costs of broiler production increased dramatically but the profit margins of smallholder farmers have been reduced, exerting greater pressure on the smallholder broiler industry.

Improving the profitability of village broiler farming through the use of locally available feedstuffs has been a high priority research area for the National Agricultural Research Institute (NARI) in Papua New Guinea (PNG) and its partners, with funding provided by the Australian Centre for International Agricultural Research (ACIAR) since 2001. The initial ACIAR project 'Poultry feeding systems in PNG' (LPS/2001/077) laid a solid foundation in the development of the smallholder poultry sector in PNG. This project placed a strong focus on the delivery of feeding strategies to village farmers (Glatz et al. 2010, 2013).

It is with this background that the ACIAR project 'Improving the profitability of village broiler production in Papua New Guinea' (ASEM/2005/094) has been implemented since 2005, with the aim of developing lower cost broiler feeding strategies.

High feed intake is as important as nutrient digestibility for rapid growth of commercial broilers (Klasing 2007), and feed intake is perhaps the single most important factor determining feed efficiency for broilers (Bao and Choct 2010). Physical feed quality is considered to have a very significant effect on broiler growth (Jafarnejad et al. 2010) and the costs associated with feed processing represent a significant portion of feed costs (Behnke and Beyer 2002).

Apart from pellets, broiler diets can take the form of dry or wet mash or crumble. Dry mash is a finely ground and mixed feed. Due to its dustiness, its palatability is poor. Wet mash is made by mixing high-moisture ingredients, such as boiled sweetpotato and cassava tubers, with protein concentrates. Crumble feed results from crushing pelleted feed to a consistency coarser than mash. It is generally accepted that the feeding of pellets improves broiler growth rate compared to mash (Choi et al. 1986; Nir et al. 1994). Reasons for this may include decreased ingredient segregation, increased digestibility, reduced energy during prehension, thermal modification of starch and protein, and increased palatability (Behnke 1994, 1998).

This experiment was conducted as part of the ACIAR project ASEM/2005/094 to develop lower cost broiler feeding options using sweetpotato and cassava as energy sources and a substitute for imported grains. Both sweetpotato and cassava are among the staple crops in PNG and are usually readily available from food gardens. Processes involved in the preparation of tubers for feed include washing, peeling (cassava only), chopping or grating, and boiling. After cooling, the boiled tubers are either used directly as mash or dried and milled before being mixed with matching protein concentrates at the right ratio to make up the complete diet.

In the context of smallholder broiler farmers, who find it practical to prepare smaller quantities of the feed, diets can be prepared in both wet mash and dried milled forms. Wet mash is straightforward and convenient to feed fresh on a daily basis. Converting the boiled tubers into a milled or even a pelleted product is less straightforward, and requires a desiccator or oven, a hammer milling machine and (if pelleting) a pelleting machine. However, wet mash is perishable and liable to spoilage, whereas dried milled products can be kept longer without spoilage and are convenient for industrial-scale processing. Smallholder broiler farmers are more likely to use the boiled sweetpotato tuber as a wet mash than converting it into a dried and milled product.

The performance of broilers fed diets based on dry milled or boiled mashed sweetpotato was investigated. This experiment tested the hypothesis that the form of presentation for the main starch ingredient in the balanced broiler finisher does not affect bird growth performance.

MATERIALS AND METHODS

Location

This study was conducted during April and May 2009 at NARI's Livestock Research Station at Labu (6°40′27″ S, 146°54′33″ E), which is located about 16 km north of the city of Lae. The climate is typically warm and wet with an average daily temperature of about 30°C and 84% relative humidity.

Housing and husbandry

The experiment was conducted in an open-sided poultry house with a deep litter floor and 20 feeding pens, each 4 × 2.5 m. Wood shavings were provided as bedding for the birds and florescent tubes were used for lighting at night. Each pen was provided with one hopper feeder and one bell drinker for a total of six female and six male birds. Average stocking density was 1.2 m^2 per bird. The broiler chicks were randomly allocated to the experimental pens and raised on a local standard commercial starter diet (Flame) from 0 to 21 days of age (end of week 3) before they were introduced to the experimental diets. Feeding of treatment diets was terminated on day 49 (end of week 7).

Experimental birds and diets

A total of 240 sexed day-old Ross strain commercial broiler chickens supplied by Niugini Tablebirds Ltd. were used in a randomised complete block design with five treatments, two blocks (sides of the experimental shed) and two replicates. Each replicate comprised a separate pen housing six female and six male birds. Feed and town drinking water were offered ad libitum. The treatment diets were as follows:

- 50% milled sweetpotato mixed with low-energy concentrate (LEC);
- 50% mashed sweetpotato mixed with LEC;
- 70% milled sweetpotato mixed with LEC;
- 70% mashed sweetpotato mixed with LEC;
- a local standard commercial broiler finisher (Flame) as control.

The LEC was designed to provide a balanced broiler finisher diet with metabolisable energy 15.0 MJ/kg and crude protein 22.7% when mixed at the recommended ratio with sweetpotato.

Data collection and analysis

Live body weights were recorded at the start and end of the experiment and at the end of each week. An inventory of birds was taken on a weekly basis. Feed intake per pen was calculated on a dry matter basis as the difference between feed offered and feed refused. Feed conversion ratio (FCR) was calculated weekly for each replicate using the current inventory. The data were fitted to the following statistical model (equation 1) to partition observed variance into treatment, block and random effects.

$$Y = T_i + B_j + e_{ij} \qquad (1)$$

where *Y* is the dependent variable (feed intake, body weight, or feed conversion ratio), *T* is the effect of the *i*th treatment (i = 1, 2, 3, 4, 5), *B* is the effect of the *j*th block (j = 1, 2), and *e* is the random error.

Data were analysed using a one-way analysis of variance of GenStat Release 7.22 (VSN International Ltd. (2008).

RESULTS

Mortality during the experiment was 3.75% overall, with birds fed the control diet having a mortality of 8.3% and birds fed the wet mash having a mortality of 6.3%. No birds from the groups fed on the milled feed formulations died.

Birds fed the control diet had higher weekly dry matter feed intake during weeks 5 and 6 but feed intake dropped in week 7 (Table 1). Birds fed on the mashed diets tended to catch up to the feed intake of birds fed the other diets during weeks 6 and 7. For the milled diets, birds fed the 50% diet maintained a better feed intake than birds fed the 70% sweetpotato diet.

The body weights of birds fed the control versus milled and wet mash diets showed significant differences from week 5, with birds fed the standard finisher diet performing the best, as expected, followed by the birds fed the 50% milled and mashed formulations ($P < 0.001$; Table 2). Weekly body weights were similar for the birds fed the 50% mashed and 50% milled diets ($P > 0.05$). The same was true for birds fed the 70% mashed and 70% milled diets during week 5 and week 6 but not during week 7 (Table 2).

During week 5, birds fed the standard finisher had a better FCR (Table 3) followed by birds fed the 50% mashed and then birds fed the 50% milled diet ($P < 0.001$). Birds fed on both of the 70% formulations performed poorly. During week 6, birds fed the 50% mashed formulation had superior FCR than birds fed the other diets, even better than the birds fed the standard diet.

Overall, growth performance of birds was similar when fed the sweetpotato mashed and milled formulations. Birds were fed ad libitum but the mashed formulation had to be processed twice a day to avoid spoilage and spillage. Under these circumstances, the high moisture content of mashed diets did not affect average feed intake and FCR of birds.

Table 1. Weekly feed intake (kg dry matter basis, per pen) for birds fed the different diets (mashed or dried and milled sweetpotato 50% or 70%, mixed with low-energy concentrate, or control).

	Week 4 (day 28)	Week 5 (day 35)	Week 6 (day 42)	Week 7 (day 49)
P value	0.875	<0.001	<0.001	<0.001
Overall mean	9.58	12.12	14.30	14.27
Milled 50% + LEC	9.57	13.84b	16.97c	15.28b
Mashed 50% + LEC	9.50	11.31a	13.34a	17.69c
Milled 70% + LEC	9.51	11.54a	13.55a	11.84a
Mashed 70% + LEC	9.68	10.51a	12.71a	15.63b
Standard broiler grower	9.62	13.41b	14.92b	10.92a
LSD 5%	ns	0.86	1.27	1.13
CV %	2.8	4.7	5.8	5.2

Means followed by the same letter within a week are not significantly different ($P > 0.05$). LEC = low-energy concentrate; LSD = least significant difference; CV = coefficient of variation; ns = not significant.

Table 2. Body weights (kg) of birds fed the different diets (mashed or dried and milled sweetpotato 50% or 70%, mixed with low-energy concentrate, or control).

	Week 4 (day 28)	Week 5 (day 35)	Week 6 (day 42)	Week 7 (day 49)
P value	0.22	<0.001	<0.001	<0.001
Overall mean	1.24	1.58	2.03	2.45
Milled 50% + LEC	1.25	1.61b	2.11b	2.54c
Mashed 50% + LEC	1.24	1.56b	2.08b	2.63c
Milled 70% + LEC	1.24	1.44a	1.77a	2.08a
Mashed 70% + LEC	1.25	1.46a	1.82a	2.23b
Standard broiler grower	1.24	1.81c	2.36c	2.77d
LSD 5%	ns	0.08	0.12	0.13
CV %	0.8	3.3	4	3.6

Means followed by the same letter within a week are not significantly different ($P > 0.05$). LEC = low-energy concentrate; LSD = least significant difference; CV = coefficient of variation; ns = not significant.

Table 3. Feed conversion ratio (dry matter basis) for birds fed the different diets (mashed or dried and milled sweetpotato, 50% or 70%, mixed with low-energy concentrate, or control).

	Week 4 (day 28)	Week 5 (day 35)	Week 6 (day 42)	Week 7 (day 49)
P value	0.2	<0.001	<0.001	0.911
Overall mean	1.90	3.46	2.84	3.32
Milled 50% + LEC	1.85	3.19b	2.85b	2.99
Mashed 50% + LEC	1.90	3.00b	2.25c	3.23
Milled 70% + LEC	1.94	4.88a	3.47a	3.14
Mashed 70% + LEC	1.85	4.21a	2.95b	3.68
Standard broiler grower	1.93	2.01c	2.68b	3.58
LSD 5%	NS	0.70	0.40	ns
CV %	3.3	13.3	9.3	36

Means followed by the same letter within a week are not significantly different ($P > 0.05$). LEC = low-energy concentrate; LSD = least significant difference; CV = coefficient of variation; NS = not significant.

DISCUSSION

The higher feed intake and body weight observed during weeks 5 and 6 for birds fed on the standard pellet diet is expected for the high genetic potential broiler birds (Behnke 1994; Behnke and Beyer 2002; Jahan et al. 2006). The improved feed intake of birds fed on the mashed diet during weeks 6 and 7 can be explained by better adaptability of the birds to this form of feed presentation. Dry milled diets are known to have problems with dustiness, which can be managed by splashing some water on the food and reducing the size of offerings.

In another study that compared the effect of pellet and mash on growth performance and carcass characteristics of commercial broilers, live body weight, weight gain, feed intake and FCR were not affected ($P > 0.05$) by the different forms of diet (Ahmed and Abbas 2012). This was attributed to similarities in growth response between mash, mixed and pellet-fed birds. Parsons et al. (2006) also reported similar comparable levels of feed intake between pellet and mash diets and indicated that the mash diet was associated with excessive feed waste. Jahan et al. (2006) compared performance of broiler chickens fed on mash and crumble and found that body weight gain was higher in crumble diets. Higher FCR value was observed for the mash group, which indicated lower feed conversion efficiency.

The advantages of pellet feeds over mash diets include non-selective feeding and high nutrient density. The added cost of pelleting is a disadvantage. Pellets are also known to increase water consumption and droppings become wetter. The mash formulation is relatively cheap to prepare at the household level, and the lower cost of production can appeal to broiler farmers, especially when the broiler chickens are sold on a body weight basis.

CONCLUSION

Overall, growth performance of birds was similar on mashed and milled formulations of the sweetpotato diets. The high moisture content of the mashed diets did not affect average intake and FCR. It was therefore concluded that both milled and mashed formulations can be promoted, milled formulation through mini-mills and the mashed formulation for preparation by individual smallholder farmers. Importantly, farmers will not lose out in terms of growth and FCR when they use mashed formulations.

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