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Pilot study on nutritional manipulation of odour emissions from poultry

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Executive Summary

Odour emissions are a normal part of chicken meat production but they potentially threaten the sustainable development of the chicken meat industry. There are currently no effective methods to reduce odour emissions that are practical or affordable for use in commercial farms. Diets can be formulated to more closely meet the bird's nutritional requirements to avoid overfeeding and to reduce the excretion of undigested components. This will decrease the amount of substrates that the microbes metabolize to odorous compounds. Diet can affect water intake, water to feed intake ratio, litter moisture, litter pH and litter water activity, all of which may affect the emission of odorants from litter. This research project investigated the role of diets on odour emission from chicken meat production.

In the introduction part of this report, a comprehensive review has been done on the key odorants from chicken meat production, their origin, analytical techniques for odour measurements and the nutritional effects on odour emissions. The five experiments that follow the introduction part investigate the role of phytase enzyme (experiment 1), protein sources (experiment 2), protein levels (experiment 3), probiotic and saponin (experiment 3), litter condition (experiments 3 and 4), necrotic enteritis and high sodium diet (experiment 4) and *Clostridium perfringens* culture (experiment 5) on odour emissions. Nutritional strategies such as partial replacement of soybean meal with meat meal in the diet, use of low protein diet, *Bacillus subtilis* based probiotic and saponin blend may lower odour emission from chicken meat production. Control of litter conditions by lowering litter moisture and litter water activity, and prevention of necrotic enteritis in meat chickens can alleviate possible odour problems around the meat chicken farms.

It should be noted that this research formed the basis of Nishchal K. Sharma's PhD program, and more information on this research can be found in the thesis if required.

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1. Introduction

1.1 Background information

Global chicken meat production has increased by 18 percent over the past 10 years and it is estimated that chicken meat production will account for more than half of the world's additional total meat output over the next decade (OECD/FAO, 2015). This growth will be supported by increased meat consumption globally as diets shift toward greater protein intake due to rising incomes, especially in the developing countries. Over the last decade, per capita consumption of chicken meat has increased from 26 to 29 kg globally and from 35 to 44.8 kg in Australia (ABARES, 2015). This steady increase in demand for chicken meat requires more farming in future. Meat chicken farms are often located near to urban and rural residential areas and expansion in their capacity may increase the risk of odour nuisance. Impacts of odours on nearby residences, communities and the environment have been recognized by the chicken meat industry and regulatory authorities as a cause of concern. Some odorants such as hydrogen sulfide (H_2S), dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide and methyl mercaptan released from meat chicken farms have been reported to produce an adverse effect on the health of humans and birds (Wadud, 2011). These concerns may prevent the establishment of new meat chicken farms or limit the expansion of existing ones, thus restricting development of the industry. Abatement of odour emissions has become important to the chicken meat industry.

In an effort to address odour issues from meat chicken farms, there have been attempts to develop mitigation strategies including litter treatments, biofilters, neutralising agents, air scrubbers, ozone treatment, windbreak walls and short stacks but these techniques are generally costly or impractical due to the required high ventilation rates in poultry farms (Dunlop and Gallagher, 2011). The requirement for "dry and friable litter condition" by RSPCA (2013) in meat chicken farms may partly address odour issues as it has been reported that lower litter moisture and higher litter pH (pH > 7.5) could effectively reduce odour emission from meat chicken farms by inhibiting anaerobic bacterial activity (Jiang and Sands, 2000). However, drying litter by increasing the ventilation rate is a costly process. Feed and gastrointestinal problems can exacerbate water excretion and contribute to wet litter condition. The principle of dietary manipulation is to carefully select ingredients, additives and enzymes to meet the nutrient requirements and rearing conditions of birds to reduce excess nutrients and increase digestibility of ingredients in the diet. It is suggested that lower levels of excreted nutrients will reduce the precursors for odour production. In this context, it may be easier and more practical to use dietary means to improve litter quality and odour emissions.

1.2 Odorants from chicken meat production

Odour is formed by the combination of hundreds of odorants, which may be classified as volatile organic compounds (VOC) or gases such as ammonia (NH_3) or H_2S (O'Neill and Phillips, 1992; Schiffman et al., 2001; Hobbs et al., 2004; Lacey et al., 2004). Odours are produced in chicken meat operations primarily from microbial decomposition of waste products such as excreta, feathers, dust and bedding (Dunlop

et al., 2010). Microbial decomposition in litter occurs under both aerobic (dry) and anaerobic (wet and caked) conditions (Jiang and Sands, 2000). Aerobic degradation of organic matter produces nitrogen containing odorants such as NH_3, amines, indole and skatole whereas anaerobic degradation predominantly produces sulfurous compounds such as H_2S, mercaptans (thiols), dimethyl disulfide and dimethyl trisulfide (Jiang and Sands, 2000). In addition, odours may also be emitted from the birds themselves (Lacey et al., 2004).

An early review paper of O'Neill and Phillips (1992) listed 168 compounds associated with livestock wastes of which 30 had very low odour detection thresholds of ≤ 0.001 mg/m^3 (1 ug/m^3 = 1 ng/l = 1 part per trillion). They found that six of the ten compounds with the lowest odour detection thresholds contained sulfur. This may be the reason that foul odour have often been described as a sulfury or a rotten egg like. Banwart and Bremmer (1975) reported that production of methyl mercaptan exceeded that of H_2S in poultry manure (excreta). Over the past 20 years there have been numerous investigations to identify key odorants specific to meat chicken farms. Jiang and Sands (2000) reported low threshold (≤ 1.3 ppb) and high threshold odorous compounds from meat chicken farms. Low threshold odorants included methyl mercaptan, ethyl mercaptan, propyl mercaptan, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, butyric acid, indole and skatole. High threshold odorants included acetic acid, acetone, acetaldehyde, carbon disulfide and methanol. Their study suggested that dimethyl disulfide was the dominant odorant in meat chicken farms. Trabue et al. (2008a) demonstrated that methyl mercaptan was the principal sulfur-containing odorant in poultry litter and because it had the lowest odour threshold level among other odorants, it could highly contribute to foul odour even at a very low concentration. Later, Trabue et al. (2008b) also showed that butanoic acid, 3-methylbutanoic acid and 4-methylphenol were major constituents of poultry odour. More recently, Dunlop and Gallagher (2011) identified 13 major odorous compounds out of several non-methane volatile organic compounds (NMVOC) and volatile organic sulfur compounds (VOSC) frequently occurring from meat chicken farms. These included acetone, 2,3-butanedione, 3-hydroxy-2-butanone, dimethyl disulfide, 1-butanol, 3-methyl-butanal, heptanal, octanal, benzene, toluene, dimethyl trisulfide, α-pinene and β-pinene. Furthermore, Pillai (2011) identified 2-butanone, phenol, cresols, propionic acid and butanoic acid in the litter as emission gases having nauseating odour characters.

Research suggests that odorants may well be correlated with overall odour intensity and offensiveness. A report by Dunlop and Gallagher (2011) highlighted a significant positive correlation between odorant concentration and odour intensity with only nine specific odorants in piggery wastes (r^2 = 0.87). In a more recent study, Murphy et al. (2014) reported eight VOC from tunnel-ventilated meat chicken farms that were considered important predictors of odour. These were dimethyl sulfide, dimethyl trisulfide, 2,3-butanedione, 3-methyl-butanal, 1-butanol, 3-methyl-1-butanol, 3-hydroxy-2-butanone, and 2-butanone. Based on these findings, a list of selected odorants associated with meat chicken farms, along with their odour characters and threshold levels, have been compiled (Table 1.1). These odorants contribute to noxious smell from meat chicken farms and thus may be targeted by dietary means or litter treatments in odour abatement studies.

Table 1.1 List of selected odorants with their odour characters and threshold levels (odour characters and threshold levels adapted from Dunlop et al., 2016a)

Odorants	Alternative names	Odour character	Minimum odour threshold ($\mu g/m^3$)	Maximum odour threshold ($\mu g/m^3$)
Sulfur containing				
Methanethiol	Methyl mercaptan	Rotten cabbage	0.0003	82
Carbonyl sulfide		Sulfide odour except when pure	70	180
Carbon disulfide	Methyl disulfide	Herbaceous, cabbage, sweet, vegetable	24.3	2.3×10^4
Dimethyl sulfide	DMS	Rotten eggs, rotten vegetables, wild radish	0.3	160
Dimethyl disulfide	DMDS	Putrid, rotten garlic, smoke, burning, rubber, rotten cabbage, intense onion	0.1	346
Dimethyl trisulfide	DMTS	Metallic, sulfur, pungent, garlicky, onion	0.06	8.8
Ethanethiol	Ethyl mercaptan	Natural gas, garlic- like, skunk- like	0.032	92
1-propanethiol	Propyl mercaptan	Onion, characteristic cabbage odour	0.04	3.9
Hydrogen sulfide	H_2S	Decaying vegetables, rotten eggs	0.21	24.9
Alcohols				
3-methyl-1-butanol	Isoamyl alcohol	Disagreeable	80	12.6×10^4
1-butanol	n-butanol, butanol	Solvent, alcohol, sweet, rancid,	158	4.2×10^4
2-butanol	Sec-butanol	Strong pleasant, wine like odour, sweet	400	8×10^4
Aldehydes and ketones				
2-butanone	Methyl ethyl ketone	Sweet, minty, acetone-like	737.3	2.5×10^5
2,3-butanedione	Diacetyl	Butter, rancid, fat, quinone, chlorine-like, yoghurt, sour-milk, sour-cream	0.007	88
3-hydroxy-2-butanone	Acetoin	Mushroom, earth, buttery, woody, yoghurt, butter-like	n/a	n/a
3-methyl-butanal	Isovalerald ehyde	Malt, rancid, apple-like, acrid	1.6	8.1
Amines				
Methylamine	MMA	Fishy, ammonia like	1.2	1.2×10^4

Dimethylamine	-	Ammonia-like, fish-like	84.6	86.7
Trimethylamine	TMA	Fishy, cat urine, faecal	0.26	2.1×10^{3}
Nitrogen containing				
Indole	Ketole	Faecal	0.15	1.9
3-methyl-indole	Skatole	Barnyard, perfume, characteristic faecal	4×10^{-4}	268
Carboxylic acids				
Formic acid	-	na	na	na
Acetic acid	Ethanoic acid	Vinegar	25	2.5×10^{5}
Propionic acid	Methyl acetic acid	Pungent, disagreeable, rancid odour, sour, mildy cheese-like		
Butanoic acid	Butyric acid	Unpleasant, rancid, obnoxious	0.4	4.2×10^{4}
Pentanoic acid	Valeric acid	Unpleasant, similar to butyric acid	0.16	120
3-methylbutanoic acid	Isovaleric acid	Unpleasant, rancid cheese, body odour	0.2	10.3
Hydrocarbons				
Benzene	-	Sweet, solvent, aromatic, petrol-like	1495	3.8×10^{5}
Hexane	n-hexane	Petrol-like	5290	2.8×10^{5}

1.3 Collection and measurement of VOC

The concentration of odorous compounds emitted from meat chicken litter is often very low. Therefore, it is important to use proper techniques to collect, identify and quantify the odorants accurately in odour abatement studies. The concentration of odorants can be measured analytically using different instruments.

1.3.1 Solid-phase micro-extraction with gas chromatography-mass spectrometry

Solid-phase micro-extraction (SPME) with gas chromatography-mass spectrometry (GC-MS) has been used to collect and measure odorants from laying hen excreta (Cai et al., 2007), headspace air of swine, dairy manure (Koziel et al., 2010; Laor et al., 2008; Lo et al., 2008), and from beef cattle operations (Wright et al., 2005). During sample collection and measurements, the SPME fibre is exposed to the headspace air above the sample for a suitable period of time and then odorous compounds are thermally desorbed in the heated injection port of a GC-MS (Matich and Pawliszyn, 1999). Sharma et al. (2016b) used SPME-GC-MS method to collect and measure odour emissions from meat chickens reared in metabolic chambers (experiment 1).

1.3.2 Thermal desorption gas chromatography mass spectrometry

Thermal desorption gas chromatography mass spectrometry (TD-GC-MS) technique has been used to measure meat chicken farm and litter headspace concentration of odorants by a number of researchers (Dunlop et al., 2013; Parcsi et al., 2012; Pillai et al., 2012; Pillai et al., 2010). During sample collection, thermal desorption tubes (sorbent tubes) pre-concentrate the sample air through a sorbent bed such as activated carbon, graphitised carbon blacks, carbon molecular sieves or carbon based porous polymers or a multi-sorbent bed. The captured analytes are then thermally liberated in the heated injection port of a GC-MS. Odorous air samples may also be collected by means of gas bags (polyvinyl fluoride–Tedlar®; polytetrafluoroethylene–Teflon®; polyethylene tetraphthalate; aluminium foil) or a gas sampling canister. Instead of pre-concentrating the sample (as with SPME or sorbent tubes), the entire sample is collected and kept within the bag or canister (Parcsi, 2010). Problems that may occur with the use of thermal desorption tubes, gas bags or canisters include greater possibility of sample loss during collection, transportation and storage before the samples are analysed using GC-MS. The odorants such as mercaptans, which are not stable, may dimerise to form disulfides (Parcsi et al., 2010) and may not be quantified accurately whereas the concentrations of disulfides may be overestimated.

1.3.3 Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy is another instrument that has been used to measure odorant concentrations from commercial swine (van Kempen et al., 2002) and turkey farms (Witkowska, 2013). The FTIR typically operates in the mid-infrared range and all organic compounds interact with infrared radiation yielding compound specific absorption characteristics whose intensity follows Beer's law. Because the strength of the absorption at certain wavelengths is proportional to the gas concentration, FTIR spectrum can be used for calculating gas concentrations. Using FTIR, it is possible to identify and quantify the volatile compounds in air, presuming that their concentration is above the detection limit of the instrument and spectral features of different compounds can be adequately separated (van Kempen et al., 2002). Sharma et al. (2015) used FTIR to measure odour emissions from meat chickens reared in chambers (experiment 2).

1.3.4 Selected ion flow tube mass spectrometry

Selected ion flow tube mass spectrometry (SIFT-MS) is an emerging technology capable of measuring certain VOC at parts per trillion (ppt) concentrations in air in real time (Prince et al., 2010). It allows for rapid, economical and convenient quantification of VOC and certain inorganic gases such as NH_3 and H_2S, based on full mass scans in the m/z range of 15 to 250 using three different precursor ions (H_3O^+, NO^+ and O_2^+). These ions are mass selected and react with trace VOC in very well controlled ion-molecule reactions but do not react with the major components of air, allowing SIFT-MS to analyse whole air for trace VOC to pptv levels (Atzeni et al., 2016). SIFT-MS may be operated in two modes: (1) full scan mode (FSM), which allows identification and quantification of non-targeted compounds and (2) selected ion mode which targets specific compounds for highly sensitive quantitative analysis,

providing significantly lower limits of quantitation and better precision than FSM (Annett *et al*., 2002). SIFT-MS has been previously used to measure odorants from meat chickens and pig farms and was found to provide repeatable quantitation of VOC and organosulfur compounds (Van Huffel et al., 2012; Atzeni et al., 2016). The advantage of SIFT-MS over GC-MS is that with SIFT-MS the odorant concentrations in the sample can be measured in real-time without the need for sample pre-concentration. This reduces the risk of sample loss and the concentration of odorants can be quantified accurately. Sharma et al. (2016a) used SIFT-MS in a selected ion mode to measure litter headspace concentration of odorants in chicken meat production.

1.3.5 Proton-transfer-reaction mass spectrometry

Similar to SIFT-MS, proton-transfer-reaction mass spectrometry (PTR-MS) can be used for online measurement of odorants. PTR-MS has been used to measure odorants from pig farms (Feilberg et al., 2010; Hansen et al., 2012) and cattle production (Ngwabie et al., 2008). It has not been used to measure odorants from meat chicken farms yet.

Thus, there are many different ways to measure odorants analytically. The choice of the method depends on availability of the instruments and the nature of the compounds to be measured. A challenge is that compounds with high volatility and reactivity can be difficult to measure accurately. SPME-GC-MS method may be useful to determine the odorants present in the sample but it is hard to quantify them accurately. TD-GC-MS is a widely used method to measure odorant concentration but with thermal desorption tube there is a greater possibility of sample loss during collection, transportation and storage before the samples are analysed using GC. The advantage of SIFT-MS over TD-GC-MS is that with SIFT-MS the odorant concentrations in the sample can be measured in real-time without the need for sample pre-concentration. This reduces the risk of sample loss and the concentration of odorants can be quantified accurately. In this project, the instruments were chosen based on the best available at the time when the experiments were conducted.

1.4 Origin of odorous compounds

In meat chicken farms, odour is emitted mainly from the litter and the major source of odour in litter is the excreta, which in the case of meat chickens, is the mixture of faeces and uric acid that accumulates in litter over time (Jiang and Sands, 2000). The faecal portion may contain undigested dietary components (carbohydrates, proteins, fats, vitamins, minerals, etc), endogenous secretions (mucins, enzymes, sloughed mucosa, etc), microflora in the gastrointestinal tract and their metabolites. The range of nutrients in meat chicken excreta collected over eight different feeding trials, as reported by van der Hoeven Hangoor et al. (2013), contained crude protein (CP) (194 g/kg) crude fat (78 g/kg), ash (57 g/kg) and crude fibre (30 g/kg). In their study, the concentrations of Ca (8.3 g/kg), K (8.2 g/kg) and P (6.6 g/kg) in excreta were higher among other minerals. The breakdown of protein in excreta produces peptones, peptides, amino acids and finally NH_3 and odorous metabolites, including those containing sulfur. The carbohydrate portion of excreta includes low molecular weight

carbohydrates such as mono-, di- and oligosaccharides; starch, and non-starch polysaccharides (NSP). These carbohydrates can be broken down in the litter by microbial action and further fragmented into alcohols, aldehydes, ketones and organic acids. Bacteria hydrolyses fat into fatty acids and alcohols. Some of the metabolic end products of these nutrients are volatile and odorous. The fresh droppings are less odorous but as they accumulate in the litter, the odour intensity gradually increases. The generation and emission of odour from litter are regulated by numerous factors such as microbial activity, excreta properties, litter moisture content, temperature and pH of litter, bird number, bird's breathe, flatus and faeces, diet, bird age, ventilation rate, climate, dust levels, and others (Dunlop and Gallagher, 2011). Microbial diversity in litter plays a major role in odour production along with environmental factors (Wadud, 2011) and therefore the conditions that favor the microbial activity in the litter are likely to increase odour emissions. Diet regulates the amount of substrates that are deposited in the litter and thus affects the litter quality (moisture, pH) and microbial activity in the litter. There is a wide range of microbes in meat chicken litter that metabolize the undigested component in the diets to odorous metabolites. The most abundant bacterial genus includes *Lactobacillus, Staphylococcus, Brevibacterium, Brachybacterium, Jeotgalicoccus, Facklamia* and *Atopostipes* (Wadud, 2011). The biochemical basis of microbial odorant production was reviewed by Le et al. (2005), Hobbs et al. (2004), Mackie et al. (1998) and Spoelstra (1980). By understanding the origin of odorous metabolites in the gut and in the litter, it may be easier to develop odour mitigation strategies at the source.

1.4.1 Sulfur containing odorants

The major sulfur containing odorants emitted from meat chicken farms are methyl mercaptan, ethyl mercaptan, propyl mercaptan, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, carbonyl sulfide, carbon disulfide and H_2S (Jiang and Sands, 2000). The production of these sulfur compounds has been reported to occur by either metabolism of sulfur containing amino acids (methionine, cysteine and cysteine) or by sulfate reduction. When excreta is stored anaerobically, sulfur containing amino acids in the excreta are broken down by micro-organisms into sulfides and mercaptans (Mackie et al., 1998). Dimethyl disulfide is the oxidation product of methyl mercaptan and dimethyl trisulfide is produced by the oxidation of H_2S and methyl mercaptan (Chavez et al., 2004). Microbes can also utilize cysteine to form carbonyl sulfide (Banwart and Bremner, 1975). Recently, Wadud (2011) reported a strong positive correlation of *Lactobacillus spp.* with the production of methyl mercaptan and dimethyl disulfide in the litter which is thought to occur by the catabolism of methionine (Seefeldt and Weimer, 2000). Similarly, *Proteus mirabilis* and *Staphylococcus cohnii* were found to produce dimethyl disulfide.

Sulfates may be supplied as additives in meat chicken diets (eg. the use of copper sulfate as a growth promotor, calcium sulfate as an acidifying agent or lysine sulfate as a source of synthetic amino acid) or by depolymerisation and desulfation of endogenously produced sulfated glycoproteins such as mucins or glucosinolates in canola meal and other non-sulfate compounds with sulfur. During sulfate reduction, H_2S is produced by either an assimilatory or a dissimilatory pathway. In the assimilatory process, bacteria produce enough reduced sulfur for the biosynthesis of cysteine and methionine whereas, in the dissimilatory process, sulfate is used as an

electron acceptor to produce sulfides (Mackie et al., 1998). The bacteria that are sulfate reducers belong to the genera *Desulfovibrio, Desulfotomaculum, Desulfobacter, Desulfococcus, Desulfonema, Veillonella, Megasphaera,* and *Enterobacteria (E.coli* and *Salmonella)* (Mackie et al., 1998). According to Spoelstra (1980), sulfate-reducing bacteria also produce trace amounts of carbonyl sulfide, carbon disulfide, and methyl, ethyl and propyl mercaptans.

1.4.2 Indoles and phenols

Phenol, p-cresol, 3-methyl phenol (m-cresol), and 4-ethylphenol are important representatives of phenolic compounds, whereas indole and skatole (3-methyl-indole) are indolic compounds. The fermentation of tyrosine, which is a non-essential amino acid, gives rise to phenol, p-cresol, 4-ethyl phenol. Similarly, fermentation of phenylalanine produces phenyl acetate and phenyl propionate whereas indole and 3-methyl indole are the principal end products of tryptophan metabolism (Macfarlane and Macfarlane, 1995). Various intestinal microbes are involved in the metabolism of these aromatic amino acids which includes the members of the genera *Bacteroides, Lactobacillus, Clostridium, and Bifidobacterium.* Wadud (2011) showed that *Atopostipes spp.* are positively correlated with the production of phenol in wet litter conditions.

1.4.3 Volatile amines and ammonia

The volatile amines emitted from meat chicken farms include methyl amine, dimethyl amine and trimethyl amine. Deamination and decarboxylation of amino acids give rise to amines and NH_3. In the absence of a carbohydrate source in the caecum of meat chickens, sources of protein material are fermented and metabolized to salvage energy but the fermentation of protein/amino acids also produce odorous metabolites (Mackie et al., 1998). In the gastrointestinal tract and in excreta at a pH of 6-7, deamination of amino acids may occur by the bacteria belonging to the genera *Bacteroides, Prevotella, Selenomonas, Butyrivibrio, Lachnospira, Eubacterium, Fusobacterium, Clostridium, Peptostreptococcus, and Acidaminococcus* (Mackie et al., 1998). Decarboxylation of amino acids occurs at a pH of 5-6 by the bacteria belonging to the genera *Bacteroides, Bifidobacterium, Selenomonas, Streptococcus,* and *Enterobacteria.* Ammonia can be odorous near the litter surface or inside the farm but it is highly volatile and easily gets diluted in the atmosphere once it is emitted out of the farm. Thus, ammonia is not generally considered as a significant component of odour downwind from a meat chicken farm.

1.4.4 Volatile fatty acids, alcohols, aldehydes and ketones

The major odorous volatile fatty acids produced in the caeca and in the litter of meat chickens include acetic acid, propionic acid, butyric acid, isobutyric acid and isovaleric acid. In a review by Svihus et al. (2013), the function and nutritional roles of the avian caeca has been described. The undigested nutrients in the small intestine such as starch, protein and fibre, when in a suitable form, may pass into the caeca and get fermented by the bacteria to produce volatile fatty acids. These undigested nutrients may further get excreted and fermented to produce volatile fatty acids from the litter.

The major odorants belonging to the group of alcohols include 1-butanol, 2-butanol, 3-methyl-1-butanol and those belonging to the group of aldehydes and ketones include 2-butanone, 2,3-butanedione, 3-hydroxy-2-butanone and 3-methyl-butanal. Most of these metabolites including acetic acid, propionic and butyric acids are carbohydrate fermentation products produced in the litter by *Lactobacillus spp.* (Wadud, 2011). The production of butyric acid with *Clostridium spp.* as a major end product of sugar fermentation has also been reported (Zhu, 2000). Butyric acid and 3-hydroxy-2-butanone have also been reported to be produced by *Clostridium spp.* in the litter (Wadud, 2011). Among *Bacillus spp., Bacillus hackensackii* has been reported to produce 2,3-butanedione and 3-hydroxy-2-butanone during 2,3-butanediol fermentation; *Bacillus subtilis* to 2-butanone, acetic acid and butyric acid; and *Bacillus amyloliquefaciens* to 2,3-butanedione, 3-hydroxy-2-butanone and butyric acid. Similarly, *Proteus mirabilis* was found to produce 2,3-butanedione, 3-hydroxy-2-butanone and 3-methyl-1-butanol (Wadud, 2011).

Taken together, there is a diverse microbial community in meat chicken litter and these microbes metabolize the undigested nutrients in litter to volatile organic and inorganic compounds some of which are odorous.

1.5 Nutritional manipulation of odour emissions

Odour emissions are a normal part of chicken meat production but they potentially threaten the sustainable development of the chicken meat industry. There are currently no effective methods to reduce odour emissions that are practical or affordable for use in commercial farms. Diets can be formulated to more closely meet the bird's nutritional requirements to avoid overfeeding and to reduce excretion of undigested components. This will decrease the amount of substrates that the microbes metabolize to odorous compounds (Mackie et al., 1998). Thus, diet can play a significant role in controlling odour issues from meat chicken farms (McGahan et al., 2002) by reducing the production of odorous metabolites in the gut and in the litter. Diet can also affect gut microflora (Yang et al., 2009), faecal microflora (Jung et al., 2008), litter moisture content (Francesch and Brufau, 2004), litter pH (Ferguson et al., 1998) and litter water activity (van der Hoeven-Hangoor et al., 2014) all of which may affect the emission of odorants from litter.

1.5.1 Supplemental methionine source, dietary sulfur content and odour

Methionine is an essential sulfur containing amino acid and is the first limiting amino acid in meat chicken diets. All commercial diets have an added synthetic methionine to satisfy the methionine (or methionine+cysteine) need of birds. The sulfur containing amino acids are also the major source of sulfur in the diet. As summarised in Table 1.1, most of the sulfur compounds have very low odour detection threshold and it is logical to assume that any changes in methionine source, methionine level or sulfur level in diets will affect the emissions of sulfur containing odorants and overall odour intensity. Chavez et al. (2004) reported the role of supplemental dietary methionine sources on odour emissions from meat chicken excreta using GC-MS. They compared a total of 5 treatment diets, one with no supplemental methionine and the others with supplemental dl-methionine or dry methionine hydroxy analogue (MHA) or liquid

MHA or sodium methioninate aqueous solution (NaMet). They found that the supplemental methionine sources significantly influenced sulfur containing odorants in the excreta. The concentrations of H_2S, carbonyl sulfide and dimethyl disulfide emissions were higher in the birds fed NaMet as compared to those fed D,L-methionine or dry methionine hydroxy analogue (MHA) or liquid MHA. Liquid MHA produced lower emissions of methyl mercaptan and dimethyl disulfide compared to other supplementary methionine sources. These results suggest that NaMet may not be desirable and that liquid MHA may reduce odour impacts in meat chickens. It may be speculated that the diets which contained NaMet had higher Na level that increased the dietary electrolyte balance resulting in higher litter moisture and emission of sulfur compounds. Unfortunately, the Na level in the diets were not measured in their study but there was no difference in excreta moisture content between the treatments. Additional studies on methionine sources may be necessary to investigate other possible odorants including the sulfur compounds. In an experiment with growing pigs, Le et al. (2007a) found that supplementing sulfur containing amino acids (methionine and cysteine) in surplus of the requirement increased odour emissions, odour intensity and reduced odour hedonic tone (more unpleasant) from the air above the manure pits. High odour emissions due to increased level of sulfur amino acids were thought to be due to the availability of precusors for the formation of H_2S, methyl mercaptan, dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide in manure and in air.

Shurson et al. (1999) studied the effect of using low and high sulfur diets for pigs and found that low sulfur diets reduced the emission of H_2S by 30 % without affecting the performance. Similarly, Whitney et al. (1999) reported the tendency of a low sulfur piglet diet to reduce H_2S emissions from the stored manure. These findings are in line with the finding of Wu-Haan et al. (2007) who observed increased production of H_2S from laying hen manure and that was thought to be due to the use of calcium sulfate as an acidifying agent in the diet. These experiments suggest that both methionine source and sulfur content in the diets may have an impact on odour emissions. However, different results were obtained by Wu-Haan et al. (2010) in an experiment with distillers dried grains with solubles (DDGS) as a feed ingredient in laying hens. In that study, laying hens were fed 0, 100, and 200 g/kg DDGS diets that contained 2.2, 2.7, and 4.2 g/kg sulfur respectively. The results showed that feeding the diet containing 200 g/kg DDGS to laying hens at 21 to 26 weeks of age reduced daily NH_3 emissions (mg/g of N intake) by 24 % and H_2S emissions (mg/g of S intake) by 58% compared with emissions from hens fed diets containing 0 to 100 g/kg DDGS although total sulfur content in excreta was higher in the hens fed 20% DDGS diet. This suggests that a high sulfur diet (200 g/kg DDGS diet) fed to laying hens for a short period of time (5 weeks) will decrease NH_3 and H_2S emissions. However, the effect of feeding higher DDGS to laying hens on long term emissions from stored manure remains to be determined. In meat chickens and laying hens, the requirements of dietary sulfur have not been established. It is thus important to produce a reliable database of sulfur content in feed ingredients and run experiments with graded levels of dietary sulfur to investigate its effect on performance, litter quality and odour. The use of low sulfur feed ingredients, monitoring of sulfate content in drinking water, replacement of supplemental lysine sulfate by lysine hydrochloride, copper sulfate by copper chloride, calcium sulfate by other acidifying agents may be ways to reduce odour emissions. Thus, there may be an opportunity to reduce sulfur and sulfate content in diets and

drinking water to minimize the emissions of sulfurous compounds from meat chicken excreta.

1.5.2 Enzymes and odour

Enzymes are used in commercial feed to enhance the digestion of nutrients, improve utilization of phytate P and lower the excretion of nutrients in the environment. The benefits of adding pentosanase (xylanase) to meat chicken diets based on viscous grains, such as wheat, barley and rye, to improve their nutritive values have been well known (Choct, 2006). The non-starch polysaccharides (NSP) in wheat, particularly the arabinoxylan component, depress the digestion of starch, protein and fat (Choct and Annison, 1992) and reduce the nutritive value of these diets. The enzymes cleave the large molecules of NSP into smaller polymers within the gut of meat chicken, thereby reducing the thickness of gut contents and increasing the nutritive value of the feed (Bedford et al., 1991; Choct and Annison, 1992). Some forms of xylanase can lower the excreta moisture content (Choct et al., 2004). Owing to these properties of xylanase, it can be argued that the addition of xylanase in the diets rich in NSP reduces nutrient excretion and odour emissions. In agreement with this hypothesis, McAlpine et al. (2012) reported that the pigs offered wheat based diets containing xylanase had a reduced total VFA concentration, acetic:propionic acid ratio and manure odour emissions decreased by 54 % compared to those fed diets without xylanase. In another study, the combined effect of xylanase and β-glucanase supplementation in diets for growing-finishing pigs was investigated by Garry et al. (2007). In their study, the addition of enzyme increased odour emissions but had no effect on NH_3 emissions in the barley-based diet but the enzyme combination had no effect on odour but decreased NH_3 emissions in the wheat based diet. Taken together, the studies on the effect of xylanase enzyme on odour emissions have only been conducted in pigs and the results so far are inconsistent. A recent study has shown that a combination of xylanase, amylase and protease enzymes reduced greenhouse gas emissions by 5-9 % from chicken meat production (Bundgaard et al., 2014).

Proteases are used singly or in combination with other enzymes in meat chicken diets. The use of proteases in meat chicken diets has been reported to increase the apparent ileal digestibilities of energy, fat, CP and amino acids (Dosković et al., 2013) thus resulting in less excretion of nutrients. Proteases may affect efficacy of other enzymes present in the diet and *vice-versa*. It was reported that pigs offered diets containing protease had increased molar proportions of isobutyric acid, isovaleric acid, and branched-chain fatty acids and NH_3 emissions from excreta compared to those without protease supplementation. But when protease and xylanase were added together in the diet, there was no effect on NH_3 emission (McAlpine et al., 2012). It is therefore important to conduct research using both single enzyme and combinations of enzymes to study odour emissions. Enzymes indeed improve digestion of feedstuffs and thus will reduce the excretion of nutrients allowing fewer substrates to be available for microbes to convert them to odorous metabolites. More research is warranted to fully understand the role of enzymes on odour emissions from meat chickens.

It is a common practice to use phytase enzyme in meat chicken diets to improve nutrient utilization. Phytase is commonly used in meat chicken diets to degrade the

phytate content, thus reducing its anti-nutritional effects, which include reduced nutrient digestion, increased endogenous losses of nutrients and decreased performance (Woyengo and Nyachoti, 2013). Phytase at higher than usual levels produces extra-phosphoric effects in meat chickens, such as improved energy, amino acid and P digestibility (Amerah et al., 2014, Cowieson et al., 2011), and enhanced growth and feed efficiency (Walk et al., 2014). However, phytase has sometimes been associated with increased litter moisture and reduced litter quality. According to Debicki-Garnier and Hruby (2003), fungal phytase at 1000 FTU/kg increased litter moisture at both 7 days and 21 days post-hatch. Phytase may increase water consumption and contribute to wet droppings when formulated into diets without using appropriate nutrient matrix values. Phytase is normally assigned nutrient matrix values for amino acids/protein and energy in addition to Ca and P [*and Na?*] to incorporate it into least cost formulations (Shelton et al., 2004). The level of Na in basal diets and Na matrix for phytase are often overlooked during formulation. High dietary Na and/or Ca levels may trigger increased water consumption and hence diuresis and wet litter (Collett, 2012). Phytase added at higher rates without lowering dietary Na and/or Ca may consequently affect litter quality. It is, therefore, important to study the effect of phytase on litter quality [*or on water consumption?]* and odour emissions together with performance, especially when used at higher levels. In experiment 1, an experiment is described that investigated the effects of graded levels of phytase in nutritionally adequate and lower specification ('downspec') wheat based meat chicken diets on performance, excreta/litter quality and odour emissions (Sharma et al., 2016b).

1.5.3 Ingredient source and odour

It may be argued that change in protein source in meat chicken diets can affect odour emissions partly because of the difference in digestibilities of protein meals that can affect the post excretion decomposition pattern. There is scant information available on the effects of different protein sources in meat chicken diets on odour emissions. In a study with growing–finishing pigs, van Heugten and van Kempen (2002) reported a high manure odour concentration with the addition of feather meal up to 120 g/kg in the diet that contained fish meal. This may be related to reduced digestibility and higher sulfur content of feather meal that contributed to a higher dietary total sulfur content. Soybean meal (SBM), canola meal (CM) and meat and bone meal (MBM) are three commonly used protein sources in meat chicken diets worldwide. Soybean and CM contain antinutritional factors, such as indigestible oligosaccharides, phytate and glucosinolate, that impede the digestion of nutrients. There are reports that show increased litter moisture with diets based exclusively on vegetable protein sources (Eichner et al., 2007; Hossain et al., 2013). Similarly, MBM is an animal by-product that varies widely in nutritional composition, contains a lower level of digestible protein and amino acids than SBM and has an unpleasant smell that could contribute to odour. It is also known that CM contains high levels of sulfur compared to SBM and MBM and the high inclusion of CM in the diet can increase total dietary sulfur and may partially contribute to sulfurous odour as described before. Thus, it is of interest to study and compare litter odorants associated with diets varying in protein sources. The effect of using high levels of SBM, CM and MBM in meat chicken diets on odour emissions has been investigated (Sharma et al., 2015) and is described in experiment 2. The influence of dietary feather meal inclusion on odour has not been reported.

DeCamp et al. (2001) reported a 32% reduction in H_2S and an 11% reduction in odour concentration when soybean hulls were added to pig diets. Gralapp et al. (2002) reported no difference in odour concentration when finishing pigs were fed diets added with 100 g/kg DDGS compared to those that were fed diets with either 0 or 50 g/kg DDGS. Pigs offered oat-based diets exhibited 56% reductions in manure odour emissions compared to pigs offered barley based diets (O'Shea et al., 2010). It has been suggested that increasing saccharolytic bacterial species such as *Bifidobacterium* and *Lactobacillus spp*. in the caecum and colon of pigs may suppress the formation of odorous metabolites of protein degradation (O'Shea et al., 2010). These findings may have implications in poultry nutrition as the protein content in meat chicken diets are higher than pigs and thus the inclusion of some forms of additives in meat chicken diets to promote *Bifidobacterium* and *Lactobacillus spp*. may reduce odour emissions. Thus, it is plausible that ingredient selection while formulating diets may promote or prevent the formation of specific odorants in excreta.

1.5.4 Low protein diet and odour

There has been some renewed interest in feeding low CP diets to meat chickens. This is mainly because of the availability of synthetic amino acids that are limiting in low protein diets such as arginine, isoleucine, valine and tryptophan at competitive prices. In addition, feeding low CP diets decrease nitrogen excretion and NH_3 emissions, reduce litter moisture and decrease litter pH (Ferguson et al., 1998; Gates 2000; Bregendahl et al., 2002). These conditions reduce footpad dermatitis (Nagaraj et al., 2007), nasal and eye lesions and improve bird welfare. Studies have indicated that dietary CP levels could be safely reduced by 20 g/kg in starter, grower and finisher phases without affecting the performance of birds when crystalline essential amino acids are used (Aftab et al., 2006). But price and availability of crystalline amino acids (arginine, isoleucine and valine) were major factors during the last decade that affected commercial acceptance of low CP diets. The CP levels in the diet may further be reduced beyond 20 g/kg without affecting meat chickens performance with the addition of specific non-essential amino acid glycine as reported by Dean et al. (2006) and Fatufe and Rodehutscord (2005). All these benefits may add to the future commercial potential of feeding low CP diets to meat chickens.

There is hardly any information on the effect of feeding low CP diets on odour emissions from meat chickens. Studies in pigs have shown reduced methyl sulfide, carbon disulfide, ethanethiol, phenol, 4-ethyl phenol, indole and 3-methylindole concentrations in manure by lowering CP in the diet (Le et al., 2007b). Similarly, Hobbs et al. (1996) observed that five out of ten odorous compounds in the manure of growing pigs and nine out of ten odorous compounds in the manure of finishing pigs declined when pigs were fed reduced CP diet with supplemented AA, compared with pigs fed a standard commercial diet. Cho et al. (2015) found decreased levels of phenols, indoles, short chain fatty acids (SCFA) (acetic acid, propionic acid, butyric acid) and branched chain fatty acids (isobutyric acid and isovaleric acid) in manure of the pigs fed 150 g/kg CP diet compared to those fed 200 g/kg CP diet. However, the response of malodour with a change in dietary CP level has been variable in pigs. Otto et al. (2003) reported no reduction in manure odour but ammonia concentration was reduced by feeding a low CP diet to pigs. An argument can be made that when low CP

diets are formulated with highly digestible protein meals (eg. SBM) odour emissions are reduced but when low CP diets are formulated with less digestible ingredients (eg. DDGS, cottonseed meal, sunflower meal, copra meal, palm kernel cake, canola meal, etc), odour emissions may increase. Thus, ingredient effects may be more pronounced than CP levels on odour emissions. This was also speculated by Jha and Berrocoso (2016) who mentioned that factors other than dietary CP levels such as protein and starch fermentation and dietary fibre source might modify the production of odorants from swine manure. Thus, when low CP diets are formulated with less digestible ingredients, it may be desirable to use a suitable combination of enzymes that can degrade fibre and protein fractions to a greater extent. It is also important to apply appropriate nutrient matrix values for those enzymes to provide desired nutrients to the birds without excesses.

Low CP diet studies in meat chickens are mainly focused on NH_3, which as previously described, is not a significant contributor of odour but is rather an irritant for shed operature and birds. It is well understood that a low CP diet fed to meat chickens reduces the concentration of putrefactive metabolites in the caeca such as NH_3, amines, phenols, indoles, skatole, cresol and branched chain fatty acids (Qaisrani et al., 2015). A low CP diet formulated to provide all the required amino acids without excesses may reduce putrefaction of animal waste and hence the production of toxic and odorous metabolites in the litter. The effect of feeding a low CP diet supplemented with crystalline amino acids on odour emissions in meat chickens has been investigated (Sharma et al., 2016b) and is described in experiment 3.

1.5.5 Dietary minerals, enteric disease, wet litter and odour

Litter is considered to be the primary source of odour because the majority of odorants are released during the decomposition of organic matter (Hobbs et al., 2004; Mackie et al., 1998). Dry and friable litter conditions are desirable in commercial poultry farms (Dunlop et al., 2016a). When the litter is wet or birds are infected with enteric disease or both, there are more complaints on odour (Poernama, personal communication, 2015). Dr Poernama is Techncial Director for Japfa Comfeed, Indonesia. The common enteric diseases in meat chickens are *Clostridium perfringens* (*Cp*) induced necrotic enteritis (NE), *E. coli*, coccidiosis and other forms of dysbacteriosis. Dietary factors such as energy source (cereal type), protein source and protein levels may predispose birds to NE (Annett et al., 2002; Drew et al., 2004). Sub-clinical forms of NE can lead to sticky droppings and dark and moist litter (Kaldhusdal and Hofshagen, 1992). Acute forms of NE may lead to diarrhea (Helmboldt and Bryant, 1971) and if NE affected birds produce sticky droppings, diarrhea and wet litter, then NE may exacerbate odour emissions. The odorants that are emitted from wet litter or NE affected flock have not been investigated while this is important to allow nutritionists to target combating their negative effects through using feed or litter additives in odour abatement studies. In experiment 3, the correlation study between litter moisture content and odorants (Sharma et al., 2016a) has been described, whereas the effect of feeding high sodium diet (wet litter condition) and *Cp* challenge on odour emissions in meat chickens has been described in experiment 4. It was also hypothesized that *Cp* itself produce a wide range of volatile odorous metabolites in different growth media. Thus, an in-vitro study was carried out (experiment 5) to test this hypothesis. In this experiment, *Cp* was cultured in two commonly used broth media to study the volatile odorous metabolites produced by it.

1.5.6 Feed additives and odour

Feed additives such as in-feed antibiotics, probiotics, prebiotics, synbiotics, saponins, essential oils and organic acids are commonly used in meat chicken diets. The use of in-feed antibiotics in animal production has a long history of about 50 years (Dibner and Richards, 2005). There will be challenges if the chicken meat industry moves towards antibiotic free chicken meat production and decreases reliance on animal origin feedstuffs. There will be increased enteric disease, decreased performance, increased mortality, increased nutrient excretion, welfare issues and likely increased odour problems. One way to address these challenges is to find alternative feed additives to in-feed antibiotics.

1.5.6.1 Probiotics, prebiotics and odour

Bacillus-based probiotics have been shown to improve performance, decrease pathogen load in the gut and reduce the headspace air concentration of NH_3 and H_2S in excreta (Jeong and Kim, 2014; Zhang et al., 2013). Addition of *B. subtilis* also showed improved nutrient retention, decreased caecal *Clostridium* and *Coliform* counts (Sen et al., 2012), reduced ileal, caecal and excreta *Salmonella* populations, increased *Lactobacillus* count in the ileum, caecum and excreta, and reduced *E. coli* counts in the excreta of meat chickens (Jeong and Kim, 2014; Park and Kim, 2014). Zhang et al. (2013) showed lower concentrations of NH_3 and H_2S in excreta from meat chickens fed diets supplemented with *B. subtilits*. In contrast, Hossain et al. (2015) observed no difference in H_2S and VFA emissions from excreta when meat chickens were fed diets supplemented with tri-strain probiotics consisting of *Bacillus subtilis*, *Clostridium butyricum*, and *Lactobacillus acidophilus*. Similarly, Upadhaya et al. (2015) observed no difference in faecal concentration of H_2S, total mercaptan and VFA in growing-finishing pigs when diets were supplemented with *Bacillus* based feed additive. However, NH_3 emissions were reduced in all these studies by the addition of probiotics. The difference in odour emissions other than NH_3 observed in these studies may be due to the difference in strains of bacteria used as probiotics and techniques used to measure odour. In meat chickens, it may concluded with confidence that *Bacillus subtilis* based probiotics can reduce NH_3 emissions but their effect on other odour emissions need further investigation. In experiment 3, the effect of feeding *Bacillus subtilis* based probiotic to meat chickens on odour emissions has been described. Chang and Chen (2003) reported the benefits of adding *Lactobacillus spp.* containing probiotics to lower meat chicken farm malodours. They found lower emission of 2-butanone, 1-butanol, dimethyl disulfide and dimethyl trisulfide in diets supplemented with lactobacillus containing probiotics as measured by GC-MS. Li et al. (2008) used synbiotics (combination of fructooligosaccharides and *Bacillus subtilis*) in a corn-soybean diet and found that diarrhoea in meat chickens was reduced by 58%. Thus, these in-feed additives have the potential to reduce odour emissions.

1.5.6.2 Saponins and odour

Saponins are natural detergents or surfactants found in a wide variety of plants. The major commercial saponin-containing products are those derived from *Yucca schidigera* and *Quillaja saponaria* (Cheeke, 2009). A blend of *Yucca* and *Quillaja* saponin improved growth and feed efficiency in meat chickens (Cheeke, 2000) and reduced NH_3 emissions in laying hens (Chepete et al., 2012). It has been reported that *Yucca* extract reduced faecal odours in dogs and cats and altered the chemical array of faecal volatiles (Lowe and Kershaw, 1997; Lowe et al., 1997). The researchers mentioned that some components of *Yucca* extracts may directly bind odorants. They also noted that the addition of *Yucca* extracts to dilute aqueous solutions of dimethyl sulfide, dimethyl disulfide, indole and skatole reduced the degree of offensiveness. Cheeke (2009) reported that the polyphenol component of *Yucca* was capable of directly binding NH_3 and H_2S thus reducing their emissions in animal production facilities. Saponin has also been reported to inhibit microbial fermentation of protein (Cheeke, 2000). All these properties of saponin may reduce odour emissions in chicken meat production. Experiment 3 describes the effect of feeding saponin on odour emissions in meat chickens.

1.5.6.3 Phytogenic feed additives, fermented products and disinfectants on odour

Other additives have been investigated as alternatives to in-feed antibiotics for their growth promoting effect and odour reduction potential. Li et al. (2015) investigated the effect of dietary phytonsine, phytogenic feed additive extracted from Korean pine, as an alternative to tylosin on growth performance and excreta gas emissions in meat chickens. The gaseous emissions were measured using a gas sampling pump (Model GV-100, GASTEC Corp. Japan). The results showed that phytonsine improved meat chickens performance and decreased the concentration of excreta NH_3, total mercaptans, acetic acid and H_2S linearly with its increasing levels. Based on these findings, the authors suggested that phytonsine could be used as an alternative to tylosin to improve performance and decrease excreta gas emission in meat chickens.

Williams et al. (2013) reported that the addition of rice-soy fermentate products (BioWISH odour and BioWISH aqua, BioWISH technologies, Naperville, IL, 60563) at 900g/ton of meat chicken diets resulted in lower emissions of 4-ethyl phenol, para-cresol, indole, skatole, valeric acid, and hexanoic acid as compared to the diet that did not contain rice-soy fermentate products. Chlorine dioxide (ClO_2) is a disinfectant that has been reported to be effective against bacterial contamination of meat chicken carcasses (Berrang et al., 2011). The bactericidal action of ClO_2 is related to its ability to alter or disrupt the cell membrane permeability and penetrate into the cell to disrupt the protein synthesis (Noszticzius et al., 2013). Recently, Ahmed et al. (2015) reported that the supplementation of 1 g/kg ClO_2 in meat chicken diets resulted in reduced excreta pH and lower emissions of H_2S, sulfur dioxide and mercaptans. The reduction in emissions of these sulfurous odorants was thought to be due to the reduction in the number of sulfate-reducing bacteria such as *E. coli* and *Salmonella* in the excreta. In their study, there was no effect of ClO_2 on body weight gain of birds but feed intake (FI) decreased resulting into better feed conversion ratio (FCR). Thus, feed additives may be useful for reducing odour emissions in chicken meat production.

2. Objectives

The aim of this project was to determine the role of diets on odour emissions from meat chickens. The key areas of research in this study were:

1. To examine the effect of phytase enzyme on performance, water to feed intake ratio, litter quality and odour emissions in meat chickens.
2. To examine the effect of different protein source in diets on water to feed intake ratio, litter quality and odour emissions in meat chickens.
3. To examine the effect of protein levels in diets on performance, litter quality and odour emissions in meat chickens.
4. To examine the effect of feed additives (antibiotic, probiotic and saponin) on performance and odour emissions in meat chickens.
5. To examine the effect of high salt diet (wet litter) and necrotic enteritis challenge on odour emissions in meat chickens.
6. To examine the odorants generated by *Cp* cultured in two broth media.

3. Methodology

3.1 Collection and measurement of odorants

The project explored various odour measuring technologies and developed suitable procedures and methods for odour collection and measurements. As the project progressed, more sophisticated equipment was used to measure specific odorants in real time. An initial pilot study was conducted in collaboration with University of New South Wales (UNSW) using thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS). The TD-GC-MS method was not effective as there was potential for sample loss during collection, storage and long distance transport with long delays in reporting of analytical results. The results of this pilot study are presented in Appendix section at the end of this report.

For subsequent experiments, odorants were measured using SPME-GC-MS (experiment 1), FTIR (experiment 2), SIFT-MS (experiments 3, 4 and 5). The detailed methods of odour measurements are described in the "materials and methods" sections of each experiment.

3.2 Effect of diets on odour emissions

Four experiments were conducted to study the effect of diets on odour emissions. The fifth experiment was conducted to study the odorants generated by *Cp* cultured in two broth media. The methods used in these experiments are described in the "materials and methods" sections of each experiment.

4. Experiment 1

Title: Performance, litter quality and gaseous odour emission of meat chickens fed phytase supplemented diets

4.1 Introduction

Phytase is commonly used in meat chicken diets to degrade the phytate content, thus eliminating its anti-nutritional effects, which include reduced nutrient digestion, increased endogenous losses of nutrients and decreased performance (Woyengo and Nyachoti, 2013). The degradation of phytate at ileal or total tract level has been shown to be incomplete at the standard 500 FTU/kg inclusion rate of phytase (Selle and Ravindran, 2007). Phytase at higher than usual levels produces extra-phosphoric effects in meat chickens, such as improved energy, amino acid and P digestibility (Amerah et al., 2014; Cowieson et al., 2011), and enhanced growth and feed efficiency (Walk et al., 2014). A recent study showed improvements in litter quality with higher doses of phytase (Bedford et al., 2015), although some reports suggest that phytase is associated with wet litter (Debicki-Garnier and Hruby, 2003; Bedford et al., 2012). Phytase may increase water consumption and contribute to wet droppings when formulated into diets without using nutrient matrix values. Phytase is normally assigned nutrient matrix values for amino acids/protein and energy in addition to Ca and P to incorporate it into least cost formulations (Shelton et al., 2004). The level of Na in basal diets and Na matrix for phytase are sometimes overlooked during formulation. High dietary Na and/or Ca levels may trigger increased water consumption and hence diuresis and wet litter (Collett, 2012). Phytase added at higher rates without lowering dietary Na and/or Ca may consequently affect litter quality.

Wet litter and odour issues from poultry farms have become prominent worldwide. It is important to maintain litter quality to promote bird health and welfare and to minimize odour emissions. Litter quality may deteriorate due to increased excretion of minerals by birds (van der Hoeven-Hangoor et al., 2013). Phytate can bind with minerals and protein in the diet making them unavailable for digestion (Cowieson et al., 2006; Selle et al., 2012). Phytase may therefore potentially improve litter quality by increasing the bioavailability of minerals and protein. Excreta/litter quality is generally measured by water activity, moisture and free water contents (van der Hoeven-Hangoor et al., 2014). The amount of water in the litter has been found to affect microbial activity (Himathongkham et al., 1999; Wadud et al., 2012), NH_3 emissions (Miles et al., 2011) and odorant emissions (Sharma et al., 2016). It is, therefore, important to study the effect of phytase on litter quality and odour emissions together with performance, especially when used at higher levels. The aim of this study was to investigate the effects of graded levels of phytase in nutritionally adequate and downspec wheat based meat chicken diets on performance, excreta/litter quality and odour emissions.

4.2 Materials and methods

4.2.1 Animal ethics

All the experimental procedures were approved by the University of New England, Australia animal ethics committee (AEC15-011).

4.2.2 Bird husbandry, experimental design and diets

A total of 720 d-old Ross 308 male broiler chicks were assigned to eight dietary treatments with six replicates of 15 birds/pen in a 2 × 4 factorial arrangement with two diet types, positive and negative controls (PC, NC), and four doses of phytase (0, 500, 1,000 and 1,500 FTU/kg) (*Buttiauxella* phytase, Axtra PHY10000 TPT, Dupont Animal Nutrition). This phytase is thermostable up to 95°C during pelleting according to the supplier's information (Feedworks, Australia). The replications were allocated in a completely randomized design. The chicks (average weight of 37 ± 1 g) were weighed before placement to ensure no significant difference between treatments. Each pen measured 1.2 m × 0.76 m and consisted of a feeder and a double outlet cup drinker. Fresh pine shavings (Hysorb wood shavings, ECW, Australia) were used as bedding material and were added at 10.35 kg/pen. The PC diet was formulated to meet the 2014 Ross 308 nutrient specifications (Aviagen, 2014), whereas the NC diet was formulated to give the same nutrient specifications of the PC diet when using nutrient matrix values for 500 FTU phytase as recommended by the phytase supplier. The lower specifications applied to the NC diet was as follows: Ca (-1.4g/kg), available P (av. P, -1.5 g/kg), Na (-0.3 g/kg), amino acids (dig Lys -0.2 g/kg, dig M+C -0.17 g/kg, dig Thr -0.17 g/kg, dig Arg -0.16 g/kg) and MEn (-0.28 MJ/kg). These down-specifications were applied across the starter (0-10 d), grower (11-24 d) and finisher (25-35 d) periods. The composition of experimental diets and their calculated and anlaysed nutrients are presented in Tables 4.1 and 4.2. Zinc bacitracin (ALBAC 150, Zoetis) was included at the rate of 330 g/t of feed resulting in an active dose of 50 mg/kg during the starter period only. Salinomycin (SACOX 120) was included as a coccidiostat at 500 g/t of feed at all stages of growth resulting in a salinomycin activity level of 60 mg/kg feed. Phytase enzyme was added to both the PC and NC diets at four levels: 0; 50 (500 FTU/kg); 100 (1,000 FTU/kg); and 150 g/t (1,500 FTU/kg). Feed was mixed and pelleted at 65 °C at the University of New England, Australia. All feeds were fed in crumble form to 10 d and as 3 mm diameter pellets thereafter until finishing the 35 d study period. Feed and water were provided *ad libitum* throughout the study. Lighting program was provided according to the Ross 308 breed management manual (Aviagen, 2014).

Table 4.1 Ingredient composition and calculated nutrients of the experimental diets (as fed basis)

Item	Positive control			Negative Control		
	Starter	Grower	Finisher	Starter	Grower	Finisher
Ingredients, g/kg						
Wheat	572	587	645	607	621	675
Soybean meal	279	223	175	269	213	173
Meat meal	45	30	30	45	30	22
Canola meal	40	80	80	40	80	80
Canola oil	33	45	44	16	29	29

Dicalcium phosphate	8.5	8.7	6.8	0.3	0.6	0.3
Limestone	6.3	7.1	6.3	7.2	7.9	8.2
D,L-methionine	3.41	2.74	2.31	3.20	2.53	2.10
L-lysine HCl	3.03	2.70	2.50	2.95	2.62	2.40
NaCl	2.72	1.86	1.86	2.05	1.20	1.30
Premix[1]	2.00	2.00	2.00	2.00	2.00	2.00
L-threonine	1.96	1.47	1.17	1.83	1.33	1.02
Na bicarbonate	1.50	2.00	2.00	1.50	2.00	2.00
Choline Cl, 60%	0.69	0.69	0.57	0.65	0.65	0.54
Xylanase powder[2]	0.50	0.50	0.50	0.50	0.50	0.50
Salinomycin[3]	0.50	0.50	0.50	0.50	0.50	0.50
Zn bacitracin[4]	0.33	-	-	0.33	-	-
Titanium dioxide	-	5.0	-	-	5.0	-
Calculated nutrients, g/kg						
MEn, MJ/kg	12.55	12.97	13.18	12.27	12.69	12.90
Crude protein	233	213	197	232	212	194
dig lysine	12.8	11.5	10.3	12.6	11.3	10.1
dig met + cys	9.5	8.7	8.0	9.3	8.5	7.8
dig leucine	14.6	13.4	12.4	14.6	13.4	12.3
dig arginine	13.7	12.3	11.0	13.5	12.1	10.8
dig isoleucine	8.9	8.2	7.5	8.9	8.1	7.5
dig threonine	8.6	7.7	6.9	8.4	7.5	6.7
dig valine	9.9	9.1	8.3	9.8	9.0	8.2
Ca	9.6	8.7	7.9	8.2	7.3	6.5
Total P	7.7	7.1	6.6	6.2	5.6	5.0
Av. P	4.8	4.3	3.9	3.3	2.8	2.4
Na	2.1	1.8	1.8	1.8	1.5	1.5
K	9.4	8.6	7.8	9.3	8.5	7.8
Cl	3.0	2.3	2.3	2.6	1.9	1.9
Mg	2.0	2.0	1.8	1.9	1.9	1.9
S	2.3	2.3	2.1	2.3	2.3	2.1
dEB[5], mEq	267	251	229	263	247	227
dEB[6], mEq	287	274	252	280	267	249

[1]Vitamin-Mineral concentrate supplied per kilogram of diet: retinol, 1,2000 IU; cholecalciferol, 5,000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamine, 16 µg; biotin, 200 µg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg; Cu (sulfate), 16 mg; Fe (sulfate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulfate and oxide), 120 mg; Zn (sulfate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg.
[2] Feedzyme XBC 1000 (Feedworks, Australia), [3] Sacox 120 (coccidiostat), [4] Albac 150 (Zoetis), [5] dEB= Na^++K^+-Cl^-, [6] dEB= Na^++K^++Mg^{2+}-Cl^--S^{2-}.

Table 4.2 Analysed nutrient composition (g/kg) of the experimental diets

Nutrients	Positive control			Negative Control		
	Starter	Grower	Finisher	Starter	Grower	Finisher
Dry matter	90.1	90.4	91.1	90.1	90.3	90.9
Gross energy, MJ/kg	17.36	17.53	17.71	17.15	17.24	17.52
Crude protein	242.8	220.9	210.6	237.9	217.9	210.4
Crude fat	64.7	57.4	71.6	37.4	89.7	102.2
Crude fibre	24.4	25.6	25.6	26.3	24.9	24.9

Ca	10.8	10.4	10.0	8.7	8.1	7.8
Total P	7.2	7.1	6.2	5.8	5.4	5.2
Na	2.2	1.7	1.7	1.5	1.3	1.3
K	9.6	9.1	8.0	9.6	8.6	8.5
Cl	2.2	2.0	1.9	2.0	1.9	1.9
Mg	1.8	1.9	1.8	1.8	1.9	1.8
S	3.0	3.0	2.8	3.1	2.9	2.8

In short, a continuous lighting was provided during the first 48 hours of chicks arrival followed by a 1-hour darkness each day up to d 7 and gradually increased to 6 hours darkness from d 10.

4.2.3 Performance of birds

Birds, feed and water were weighed in all the pens on d 0. They were again weighed on d 10, 24 and 35 to calculate feed intake, water to feed intake ratio, weight gain and FCR (corrected for mortality). Water intake was measured by subtracting the final and initial weight of water in the 20 litre water tank in each pen. Two birds from each pen were euthanized by cervical dislocation on d 24 and the left tibias were removed to measure tibia weight, length, diameter and ash content. The tibia was defleshed according to the autoclave method as described by Hall et al. (2003) and was oven dried overnight at 105 ºC. Dried tibia samples were weighed and the length and diameter measured using a micrometer (Mitutyo No. 2050-08, Japan). Tibia were ashed at 600 °C to constant weight to calculate percentage tibia ash. Liver, spleen, bursa and small intestine were collected from the same birds to measure their weights. On d 35, 2 birds were sampled from each pen to measure the weights of breast meat, abdominal fat and empty small intestine (duodenum, jejunum and ileum).

4.2.4 Excreta and litter characteristics

Litter quality was observed and scored on d 35. Each pen was divided into two halves and litter quality was scored for each half of the pen and averaged. Three independent observers were involved in the litter scoring process to obtain the average value. Litter quality scores were taken visually, which ranged from 1-5, with 1 being extremely dry and no caked litter and 5 being total pen coverage of caked litter. On d 10, a total of 96 birds (2 birds/pen) were selected and transferred to metabolic cages in a climate controlled room with six replications of two birds per cage for each of the eight treatments. The birds were adapted in the cages for eight days and on d 18 fresh excreta samples were collected four times at two-hour intervals to measure moisture and free water content. The moisture content from fresh excreta was measured by subjecting 10 g of samples to forced air at 105 ºC for 48 h and measuring the weight difference before and after drying. Excreta free water content was measured according to the procedures described by van der Hoeven-Hangoor et al. (2013). In short, approximately 22 ± 0.3 g of pooled excreta was weighed in a pre-weighed centrifuge tube and centrifuged at 2,230 × *g* at 5 °C for 24 minutes (min). The supernatant was manually removed using a pipette and the tube was re-weighed. Excreta free water was calculated as the difference between the initial weight and weight post removal of the

supernatant and expressed as a percentage of the original excreta weight. Excreta was also collected daily from d 19-21 to measure the moisture content by following the same procedure as described above. In both cases, excreta was refrigerated at 4 °C immediately upon collection. Excreta pH was determined by mixing excreta and de-ionised water in the ratio of 1:5 and measuring the pH with a pH meter (EcoScan 5/6 pH meter, Eutech Instrument Pte Ltd; Singapore).

4.2.5 Solid phase micro-extraction (SPME) measurement of odorants

After the studies were finished with the cages, the same birds were sampled to study odorant emissions. At the age of 22 d, 45 birds of uniform body weight were selected out of 96 birds from the cages and adapted for 3 d to metabolic chambers to measure odour emissions (Sharma et al., 2015) and fed their respective test diets. The experiment was conducted as a completely randomized design with five treatments and three replications of three birds/replicate. The treatments were: PC, NC, NC + 500 FTU/kg, NC + 1,000 FTU/kg and NC + 1,500 FTU/kg phytase. The experiment started at d 25 and emissions were measured on d 39. Headspace samples from birds and litter were collected with 85 µm carboxen/polydimethylsiloxane SPME fibres (Supelco, Sigma-Aldrich, Australia) and analysed using an Agilent HP 6890 series GC system/5973 MSD, gas chromatography-mass spectrometry (GC-MS, California, USA) for identification of odorants. New fibres were conditioned according to the manufacturer's instructions. Before each sampling, the SPME fibre was desorbed in a GC injector for 5 min at 260 ºC, and then SPME collections were performed by direct fibre exposure in the dynamic headspace of the chambers for 20 min. This sampling time was selected based on preliminary tests of control headspaces with varying SPME sampling times; the 20 min sampling time consistently resulted in detectable amounts of odorants that are associated with meat chicken litter. Immediately after the sampling, the odorants were analysed using the GC-MS equipped with a 30 m polar capillary column, 0.25 mm internal diameter and 0.25 µm film thickness (Zebron capillary GC column, Phenomenex, Australia). The injector was set at 260 °C and the column was set at an initial temperature of 40 °C and final temperature of 240 °C. The carrier gas was helium. Mass/charge (m/z) ratio range was set between 40 and 300 amu (atomic mass unit). Spectra were collected at 5.36 scans/sec and electron multiplier voltage was 1,224 V. The detector was auto-tuned daily. The VOC were identified by matching mass spectra of unknown compounds with the spectra from the National Institute of Standards and Technology (NIST, USA) commercial MS library. Only the important odorous VOC in meat chicken farm that were reported by Murphy et al. (2014) were targeted.

4.2.6 Chemical analyses

Proximate analyses were conducted using AOAC methods (AOAC, 1990). Dry matter content of the diets and litter were determined by placing duplicate samples in a drying oven at 105 ºC for 24 h (AOAC, 1990). Gross energy content of the diets was determined on a 0.5 g sample using an adiabatic bomb calorimeter (IKA Werke, C7000, GMBH and Co., Staufen, Germany) with benzoic acid as a standard. Nitrogen content of the diets and excreta was determined on a 0.15 g sample with a combustion anlayser (Leco model FP-2000N anlayser, Leco Corp., St. Joseph, MI) using EDTA as a calibration standard. Crude protein was calculated by multiplying percentage N

by 6.25. Minerals in the feed were analysed using inductively coupled plasma optical emission spectrometer (ICP-OES, Model- 725 radial viewed) using perchloric acid and hydrogen peroxide for digestion of the samples (Anderson and Henderson, 1986). Phytase activity in the feed was measured according to the AOAC method 2000.12 (AOAC, 2005).

4.2.7 Statistical analysis

Performance data and excreta/litter parameters were anlaysed following a 2 × 4 factorial arrangement using JMP statistical software version 8 (SAS Institute Inc, Cary, NC) to test the main effects of diets, phytase and their interaction. Data were subjected to two-way ANOVA and means were separated by Tukey's HSD test at a probability level of 0.05. Odorant concentration measurements were analysed by one-way ANOVA and means were separated by Tukey's HSD test at a probability level of 0.05. The p-values above 0.10 were shown in short form as NS in the Tables.

4.3 Results

4.3.1 Feed composition and phytase activity

The NC diet contained more limestone and much less dicalcium phosphate than the PC diet. The analysed values for Ca and total P in the diets were slightly higher than the calculated ones but the trend was similar across the treatments (Table 4.2).

Table 4.3 Analysed phytase activity (FTU/kg)[1] in the experimental diets

Experimental diets	Starter (d 0 to 10)	Grower (d 11 to 24)	Finisher (d 25 to 35)
Positive control (PC)	400	379	456
PC + 500 FTU/kg of phytase	1,005	1,041	1,063
PC + 1,000FTU/kg of phytase	1,524	1,585	1,538
PC + 1,500 FTU/kg of phytase	2,050	2,063	2,145
Negative control (NC)	434	314	425
NC + 500 FTU/kg of phytase	1,016	1,007	1,011
NC + 1,000 FTU/kg of phytase	1,552	1,540	1,573
NC + 1,500 FTU/kg of phytase	2,063	2,193	2,006

[1]One unit of phytase activity (FTU) is defined as the quantity of enzyme that liberates 1µmol of inorganic P per minute from sodium phytate at pH 5.5 and 37 °C.

The analysed values for phytase activity in the PC and NC diets were close to the expected values when the background phytase levels were taken into account (Table 4.3).

4.3.2 Growth performance and water to feed intake ratio

The overall mortality during the entire study period was less than 4% and there was no diet related mortality ($P > 0.05$, data not shown). The effect of supplementing diets with increasing levels of phytase on bird performance is shown in Tables 4.4, 4.5 and

4.6. During the starter period (Table 4.4), the birds fed the PC diet had higher FI, higher body weight gain (BWG) ($P < 0.001$) and 3 points better FCR ($P < 0.01$) than those fed the NC diet. The diets supplemented with phytase improved BWG and FCR in the birds at all the levels compared to the diets without phytase ($P < 0.001$). There was no diet × phytase interaction during the starter period.

During the period of 0-24 d (Table 4.5) and 0-35 d (Table 4.6), diet by phytase interactions were observed for FI and BWG ($P < 0.01$). The birds fed the NC diet without phytase had lower FI and BWG compared to those fed the PC diet without phytase. The addition of phytase to the NC diet increased FI and BWG in a dose-dependent manner up to the level of 1,000 FTU/kg but the increment was lower at 1,500 FTU/kg compared to 1,000 FTU/kg on d 24 ($P < 0.01$). On d 24, when phytase was added to the PC diet, there was no effect on FI at any levels but BWG was higher by 4.2% at inclusion rates of 1,000 or 1,500 FTU/kg ($P < 0.01$). The birds fed the NC diet had 2 points higher FCR at d 24 compared to those fed the PC diet ($P < 0.01$). As the main effect, phytase improved FCR by 2 and 5 points at inclusion levels of 500 and 1,000 or 1,500 FTU/kg, respectively ($P < 0.01$).

On d 35, results followed a similar pattern in that phytase had a greater impact in birds fed the NC as compared to PC diet (Table 4.6). The effect of graded levels of phytase on water to feed intake ratio (WI:FI) is shown in Tables 4.4, 4.5 and 4.6. There was an interaction between diet and phytase on the WI:FI at 0-10 d, and 0-24 d ($P < 0.01$). On d 35, there was a tendency for this interaction to persist ($P = 0.06$). The birds fed the NC diet without phytase had a higher WI:FI at all stages of growth compared to those fed the PC diet without phytase ($P < 0.01$). At 0-24 d, the addition of phytase to the NC diet at all the levels reduced WI:FI of birds by 11.5%-13% ($P < 0.01$), but WI:FI was not affected when phytase was added at any level to the PC diet.

4.3.3 Carcass yield and tibia bone characteristics

There was no effect of diet on the relative weight of the liver, spleen, bursa, small intestine, breast meat or abdominal fat (Table 4.7). On d 24, the addition of phytase to the diets increased relative weight of the spleen at 500 FTU/kg ($P < 0.05$) but there was no further increase in weight at the higher levels of phytase. The addition of phytase to the diets also decreased the relative weight of the small intestine at 1,000 or 1,500 FTU/kg by 8% on d 24 ($P < 0.05$) but there was no effect on d 35 ($P > 0.05$). Phytase tended to increase ($P = 0.08$) breast meat yield on d 35 across the diets and the increment was 4.94% at a level of 1,000 FTU/kg. Phytase had no effect on the relative weight of liver, bursa or abdominal fat. There was no interaction between diet and phytase on any carcass or visceral organ parameters measured ($P > 0.05$).

Table 4.4 Performance and WI:FI of meat chickens fed nutritionally adequate (PC) and downspec (NC) wheat based diets supplemented with increasing levels of phytase (0-10 d)

Treatments	FI, g	BWG, g	Corrected FCR	WI, g	WI:FI
Diets					

NC	296[b]	246[b]	1.207[a]	639	2.16
PC	303[a]	258[a]	1.173[b]	649	2.15
SEM	2.14	2.07	0.008	9.87	0.03
Phytase, FTU/kg					
0	294	238[b]	1.239[a]	645	2.20
500	298	253[a]	1.181[b]	637	2.14
1,000	304	261[a]	1.167[b]	665	2.19
1,500	301	258[a]	1.173[b]	630	2.09
SEM	3.03	2.93	0.01	13.9	0.04
Dietary treatments					
NC + 0	286	232	1.233	660[abc]	2.31[a]
NC + 500	295	245	1.204	607[cd]	2.06[cd]
NC + 1,000	307	261	1.176	696[a]	2.27[ab]
NC + 1,500	297	246	1.207	594[d]	2.00[d]
PC + 0	303	244	1.242	630[bcd]	2.09[cd]
PC + 500	301	261	1.153	666[ab]	2.22[abc]
PC + 1,000	302	260	1.162	634[bcd]	2.10[bcd]
PC + 1,500	305	269	1.134	665[ab]	2.18[abc]
SEM	2.13	4.15	0.02	19.7	0.06
P-value					
Diets	<0.05	<0.001	<0.01	NS	NS
Phytase	NS	<0.001	<0.001	NS	NS
Diet × Phytase	NS	NS	NS	<0.01	<0.01

FI = feed intake; BWG = body weight gain; FCR = feed conversion ratio; WI= water intake.
[a,b,c,d] Means in the same column with different superscripts differ significantly ($P < 0.01$).

Table 4.5 Performance and WI:FI of meat chickens fed nutritionally adequate (PC) and downspec (NC) wheat based diets supplemented with increasing levels of phytase (0-24 d)

Treatments	FI, g	BWG, g	Corrected FCR	WI, g	WI:FI
Diets					
NC	1,620	1,262	1.281[a]	3,555	2.20
PC	1,690	1,336	1.260[b]	3,593	2.13
SEM	9.92	7.70	0.003	38.74	0.02
Phytase, FTU/kg					
0	1,575	1,212	1.297[a]	3,573	2.27
500	1,664	1,295	1.281[b]	3,560	2.14
1,000	1,704	1,357	1.254[c]	3,638	2.14
1,500	1,677	1,333	1.251[c]	3,524	2.10

SEM	14.02	10.89	0.004	54.78	0.03
Dietary treatments					
NC + 0	1,485[d]	1,129[d]	1.315	3,601	2.42[a]
NC + 500	1,632[c]	1,256[c]	1.299	3,495	2.14[b]
NC + 1,000	1,712[a]	1,362[a]	1.257	3,650	2.14[b]
NC + 1,500	1,650[bc]	1,302[b]	1.267	3,473	2.10[b]
PC + 0	1,665[abc]	1,296[bc]	1.285	3,545	2.13[b]
PC + 500	1,696[ab]	1,333[ab]	1.272	3,626	2.14[b]
PC + 1,000	1,695[ab]	1,351[a]	1.255	3,626	2.14[b]
PC + 1,500	1,703[ab]	1,364[a]	1.249	3,575	2.10[b]
SEM	19.83	15.39	0.005	77.48	0.04
P-value					
Diets	<0.01	<0.01	<0.01	NS	<0.05
Phytase	<0.01	<0.01	<0.01	NS	<0.01
Diet × Phytase	<0.01	<0.01	NS	NS	<0.01

FI = feed intake; BWG = body weight gain; FCR = feed conversion ratio; WI= water intake.
[a,b,c,d] Means in the same column with different superscripts differ significantly ($P < 0.01$).

The effect of graded levels of phytase on the length, weight, diameter and ash content of tibia bones of meat chickens are shown in Table 4.8. The birds fed the PC diet had an average of 1.4 mm longer tibias than those fed the NC diet ($P < 0.01$). Phytase increased tibia length by 2.7% and 3.4% when included in the diets at the levels of 500 and 1,000 FTU/kg, respectively ($P < 0.01$). There was a tendency of diet × phytase interaction ($P = 0.06$) on tibia length with a bigger response when phytase was added to the NC diet than to the PC diet. Neither diet nor phytase affected the diameter of the tibia bone ($P > 0.05$). There was an interaction between diet and phytase on percentage tibia ash ($P < 0.001$) and tibia ash weight ($P < 0.05$). The birds fed the NC diet without phytase had lower tibia ash weight ($P < 0.05$) and percentage tibia ash ($P < 0.001$) compared to those fed the PC diet without phytase ($P < 0.001$). The addition of phytase to the NC diet increased percentage tibia ash at all levels equivalent to that of birds fed the PC diet ($P < 0.001$) but there was no effect of phytase on percentage tibia ash when it was added to the PC diet. Phytase also increased tibia ash weight of birds when it was added to the NC diet at the levels of 1,000 or 1,500 FTU/kg ($P < 0.05$) but no such effect was observed when phytase was added to the PC diet.

Table 4.6 Performance and WI:FI of meat chickens fed nutritionally adequate (PC) and downspec (NC) wheat based diets supplemented with increasing levels of phytase (0-35 d)

Treatments	FI, g	BWG, g	Corrected FCR	WI, g	WI:FI
Diets					
NC	3481	2460[b]	1.388[a]	7580	2.18[a]
PC	3548	2538[a]	1.367[b]	7530	2.12[b]
SEM	36.69	26.18	0.005	116.1	0.02
Phytase, FTU/kg					
0	3367	2364	1.397[a]	7446	2.21[a]
500	3560	2527	1.383[ab]	7700	2.16[ab]

1,000	3573	2555	1.365[b]	7543	2.11[b]
1,500	3559	2549	1.364[b]	7531	2.12[b]
SEM	51.89	37.03	0.007	164.2	0.03
Dietary treatments					
NC + 0	3061[b]	2125[b]	1.440	7071[b]	2.31[a]
NC + 500	3625[a]	2555[a]	1.419	7927[a]	2.19[b]
NC + 1,000	3674[a]	2590[a]	1.419	7736[a]	2.11[b]
NC + 1,500	3565[a]	2569[a]	1.388	7585[ab]	2.13[b]
PC + 0	3474[a]	2460[a]	1.412	7400[a]	2.13[b]
PC + 500	3494[a]	2499[a]	1.398	7472[ab]	2.14[b]
PC + 1,000	3492[a]	2520[a]	1.386	7351[ab]	2.11[b]
PC + 1,500	3553[a]	2529[a]	1.405	7477[ab]	2.10[b]
SEM	73.38	52.36	0.01	232.2	0.04
P-value					
Diets	NS	<0.05	<0.01	NS	<0.05
Phytase	<0.05	<0.01	<0.01	NS	<0.05
Diet × Phytase	<0.01	<0.01	NS	0.05	0.057

FI = feed intake; BWG = body weight gain; FCR = feed conversion ratio; WI= water intake.
[a,b,c,d] Means in the same column with different superscripts differ significantly ($P < 0.01$).

Table 4.7 Carcass yield and relative weights of visceral organs (g/kg) of meat chickens fed nutritionally adequate (PC) and downspec (NC) wheat based diets supplemented with increasing levels of phytase

Treatments	Liver	Spleen	Bursa	Small intestine[1]		Breast meat	Abdominal fat
		d 24		d 24	d 35	d 35	d 35
Diets							
NC	26.2	0.79	1.77	31.3	21.3	201.2	9.65
PC	26.0	0.81	1.75	31.0	21.6	199.8	10.30
SEM	0.31	0.03	0.07	0.51	0.54	2.39	0.292
Phytase, FTU/kg							
0	26.1	0.70[b]	1.72	32.3[a]	22.9	194.5	9.70

500	26.3	0.84[a]	1.75	32.7[a]	21.0	198.5	9.90
1,000	26.1	0.87[a]	1.85	29.9[b]	20.7	204.1	10.1
1,500	25.7	0.79[ab]	1.74	29.9[b]	21.2	205.0	10.2
SEM	0.44	0.04	0.10	0.75	0.76	3.68	0.41
P-value							
Diet	NS	NS	NS	NS	NS	NS	NS
Phytase	NS	<0.05	NS	<0.05	NS	0.08	NS
Diet × Phytase	NS	NS	NS	NS	NS	NS	NS

[a,b] Means in the same column with different superscripts differ significantly ($P < 0.01$).
[1]Empty weight of duodenum, jejunum and ileum relative to live body weight.

4.3.4 Excreta and litter characteristics

The effect of diet or phytase on excreta moisture, pH, excreta free water and litter score is presented in Table 4.9. The moisture content of fresh excreta at d 18 was 11.3% higher than the excreta collected for three days from d 19-21 ($P < 0.001$). Neither diet nor phytase had an effect on excreta moisture content on d18 or d21. There was a tendency for a phytase × diet interaction ($P = 0.07$) with the lowest value for excreta moisture found with the inclusion of 1,500 FTU/kg in the NC diet. Phytase at the level of 500 FTU/kg decreased excreta free water content by 32.5% ($P < 0.05$). The addition of phytase to the diets had no effect on excreta pH ($P > 0.05$) but the birds fed the NC diet produced 0.22 points lower excreta pH than those fed the PC diet ($P < 0.01$). There was an interaction between diet type and phytase on litter score at d 35 ($P < 0.05$). Phytase improved litter quality in the birds fed the NC diet but not those fed the PC diet, but NC diet fed birds produced poorer litter quality compared to the PC diet fed birds when phytase was not added.

Table 4.8 Tibia bone characteristics of meat chickens fed nutritionally adequate (PC) and downspec (NC) wheat based diets supplemented with increasing levels of phytase (d 24)

Treatments	Tibia bone characteristics			
	Ash, %	Length, mm	Ash weight, g	Diameter, mm
Diets				
NC	49.4	71.4[b]	1.36	6.1
PC	50.6	72.8[a]	1.48	6.2
SEM	0.23	0.31	0.02	0.07
Phytase, FTU/kg				
0	48.3	70.6[b]	1.30	6.2
500	50.0	72.5[a]	1.37	6.1
1,000	50.5	73.0[a]	1.52	6.3
1,500	51.0	72.2[ab]	1.47	6.1

SEM	0.33	0.45	0.04	0.10
Dietary treatments				
NC + 0	46.3[b]	69.0	1.15[b]	6.2
NC + 500	49.5[a]	71.5	1.33[ab]	5.9
NC + 1,000	50.6[a]	72.9	1.52[a]	6.3
NC + 1,500	51.0[a]	72.1	1.42[a]	6.0
PC + 0	50.2[a]	72.2	1.45[a]	6.2
PC + 500	50.5[a]	73.5	1.42[a]	6.3
PC + 1,000	50.5[a]	73.1	1.53[a]	6.3
PC + 1,500	51.1[a]	72.3	1.51[a]	6.2
SEM	0.47	0.63	0.05	0.14
P-value				
Diets	<0.001	<0.01	<0.001	NS
Phytase	<0.001	<0.01	<0.001	NS
Diet × Phytase	<0.001	NS	<0.05	NS

[a,b] Means in the same column with different superscripts differ significantly ($P < 0.01$).

4.3.5 Volatile organic compound emissions measured by SPME-GC-MS

More than 50 VOC were detected with SPME-GC-MS measurements from the chamber but only 18 were consistently measured from all the treatments and are presented in Figure 4.1. There was no effect of diet or phytase on the concentration of these VOC ($P > 0.05$). With the exception of dimethyl sulfone, no other sulfur compounds were detected by SPME-GC-MS technique. There was no effect of diet or phytase on the concentration of odorants such as dimethyl sulfone, 2,3-butanedione, 3-methyl-1-butanol, 3-hydroxy-2-butanone and 2-butanone ($P > 0.05$).

Table 4.9 Excreta characteristics of meat chickens fed nutritionally adequate (PC) and downspec (NC) wheat based diets supplemented with increasing levels of phytase on d 18 and d 19-21.

Treatments	Excreta moisture[1], %	Excreta pH[2]	Excreta free water[3], %	Litter score[4] d 35
Diet				
NC	76.6	5.75[b]	26.8	2.2
PC	76.7	5.97[a]	27.3	1.9
SEM	0.38	0.05	1.08	0.1
Phytase, FTU/kg				
0	77.5	5.84	30.2[a]	2.5
500	76.6	5.90	22.8[b]	2.0
1,000	76.8	5.82	28.0[ab]	1.6

1,500	75.8	5.88	27.2[ab]	2.0
SEM	0.54	0.08	1.52	0.16
Days				
18	80.8[a]			
19-21	72.6[b]			
SEM	0.38			
Dietary treatments				
NC + 0	77.6	5.69	30.6	3.0[a]
NC + 500	77.6	5.76	24.4	2.0[b]
NC + 1,000	76.4	5.75	27.3	1.6[b]
NC + 1,500	74.8	5.80	24.9	2.0[b]
PC + 0	77.5	5.99	29.7	1.9[b]
PC + 500	75.5	6.05	21.2	2.1[b]
PC + 1,000	77.2	5.88	28.8	1.7[b]
PC + 1,500	76.8	5.95	29.6	2.1[b]
SEM	0.75	0.10	1.27	0.22
P-value				
Diet	NS	<0.01	NS	0.05
Phytase	NS	NS	<0.05	<0.01
Days	<0.01			
Diet × Phytase	0.07	NS	NS	<0.05
Diet × Days	NS			
Phytase × Days	NS			
Diet × Phytase × Days	NS			

[a,b] Means in the same column with different superscripts differ significantly ($P < 0.05$)
[1] Excreta collected over 3 days (d 19-21).
[2] pH of excreta collected over 3 days (d 19-21).
[3] Excreta collected every 2 h for 4 times on d 18.
[4] Litter score from floor pens at d 35 (score 0-5). The lower score represents drier litter.

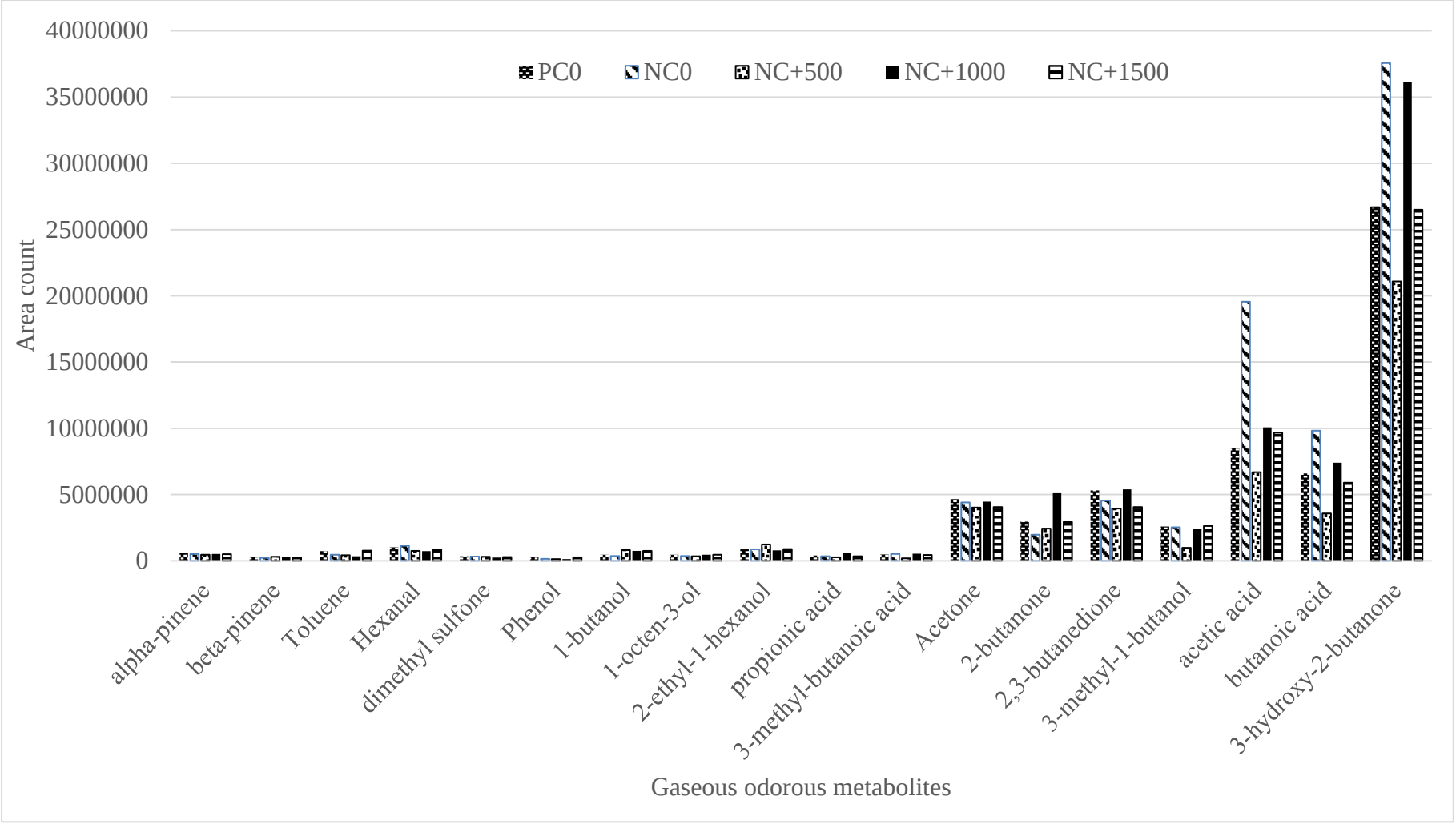

Figure 4.1 Effects of graded levels of phytase (0, 500, 1,000, 1,500 FTU/kg) on the downspec negative control (NC) compared to the positive control (PC) on gaseous odour emissions measured from the chamber with SPME-GC-MS on d 39.

4.4 Discussion

Although higher levels of phytase in diets have generally resulted in better performance in meat chickens (Walk et al., 2014), phytase has sometimes been associated with increased litter moisture and reduced litter quality. According to Debicki-Garnier and Hruby (2003), fungal phytase at 1000 FTU/kg increased litter moisture at both 7 and 21 d post-hatch. The evidence of wet litter with the use of phytase is thought to be due to the inappropriate dietary formulation (Dunlop et al., 2016) that do not use appropriate matrix values for Ca, P and Na in the formulation of phytase supplemented diets. This will result in a higher concentration of these minerals in the gut lumen that makes the intestinal contents more hypertonic to blood and reduces water absorption, thereby resulting in the wet litter (van der Klis and Lensing, 2007). This experiment was conducted to study the effect of graded levels of phytase on performance, WI:FI, litter quality and odour emissions from meat chickens when it was added in the diet without using matrix values ("over the top") or into the formulation with nutrient matrix values.

Increased Ca:av. P has been shown to reduce phytate degradation and P digestibility (Amerah et al., 2014) due to the formation of Ca-phytate complex and increase in pH of the proximal digestive tract (Selle et al., 2009). In the current study, the NC diet with no phytase had wide Ca:av. P and the birds fed NC diet had highest W:FI compared to those fed other diets. This is likely due to birds drinking more water in an attempt to dilute the higher solute concentration in the gut lumen or perhaps to remove the Ca-phytate complex that may have irritating characteristics. Phytate can also bind with protein in the diet (Cowieson et al., 2006; Selle et al., 2012) making amino acids unavailable for digestion. Both a mineral imbalance and reduction in protein digestion may lead to compromised gut integrity and reduced net water absorption from the GI tract. This increased water intake and output thus affects litter quality (van der Klis & de Lange, 2013). The addition of phytase to the NC diet released Av. P thereby restoring Ca:av. P balance and removing Ca-phytate complex. In addition, it is possible that the cell wall architecture around the aleurone layer is broken down by phytase resulting in improved digesta flow and digestibility of nutrients. This would allow more efficient recycling of water in the large intestine and caeca causing birds to consume less water per unit of FI.

The highest WI:FI in birds fed the NC diet without phytase in this study also produced poor litter quality with higher litter score compared to all other treatments. The NC diet contained more Ca from limestone than dicalcium phosphate as compared to the PC diet. There is a debate to whether limestone and dicalcium phosphate as Ca sources have any relation with wet litter (Dunlop et al., 2016) but so far there is no valid conclusion on this. High Ca in meat chicken diets has been reported to deteriorate litter quality in the previous study (Enting et al., 2009). It has been suggested that litter quality can be improved by lowering Ca (Pos et al., 2003); Ca, av. P and Na (Bedford et al., 2015) in diets when phytase is used. The improved litter score with the addition of phytase in the NC diet (with low Ca, av. P and Na) in the current study is in agreement with the above studies.

The current study shows that the effect of phytase is much greater in low av. P diets using a nutrient matrix to formulate feed as compared to adding over the top of an already av. P sufficient diet. While small performance benefits may be observed when

adding phytase to av. P sufficient diets, the benefits are not economically sound. This is evident by the results on d 24 and again on d 35 where phytase increased BWG by a small margin when added over the top of the PC diet but markedly when added to the NC diet at 500, 1,000 or 1,500 FTU/kg respectively. These results show that phytase is more effective with a lower than higher av. P level in diets to improve FI and BWG of meat chickens. Similar to our findings, Walk et al. (2013) reported the extra-phosphoric effects of using higher levels of phytase to a downspec diet and this was thought to be due to the mitigation of anti-nutritional effects of phytate rather than improved P utilization. Shirley and Edwards (2003) also reported performance benefits of using higher levels of phytase to nutritionally marginal diets. The lower tibia ash weight and tibia ash as a percent of body weight in the birds fed the NC diet compared to those fed the PC diet and the subsequent improvements after addition of phytase to the NC diet but not to the PC diet observed in this study were similar to the previous findings (Dilger et al., 2004; Walk et al., 2013). This suggests that any amount of P released in the gut by degradation of phytate in the birds fed nutritionally adequate diet with phytase would not be deposited in the bone but may get excreted.

The lower weight of small intestine on d 24 with the addition of 1,000 or 1,500 FTU/kg phytase in diet observed in this study is similar to the finding by Wu et al. (2015). It represents a more efficient intestine that uses less energy for maintenance and thus the surplus energy may be utilized for growth and absorption of nutrients. This finding may be correlated with the low FCR observed in the birds on d 24 when they were fed diets with 1,000 or 1,500 FTU/kg phytase. A recent report suggests that higher levels of phytase may modulate ileal microbiota in meat chickens (Ptak et al., 2015). It may be possible that the addition of higher levels of phytase decrease the undesirable microbial load and thus the amount of inflammation in the gut and its size.

It has been reported that high litter moisture favors the emission of odorous metabolites such as methyl mercaptan, H_2S, dimethyl sulfide, NH_3, trimethyl amine, phenol, indole, and 3-methyl-indole (Sharma et al., 2016). In the current study, dietary phytase did not reduce excreta moisture, however, litter score was improved by phytase. This was expected to reduce emissions of these odorous metabolites from the litter. Further, the possible increased amino acid digestibility by the application of higher levels of phytase (Amerah et al., 2014) was expected to reduce the amino acid fermentation products in the litter. Most of the sulfurous odorants such as methyl mercaptan, dimethyl sulfide and H_2S originate from sulfur-containing amino acids (methionine and cysteine) in excreta (Mackie et al., 1998). Similarly, phenol and cresol originate from microbial degradation of l-tyrosine; indole, and skatole originates from microbial fermentation of l-tryptophan; amines and NH_3 are produced by decarboxylation and deamination of amino acids (Mackie et al., 1998). A range of VOC was measured in this study that belongs to the group of volatile fatty acids, alcohols, aldehydes, ketones and phenols. Among these VOC, the important odorous ones included diacetyl, 3-methyl-1-butanol, 3-hydroxy-2-butanone, 2-butanone, acetic acid, propionic acid, butyric acid and phenol. These compounds are carbohydrate fermentation products and can affect the environment surrounding the meat chicken farms (Mackie et al., 1998; Wadud, 2011). However, with the SPME-GC-MS method used in this study, neither diet nor phytase affected the concentration of these odorants. This perhaps indicated that phytase did not have a profound effect on the digestion of carbohydrate rich substrates. However, the SPME-GC-MS technique may not be as sensitive as SIFT-MS to measure the odorants in meat chickens. Recently, differences in headspace

concentrations of odorants between litters varying in moisture content were found to be significant using SIFT-MS (Sharma et al., 2016). The SPME-GC-MS technique used in this study was also unable to measure sulfurous compounds such as mercaptans that are considered significant contributors of odour.

4.5 Conclusion

The results indicate that phytase has greater performance benefits when formulated using nutrient matrix values as compared to adding it over the top in an already nutrient sufficient diet. The later method would be expected to increase feed costs without concomitant performance benefits. While litter quality was improved by phytase in the downspec diet, a reduction in odour from birds and litter could not be detected using the SPME-GC-MS technique. Further work is warranted to study the effect of phytase enzyme on odour emissions using instrumentation designed to detect sulfurous compounds more highly associated with odour from meat chicken farms.

5. Experiment 2

Title: Effect of ingredient composition in diets on odour emissions in meat chicken production

5.1 Introduction

Odours from poultry farms are a potential nuisance in the surrounding community. Odours generated from meat chicken farms are a result of both microbial decomposition of excreta in litter (Jiang and Sands, 2000) as well as emissions directly from the birds (Lacey et al., 2004). Recently, Murphy et al. (2014) reported eight major VOC from tunnel ventilated meat chicken farms that were considered important predictors of odour. These were dimethyl sulfide , dimethyl trisulfide , 2,3-butanedione, 3-methyl-butanal, 1-butanol, 3-methyl-1-butanol, 3-hydroxy-2-butanone (acetoin), and 2-butanone. Previously, Jiang and Sands (2000), Dunlop et al. (2011) and Pillai (2011) reported similar odorous compounds plus mercaptans (methyl-, ethyl-, propyl-), dimethyl disulfide , phenol, cresol, acetic, propionic and butanoic acids, indole and skatole as odorous compounds from meat chicken farms. In an effort to address odour issues from farms, there have been attempts to develop mitigation strategies including litter treatments, biofilters, neutralising agents, air scrubbers, ozone treatment, windbreak walls and short stacks but these techniques are generally costly or impractical due to the required high ventilation rates in meat chicken farms (Dunlop et al., 2011). There is little information available linking diet composition to odour emissions to develop suitable odour mitigation strategies.

Diets can be formulated to more closely meet the bird's nutritional requirements to avoid overfeeding and to reduce excretion of undigested components. This will decrease the available substrates that the microbes metabolise to odour compounds

(Mackie et al., 1998). The composition of meat chicken excreta is related to the composition of the diet. Chavez et al. (2004) reported the role of dietary methionine sources in generation of odorants from poultry excreta. They found H_2S, carbonyl sulfide and dimethyl disulfide emissions as measured by gas chromatography/mass spectrometry (GC/MS) to be higher in birds fed sodium methionate as compared to birds fed D,L-methionine powder or liquid D,L-hydroxy-methyl-thio-butanoic acid (HMTBA) or its dry calcium salt. Chang and Chen (2003) reported the benefits of adding lactobacillus-containing probiotics to lower meat chicken farm malodours. They found lower emission of 2-butanone, 1-butanol, dimethyl disulfide and dimethyl trisulfide in diets supplemented with lactobacillus containing probiotics as measured by GC/MS. There is scant information available on the effects of different protein sources in meat chicken diets on odour emissions. In one study in growing-finishing pigs, van Heugten and van Kempen (2002) reported high manure odour concentration with the addition of feather meal up to 120 g/kg in the diets.

Soybean meal (SBM) is the most commonly used protein source in meat chicken diets worldwide and contains 460–480 g/kg CP and 8.37–10.47 MJ/kg metabolisable energy (ME) (Ravindran et al., 2014). Canola meal (CM) contains approximately 340–370 g/kg CP and can be used as an alternative dietary protein source to SBM for meat chickens. However, diets formulated exclusively with plant protein sources increase water consumption and elevate litter moisture content (Vieira and Lima, 2005; Eichner et al., 2007; Hossain et al., 2013). In addition, because of the presence of many anti-nutritional factors in plant protein sources, high dietary levels of SBM or CM may produce wet litter. Litter moisture content is presumed to be one of the most critical factors affecting odour production in poultry farms (Jiang and Sands, 2000; Carey et al., 2004). Meat and bone meal (MBM) is an animal by-product which is also used as a protein source in meat chicken diets at levels up to 120 g/kg. However, MBM varies widely in nutritional composition, typically contains a lower level of digestible protein and amino acids than soybean meal and has an unpleasant smell that could contribute to odour. Thus, it is of interest to study and compare litter odorants associated with diets varying in ingredients and nutrient contents.

Concentration of specific odorants can be quantified using real time gas analysers such as the Fourier transform infrared (FTIR) spectrometer. Van Kempen et al. (2002) and Witkowska (2013) successfully used FTIR to detect and quantify odours from swine and turkey houses, respectively. The objective of the current study was to use FTIR to examine odorant emissions from meat chickens fed diets differing in ingredients and nutrient composition.

5.2 Materials and methods

Two experiments were conducted to measure the effect of different diets on litter odorant emissions. In each experiment, randomly selected meat chickens were placed in specially designed chambers in a climate controlled room to measure odorants. The experiments were approved by the Animal Ethics Committee of the University of New England, Australia.

5.2.1 Metabolic chambers

The chambers that were used in this experiment were the same as the ones described by Swick et al. (2013). In short, 15 chambers made of stainless steel and equipped with a wire mesh cage were placed in a climate controlled room. Temperature and humidity in each chamber were monitored using the sensors and shown on an electronic display. The outlet in each chamber was connected to the FTIR instrument for odour measurements.

5.2.2 Experimental design and diets

Experiment 1

A total of 288 day-old Ross 308 male meat chickens were reared in floor pens with wood shavings as a bedding material. The birds were fed a common starter diet to d 10, experimental grower diets from 10–25 days and experimental finisher diets thereafter. At the age of 22 days, 12 birds of uniform body weight were selected from a pool of 288 birds and adapted to the metabolic chambers for 6 days in a climate controlled room and fed their respective test diets. Litter materials were not used in this experiement and the birds were reared on raised wire floors. The experimental collection started when the birds were 28 days old and finished when they were 42 days old. Feed and water were provided *ad libitum*. Each diet was replicated three times with two birds per chamber. Two treatment diets were formulated according to the Ross 308 nutrient specifications for digestible amino acids (Aviagen, 2007). Diets differed in ingredient composition and ME (Table 5.1).

Experiment 2

A total of 90 day-old Ross 308 male meat chickens were assigned to three dietary treatments with 30 chicks per pen reared for the first 10 days. Wood shavings were used as a bedding material. At the age of 10 days, 25 birds of uniform body weights were selected from each treatment and transferred to the metabolic chambers. Each treatment diet was replicated five times with five birds in each chamber. The litter accumulated during the first 10 days in the floor pens of respective treatments was collected in equal amounts (1.5 kg) and transferred to the chambers at the same time as the birds. Feed and water were provided *ad libitum* and intakes were recorded at d 24 and d 32. Basal diet (SBM group) contained onlySBM as a protein source. The other two diets used CM and MBM at the expense of SBM. The CM diet had 60% of the protein source as CM and the MBM diet contained 43–54% of the protein source as MBM. Wheat was included in the diets at 600–700 g/kg and cottonseed oil and synthetic amino acids were added to make the diets approximately isocaloric and isonitrogenous, and to give them similar digestible amino acid contents. The diets were formulated according to the Ross 308 nutrient specifications for digestible amino acids (Aviagen, 2007) but with slightly lower ME than Ross 308 specifications. All diets contained xylanase and phytase enzymes (Table 5.2).

Table 5.1 Composition of the wheat-soy based experimental diets for experiment 1 (g/kg, as-fed basis)

	Grower diet (10–25 days)	Finisher diet (26–42 days)

Ingredients	Wheat, canola seed (T1)	Wheat-corn no canola seed (T2)	Wheat, canola seed (T1)	Wheat-corn no canola seed (T2)
Wheat	668.4	591.1	723.6	638.9
Soybean meal	161.2	164.9	106.6	104.0
Meat and bone meal	76.3	77.3	75.2	76.9
Corn	0.0	134.0	0.0	150.0
Canola seed	60.0	0.0	60.0	0.0
Canola oil	18.0	16.2	20.0	14.6
Limestone	3.53	3.44	3.53	3.40
Xylanase powder[1]	0.05	0.05	0.05	0.05
Salt	0.48	0.37	0.47	0.36
Na bicarbonate	2.00	2.00	2.00	2.00
Vitamin mix[2]	0.50	0.50	0.50	0.50
Mineral mix[3]	0.75	0.75	0.75	0.75
Choline Cl, 70%	0.04	0.40	0.00	0.37
l-lysine HCl	3.44	3.47	3.15	3.93
dl-methionine	2.97	3.04	2.22	2.32
l-threonine	1.80	1.80	1.41	1.44
Salinomycin[4]	0.50	0.50	0.50	0.50
Calculated nutrients (g/kg)				
ME, MJ/kg	13.2	12.7	13.4	12.9
CP	210	203	190	183
dig Lys	11.0	10.6	9.55	9.76
dig M+C	8.40	8.10	7.30	7.04
dig Thr	7.30	7.04	6.30	6.07
Ca	8.40	8.27	8.19	8.10
Av. P	4.20	4.13	4.10	4.05
Na	1.60	1.54	1.60	1.54
K	7.50	7.12	6.49	6.05
Cl	1.82	1.85	1.75	1.93
dEB[5], mEq/kg	210.1	197.0	186.3	167.3
Analysed DM, g/kg	913.4	912.0	906.5	909.4

[1]Porzyme (Dupont Animal Nutrition)

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

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

[4]Sacox (coccidiostat), [5]dietary electrolyte balance ($Na^+ + K^+ - Cl^-$)

Table 5.2 Composition of the treatment diets for experiment 2 (g/kg, as-fed basis)

Ingredients	Starter diet (0–10 days)			Grower diet (10–24 days)			Finisher diet (24–32 days)		
	SBM	MBM	CM	SBM	MBM	CM	SBM	MBM	CM
Wheat	627.2	760.3	600.0	670.5	757.7	604.6	704.8	777.6	646.2
Soybean meal	291.1	91.0	125.2	254.2	117.6	117.2	227.0	112.9	100.0
Meat and bone meal	-	110.0	-	-	90.0	-	-	73.9	-
Canola meal	-	-	180.0	-	-	190.0	-	-	174.1
Cottonseed oil	42.5	16.5	53.8	33.2	11.8	50.0	35.0	18.0	50.4
Limestone	12.88	-	11.75	12.4	-	11.10	12.07	3.41	10.90
Dicalcium phosphate	11.38	-	9.74	10.2	-	8.30	8.73	-	6.94
Xylanase[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Phytase[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt	2.59	0.66	2.31	2.92	1.37	2.65	2.92	1.65	2.68
Na bicarbonate	1.50	1.50	1.50	1.00	1.00	1.00	1.00	1.00	1.00
TiO2	-	-	-	5.00	5.00	5.00	-	-	-
Vitamin mix[3]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mix[4]	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Choline chloride	0.46	0.68	0.91	1.73	2.60	0.85	1.68	2.41	1.13
L-lysine HCl	3.48	6.46	5.15	2.70	4.41	3.37	1.77	3.24	2.43
D,L-methionine	3.23	3.87	2.78	2.80	3.19	2.05	2.14	2.48	1.47
L-threonine	1.84	3.02	2.27	1.50	2.19	1.51	0.97	1.56	1.00
L-tryptophan	-	0.11	-	-	-	-	-	-	-
L-isoleucine	-	1.17	0.61	-	0.40	0.03	-	-	-
L-arginine	-	2.08	1.77		0.68	0.57	-	-	-
L-valine	-	0.85	0.34	-	0.11	-	-	-	-
Calculated nutrients (g/kg)									
MEn, MJ/kg	12.7	12.7	12.7	12.9	12.9	12.9	13.1	13.1	13.1
CP	223	224	216	200	205	202	190	192	190
dig lysine	12.7	12.7	12.7	11.0	11.0	11.0	10.2	10.2	10.2
dig met+cys	9.4	9.4	9.4	8.4	8.4	8.4	7.6	7.6	7.6
dig threonine	8.3	8.3	8.3	7.3	7.3	7.3	6.5	6.5	6.5
Ca	9.5	10.5	9.5	9.0	9.0	9.0	8.5	9.0	8.5
Av. P	4.8	6.5	4.8	4.5	5.8	4.5	4.2	5.2	4.2
Na	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
K	9.2	6.0	7.8	8.6	6.4	7.8	8.2	6.3	7.4
Cl	2.8	2.8	2.9	3.0	3.0	2.8	2.8	2.8	2.6
dEB[5],	234.7	152.8	196.1	213.7	157.4	198.9	209.1	160.5	194.3
Analysed nutrients (g/kg)									
DM	904.8	901.1	902.6	911.4	913.1	916.6	901.0	901.3	906.1
CP	222.6	224.8	222.9	214.5	216.4	209.7	181.3	184.1	184.2

[1]Feedzyme XBC 1000G (Dupont Animal Nutrition)
[2]Phyzyme (Dupont Animal Nutrition)
[3]Vitamin concentrate supplied per kilogram of diet: retinol, 12000 IU; cholecalciferol, 5000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamin, 16 µg; biotin, 200 µg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg, [4]Trace mineral concentrate supplied per kilogram of diet: Cu (sulfate), 16 mg; Fe (sulfate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulfate and oxide), 120 mg; Zn (sulfate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg, [5]dietary electrolyte balance (Na^++K^+-Cl^-)

5.2.3 Gas collection and analysis

Gas concentrations were determined by FTIR using a portable multi-component Gasmet DX-4015 analyser (Gasmet Technologies, Finland). In experiment 1, gaseous samples were measured only once at d 42 in the presence of birds and excreta without litter material (birds on raised wire floor). In experiment 2, emissions were measured at d 24 and d 32 from birds, excreta and litter. Chamber lids were closed for approximately 15 min before sample collection. Water was used to seal the chambers. At that time there was zero air exchange and odorants were allowed to concentrate prior to sampling. Carbon dioxide (CO_2) and oxygen (O_2) levels inside chambers were recorded during the period of closure and remained at levels less than 2% (CO_2) and more than 18% (O_2), respectively. The FTIR was set up as follows: flushing time, 30 seconds (s); pumping time, 1 min; measuring time, 3 min. The gas samples were drawn at a flow rate of 2 l/min with the in-built pump in FTIR (i.e. 2 litres of gases were measured from each chamber) and the exhaust from the FTIR was fed back into the chamber. Before measurements, the analyser was zero calibrated using pure dry nitrogen gas. After the measurements, sample spectra were recorded and qualitative and quantitative analyses were conducted with the use of Calcmet Professional software with a library of reference spectra for 50 gases. Calcmet is Gasmet DX-4015 proprietary software and uses modified classical least square method for analysis of data. The concentrations of chemical compounds were expressed in ppm (v/v) units and total elemental sulfur from treatment groups is calculated as mg S/m^3. After finishing measurements from one treatment, FTIR was flushed with dry nitrogen gas for 15 min before taking measurements from the next treatment group.

5.2.4 Litter moisture and pH measurements

Five litter subsamples per chamber were sampled to obtain a representative complete litter profile (caked and friable). Pooled litter subsamples were then thoroughly mixed in a 1 l plastic box and moisture content and pH were assayed. Litter pH was determined by mixing litter and de-ionised water in the ratio of 1:5 and measuring with a pH meter (EcoScan 5/6 pH meter, Eutech Instrument Pte Ltd; Singapore).

5.2.5 Crude protein and dry matter measurements

The nitrogen content of each diet was determined by the Dumas combustion technique as described in 4.2.6 and CP calculated by multiplying nitrogen by 6.25. Dry matter contents of diets and litter were determined as described in 4.2.6

5.2.6 Statistical analysis

The data were analysed by one-way analysis of variance using the general linear models procedure (SAS Institute Inc., Cary, NC). In experiment 1, differences among the treatment means were determined using the t-tests and in experiment 2, Duncan's multiple range test was used. Variability in the data is expressed as the standard error

means (SEM) and a probability level of $P < 0.05$ was considered to be statistically significant.

5.3 Results and discussion

5.3.1 Experiment 1

Altogether 24 VOC were detected and quantified. Eight odorants suggested to be most likely to contribute to odour impacts are listed in Table 5.3. Methyl mercaptan , dimethyl disulfide , 2,3-butanedione, phenol and m-cresol were measured at higher concentrations than the odour detection threshold (Schiffman et al. 2001). Methyl mercaptan has a rotten cabbage smell and was produced at higher levels ($P < 0.05$) from T1 group. Dimethyl disulfide was also detected in chamber air from both diets. Dimethyl disulfide is the oxidation product of methyl mercaptan. The results suggest that the use of 60 g/kg canola seed in the diet led to higher levels of methyl mercaptan, a sulfur containing odorant in T1 compared to T2 that did not have canola seed. The calculated digestible methionine plus cysteine were similar in both diets (7.3 g/kg vs. 7.0 g/kg). This small difference in dietary sulfur amino acid level is unlikely to produce difference in sulfur odorants among treatments. However, a higher excreta moisture content was observed in birds fed T1 ($P < 0.05$). Increased litter moisture is associated with higher concentrations of organosulfides, aldehydes and alcohols (Murphy et al. 2014) due to increased anaerobic degradation (Jiang and Sands, 2000). Therefore, the higher organosulfide emission from T1 in this study was likely related to higher excreta moisture content.

Table 5.3 Excreta moisture content and odorants emitted from meat chickens fed two commercial diets at 28–42 days in experiment 1 (in ppm, v/v)

Compounds	Wheat, canola seed (T1)	Wheat-corn no canola seed (T2)	SEM	P-value
2,3-butanedione/diacetyl	1.099[b]	2.307[a]	0.286	0.005
2-butanone	0.923	0.704	0.157	0.548
Dimethyl disulfide	3.242	3.079	0.154	0.651
Methyl mercaptan	19.393[a]	15.607[b]	0.940	0.014
2-butanol	0.000	0.344	0.109	0.116
3-methyl-butanal	0.317	0.496	0.166	0.645
Phenol	0.880[b]	0.981[a]	0.026	0.027
m-cresol	0.582[b]	1.051[a]	0.112	0.006
Excreta moisture, %	76.20[a]	68.25[b]	1.530	0.035

[a,b] Means in the same row with different superscripts differ (P<0.05) or (P<0.01).

Diacetyl (2,3-butanedione) has a rancid butter smell (Dunlop et al. 2011). This compound was produced at higher levels in T2 group ($P < 0.01$). Diacetyl is a product of fermentation and is considered an important odorant due to its low human detection threshold (Murphy et al. 2014). The T2 group produced higher levels of phenol ($P < 0.05$) and m-cresol ($P < 0.01$). Phenol originates from the microbial degradation of

tyrosine in the intestinal tract of animals and from phenolics contained in litter (Mackie et al. 1998; Le et al. 2005).

5.3.2 Experiment 2

5.3.2.1 Feed intake, water intake and litter characteristics

Feed intake (FI), water intake (WI), WI:FI, litter moisture and pH for treatment groups at various time intervals are presented in Table 5.4. At d 24, FI and WI were significantly higher in SBM and CM groups ($P < 0.01$) compared to MBM group. However, there was no significant difference in WI:FI between any treatments to d 24. From d 24–32, FI was higher in the SBM group ($P < 0.01$) but the CM group consumed more water ($P < 0.05$) and had the highest WI:FI ($P < 0.01$). During the growing period (d 10–32, SBM and CM groups had higher WI ($P < 0.01$) than MBM group. The SBM group had the highest FI followed by the CM group ($P < 0.01$). However, the WI:FI was highest in the CM group during the whole rearing period ($P < 0.05$).

Table 5.4 Water intake, FI and litter characteristics of meat chickens at various stages of growth (experiment 2)

Period	Parameters	Treatments			SEM	P-value
		SBM	MBM	CM		
10–24 days	FI, g	1271[a]	1088[b]	1191[a]	27.00	0.005
	WI, ml	2637[a]	2106[b]	2596[a]	83.71	0.002
	WI:FI	2.07	1.94	2.18	0.05	0.124
24–32 days	FI, g	812[a]	732[b]	754[b]	11.69	0.002
	WI, ml	1502[ab]	1294[b]	1663[a]	58.47	0.015
	WI:FI	1.85[b]	1.77[b]	2.21[a]	0.07	0.007
10–32 days	FI, g	2082[a]	1819[c]	1945[b]	35.51	0.0003
	WI, ml	4139[a]	3399[b]	4259[a]	125.66	0.0003
	WI:FI	1.99[b]	1.87[b]	2.19[a]	0.05	0.018
	Litter moisture, %	32.17[a]	19.36[b]	34.35[a]	2.43	0.006
	Litter pH	8.19[a]	6.76[b]	7.93[a]	0.24	0.010

[a,b] Means in the same row with different superscripts differ (P<0.05) or (P<0.01).
FI- feed intake, WI- water intake

There were no significant differences in pH or moisture contents of litter between SBM and CM groups but these were higher than that of the MBM group ($P < 0.05$). These findings were similar to that of Eichner et al. (2007) and Hossain et al. (2013) who also reported higher litter moisture with diets based exclusively on vegetable protein sources. Soybean meal has a higher K content than CM and MBM (Leeson and Summers, 2005) and this was reflected in the calculated dietary K content for SBM, MBM and CM diets, respectively (Table 5.2). As FI of the SBM and CM groups were also higher than the MBM group, the K intake further increased in these groups. Diets with increased Na and/or K result in increased WI (Smith et al., 2000) and litter moisture (Eichner et al. 2007). Because the Na contents of all the experimental diets were similar, it is likely that increased WI and litter moisture observed in the SBM group (and partly in CM group) were due to high K intakes. Canola meal has higher

sulfur content than SBM and MBM (Leeson and Summers, 2005). Thus, high dietary CM increases the total sulfur content in the diet. A high concentration of sulfur in the CM diet would have affected dietary electrolyte balance and contributed partly to wet litter. However, the commonly used dietary electrolyte balance (dEB) equation (Na^++K^+-Cl^-) doesn't take into account the sulfur content in the diet but the anion-cation ratio of the diet can be out of balance with an excess amount of sulfur if canola meal is included at high levels (Summers and Bedford, 1994).

Canola meal contains a high crude fibre compared to SBM due to a much higher content of lignin and associated polyphenols (Khajali and Slominski, 2012). The FI of the CM group was lower than that of the SBM group for the entire experimental period but there was no difference in WI between these groups. Thus, WI:FI was higher in the CM group.

5.3.2.2 Odorants

The odorants measured at d 24 and d 32 are presented in Tables 5.5 and 5.6, respectively. More odorous compounds were detected at d 32 compared to d 24. Few more odorants were detected but occassionally at d 24 and thus not included. At d 24, methyl mercaptan emission was highest in SBM group and lowest in MBM group (P = 0.01) but total elemental sulfur were higher in SBM and CM groups (P < 0.01) compared to the MBM group. High FI and WI in SBM and CM groups could have produced more excreta with high moisture. High sulfur emissions may be due to a high moisture content and a lower pH (Dunlop, personal communication). Unfortunately, litter moisture and pH were not measured at d 24 in this study.

Nine odorants were detected consistently at d 32 but no significant difference in concentration was measured between treatments. This result explains the complex nature of odour. The total elemental sulfur were higher in groups fed SBM and CM diets at d 24 but were similar in birds fed the MBM diet at d 32. A lower litter pH

in the MBM diet at d 32 was observed. While this treatment also had the lowest moisture content on d 32, differences in odorants were no longer significant as compared to measurements on d 24. A higher number and concentration of odorants was detected at d 32 suggesting that emissions would be more odorous at the later stage of growth. It is possible that the total excreta and moisture load toward the end of the growout may overwhelm any differences in odour production caused by diet. Volatile fatty acids were detected from the emissions of all treatment groups. Acetic acid, propionic acid and butyric acids are saccharolytic fermentation products, which are produced by anaerobic bacteria in the caeca of birds and in litter. It has been reported that an increase in the caecal or excreta VFA concentration will decrease in the MBM diet at d 32 was observed. While this treatment also had the lowest moisture content on d 32, differences in odorants were no longer significant as compared to measurements on d 24. A higher number and concentration of odorants was detected at d 32 suggesting that emissions would be more odorous at the later stage of growth. It is possible that the total excreta and moisture load toward the end of the growout may overwhelm any differences in odour production caused by diet.

Table 5.5 Odorous compounds emitted from meat chickens at d 24 in experiment 2 (in ppm, v/v)

Compounds	Treatments			SEM	P-value
	SBM	MBM	CM		
Dimethyl disulfide	1.533	1.568	1.780	0.068	0.295
Ethyl mercaptan	1.430	1.725	1.765	0.078	0.160
Methyl mercaptan	6.673[a]	4.503[b]	5.303[b]	0.342	0.012
Total elemental sulfur (mg S/m^3)	14.65[a]	12.28[b]	13.94[a]	0.274	0.005

[a,b] Means in the same row with different superscripts differ ($P < 0.05$) or ($P < 0.01$).

Table 5.6 Odorous compounds emitted from meat chickens at d 32 in experiment 2 (in ppm, v/v)

Compounds	Treatments			SEM	SD	P-value
	SBM	MBM	CM			
2,3-butanedione	0.418	0.178	0.293	0.068	0.236	0.392
Dimethyl disulfide	0.490	0.990	0.000	0.258	0.893	0.321
Ethyl mercaptan	1.310	1.545	1.378	0.105	0.364	0.685
Methyl mercaptan	5.553	4.793	6.705	0.404	1.401	0.301
Total elemental sulfur (mg S/m^3)	10.29	10.91	10.60	0.273	0.947	0.792
2- butanol	0.285	0.203	0.260	0.027	0.092	0.476
Phenol	0.490	0.600	0.535	0.022	0.076	0.110
Acetic acid	0.458	0.660	0.463	0.071	0.245	0.452
Propionic acid	0.300	0.430	0.255	0.069	0.240	0.608
Butyric acid	0.285	0.203	0.185	0.033	0.115	0.466

Volatile fatty acids were detected from the emissions of all treatment groups. Acetic acid, propionic acid and butyric acids are saccharolytic fermentation products, which are produced by anaerobic bacteria in the caeca of birds and in litter. It has been reported that an increase in the caecal or excreta volatile fatty acid concentration will decrease manure pH and NH_3 emissions (Canh et al., 1998). However, the effect of these volatile fatty acids on other odorous compounds and odour nuisance is inconsistent and not yet clear (Le et al.,2005). Some of the odorants were measured at higher concentrations in experiment 1 compared to experiment 2. The lower concentration of odorants measured in experiment 2 may be due to the presence of bedding materials (wood shavings) which may provide a surface for odorant adsorption or may reduce the diffusion of odorants from the litter or litter moisture difference between the expeiments. If this is the case, future research should focus on studying odour emissions from farms while paying particular attention to the properties and conditions of the litter.

This study clearly showed that diet impacts odour emissions from meat chicken production. The use of closed circuit metabolic chambers coupled with FTIR allowed accurate detection and quantification of the odorous compounds, which are of interest to poultry industry. Minor changes in diet composition were found to change the relative abundance of gases associated with odours. Further investigation is warranted to more fully understand the effect of microbial metabolism of nutrients and metabolites in the gut and litter on odour formation.

6. Experiment 3

Title: Effect of dietary protein levels and feed additives on odour emissions in chicken meat production

6.1 Introduction

Odour emissions have been identified as a potential threat for the sustainable development of the chicken meat industry. The expansion of existing farms or the establishment of new farms thus depends on effective control of dust and odour emissions. Emissions from meat chicken farms contain a large number of odorants (Murphy et al., 2014). Some of these odorants can be reduced through dietary manipulation (Chavez et al., 2004; Sharma et al., 2015). Feeding meat chickens a low protein diet reduces the concentration of putrefactive metabolites in the caeca such as NH_3, amines, phenols, indoles, skatole, cresol and branched chain fatty acids (Qaisrani et al., 2015). Some of these metabolites are toxic and odorous (Mackie et al., 1998). A low protein diet formulated to provide all the required amino acids without excesses may reduce putrefaction of animal waste and hence the production of toxic and odorous metabolites in the litter.

Feed additives such as in-feed antibiotics, probiotics and saponins are commonly used in meat chicken diets. In-feed antibiotics reduce the microbial load in the gut and decrease the production of microbial metabolites (Dibner and Richards, 2005). *Bacillus* based probiotics improved performance, decreased pathogen load in the gut and reduced the headspace air concentration of NH_3 and H_2S in excreta (Jeong and kim, 2014; Zhang et al., 2013). A blend of *Yucca* and *Quillaja* saponin improved growth and feed efficiency in meat chickens (Cheeke, 2009) and reduced NH_3 emissions in laying hens (Chepete et al., 2012). Thus, these in-feed additives may have the potential to reduce odour emissions. However, no information in the literature is available to determine whether this is the case. The objective of this experiment was to measure the effect of dietary CP level and additives on odour flux from litter at different ages of meat SIFT-MS.

6.2 Materials and methods

The experimental procedures were in accordance to the animal ethics committee guidelines of University of New England, Australia. Ethics approval was granted before the studies were conducted.

6.2.1 Bird husbandry, experimental design, and diets

A total of 180 one-day-old male Ross 308 chicks were allocated to five dietary treatments, each of which was replicated three times with 12 birds per replicate and measured at three ages. The replications were allocated in a completely randomized design. The chicks (35 ± 1 g) were weighed before placements to ensure consistency in pen weights. Each pen measuring 1.2 m × 0.76 m represented one replicate consisting of a feeder and a double outlet cup drinker. A litter collection tray measuring 0.46 m × 0.29 m × 0.065 m was placed in each pen away from the feeder and drinker before litter was spread over the pens to cover the tray. Fresh pine shavings (Hysorb wood shavings, ECW, Australia) were used as litter and added at 10.35 kg/pen. The experiment was designed according to a 5 × 3 factorial arrangement of treatments, and the main factors consisted of diet (low CP, high CP, high CP+antibiotic, high CP+probiotic, high CP+saponin) and age (15, 29 and 35 d). Two birds from each pen were culled at d 10 and 24 for other experimental purposes. The composition of experimental diets and their calculated and anlaysed nutrients are presented in Tables 6.1 and 6.2.

High CP and low CP diets differed by 5% CP in starter phase and 4.5% CP in grower and finisher phases. These were formulated to contain the same ratio of SBM, CM and MBM in all phases and similar levels of metabolisable energy (ME) and digestible amino acid contents. Low protein diets were supplemented with L-valine, L-isoleucine, L-arginine, L-lysine, D,L-methionine and L-threonine. Zinc bacitracin ALBAC 150, Zoetis) was used as the in-feed antibiotic at the rate of 330 g/t of feed (50 ppm active Zn bacitracin), a blend of three *Bacillus subtilis* strains (ENVIVA PRO, Dupont Animal Nutrition) was used as a probiotic at 500 g/t to provide 150,000 cfu/g of feed and saponin came from a blend of *Yucca schidigera* and *Quillaja saponaria* (NUTRAFITO PLUS, Desert King International) and used at 150 g/t of feed. These additives were added by replacing equal amounts of wheat in the formulation. Feed was mixed and pelleted at 65 °C at the University of New England, Australia. Feed was provided in three phases: starter (0-10 d), grower (10-24 d) and finisher (24-35 d). All feeds were in crumble form to 10 d and in pellet form thereafter until at 35 d. Feed and water were provided *ad libitum* throughout the study. The lighting program followed the recommendations set forth in the Ross 308 breed management manual (Aviagen, 2014).

Table 6.1 Ingredient composition and calculated nutrients of experimental diets (as-fed basis)

Ingredients, %	High CP			Low CP		
	Starter	Grower	Finisher	Starter	Grower	Finisher
Wheat	50.2	52.8	58.4	66.9	69.7	73.4
Soybean meal	30.2	23.7	23.1	18.6	13.9	13.4
Canola meal	9.45	13.1	8.16	5.84	7.69	4.73
Meat meal	5.40	4.08	4.26	3.34	2.40	2.47
Canola oil	3.42	4.95	4.67	1.71	2.95	3.05
Limestone	0.37	0.40	0.37	0.70	0.71	0.69
Dicalcium phosphate	0.03	0.19	0.05	1.03	0.81	0.68
Salt	0.21	0.15	0.15	0.23	0.17	0.17
Na bicarbonate	0.20	0.20	0.20	0.19	0.20	0.20
D,L-methionine	0.15	0.13	0.14	0.27	0.25	0.24
L-Lysine HCl	0.004	0.03	-	0.43	0.43	0.37
L-Threonine	0.03	0.04	0.08	0.20	0.21	0.20
L-Arginine	-	-	0.18	0.10	0.15	0.12
L-Valine	-	-	-	0.02	0.03	-
L-Isoleucine	-	-	-	-	0.02	-
Vitamin-mineral premix[1]	0.20	0.20	0.20	0.20	0.20	0.20
Choline Cl, 60%	0.06	0.06	0.04	0.09	0.09	0.07
Xylanase powder[2]	0.05	0.05	0.05	0.05	0.05	0.05
Salinomycin[3]	0.05	0.05	-	0.05	0.05	-
Calculated nutrients						
MEn, MJ/kg	12.3	12.8	13.0	12.3	12.8	13.0
Crude protein	26.0	24.0	23.0	21.0	19.5	18.4
Crude fibre	2.97	3.08	2.82	2.69	2.74	2.58
dig lysine	1.20	1.10	1.00	1.20	1.10	1.00
dig M+C	0.84	0.80	0.76	0.84	0.80	0.76
dig arginine	1.57	1.41	1.52	1.26	1.19	1.10
dig isoleucine	0.99	0.90	0.87	0.78	0.74	0.69
dig threonine	0.77	0.73	0.72	0.77	0.73	0.70
dig valine	1.14	1.05	1.00	0.92	0.85	0.79
Ca	0.95	0.85	0.80	0.99	0.85	0.80
Total P	0.79	0.76	0.71	0.81	0.72	0.68
Av. P	0.47	0.43	0.40	0.52	0.43	0.40
Na	0.21	0.18	0.18	0.21	0.18	0.18
K	1.04	0.94	0.90	0.80	0.73	0.70
Cl	0.20	0.16	0.16	0.30	0.25	0.24
dEB[4], mEq	300	273	263	211	194	189

[1]Vitamin-Mineral concentrate supplied per kilogram of diet: retinol, 12000 IU; cholecalciferol, 5000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamine, 16 µg; biotin, 200 µg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg; Cu (sulfate), 16 mg; Fe (sulfate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulfate and oxide), 120 mg; Zn (sulfate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg

[2]Feedzyme XBC 1000 (Feedworks, Australia)

[3]Sacox 120 (coccidiostat), [4]dEB (dietary electrolyte balance)= $Na^+ + K^+ - Cl^-$

Table 6.2 Analysed nutrients of experimental diets (as-fed basis)

Analysed nutrients, %	High CP			Low CP		
	Starter	Grower	Finisher	Starter	Grower	Finisher
Dry matter	91.4	90.3	91.0	91.2	91.4	91.1
Gross energy, MJ/kg	17.6	17.7	17.8	17.0	17.4	17.5
Crude protein	27.0	25.7	23.3	22.9	20.3	19.5
Crude fibre	2.99	3.32	2.99	2.97	2.68	2.85
Lysine	1.41	1.35	1.15	1.38	1.26	1.12
M+C	0.90	0.85	0.82	0.90	0.84	0.81
Threonine	0.96	0.93	0.84	0.92	0.83	0.78
Arginine	1.58	1.48	1.47	1.32	1.18	1.12
Valine	1.25	1.21	1.04	1.04	0.90	0.84
Isoleucine	1.08	1.03	0.89	0.87	0.77	0.70
Leucine	1.86	1.80	1.56	1.55	1.31	1.18
Phenylalanine	1.20	1.15	1.00	1.00	0.84	0.78
Tyrosine	0.65	0.61	0.54	0.53	0.44	0.37
Glycine	1.45	1.37	1.25	1.15	0.95	0.93
Alanine	1.19	1.13	0.99	0.94	0.79	0.68
Tryptophan	0.35	0.36	0.33	0.29	0.26	0.21
Ca	1.00	0.93	0.92	1.09	0.95	0.89
Total P	0.76	0.79	0.69	0.78	0.73	0.71
Na	0.19	0.18	0.18	0.19	0.18	0.18
K	1.11	1.05	0.93	0.84	0.75	0.73
Cl	0.15	0.15	0.15	0.30	0.25	0.25
Mg	0.23	0.24	0.21	0.20	0.20	0.19
S	0.31	0.32	0.28	0.28	0.28	0.28
dEB[1], mEq	324	304	274	213	199	194
dEB[2], mEq	320	302	273	203	189	176

[1]dEB (dietary electrolyte balance)= $Na^{+}+K^{+}-Cl^{-}$

[2]dEB= $Na^{+}+K^{+}+Mg^{2+}-Cl^{-}-S^{2-}$

6.2.2 Flux hood

A modified U.S EPA type flux hood (Figure 6.1) was constructed using a 560 ml, dome-shaped, stainless steel bowl that had an internal diameter of 156 mm that covered a surface area of 0.0191 m^2 (Kienbusch, 1986). Teflon tubes (3.18 mm outer diameter) and stainless steel fittings (Swagelok, USA) were used to construct all the inlet and outlet lines in the flux hood. For uniform airflow distribution inside the flux hood, a teflon tube, approximately 295 mm long, was positioned around the inner circumference of the hood and had four holes drilled in it. A short outlet tube (teflon tube) 260 mm long passed from the hood and connected to the SIFT-MS. A vent was constructed on top of the hood with a 55 mm length and teflon tube to prevent pressure fluctuations within the hood and to prevent outside air to enter the hood through the vent hole.


Figure 6.1 Flux hood outer view (left) and inner view (right)

6.2.3 Litter collection trays

The collection trays were carefully removed from each pen without disturbing the litter surface. The trays were covered with aluminium foil and immediately transferred to the laboratory under controlled conditions of 21 °C (±1 °C) with continuous air ventilation. Immediately before odorant analysis, the aluminium foil was removed and the sample litter tray was covered with the fabricated flux hood. The flux hood was purged with ultra-high purity N gas (N 99.99%, BOC Limited, Australia) at 500 ml/min until the gases under it reached the equilibrium concentration. The other end of the fluxhood was connected to the SIFT-MS instrument, which drew the gas sample at 14 ml/min. Measurements were taken at two different points in the tray and averaged to get a single value. The trays were put back to the respective pens immediately after odorant measurements without disturbing the litter surface. After each measurement, the SIFT-MS was flushed with the ultra-high purity N_2 gas for 8 min to prevent cross contamination within the sampling lines or instrument. Background measurements were done using the same N_2 gas.

6.2.4 SIFT-MS measurement of odorants

Emissions of odorants from the sample litter headspace were measured at 15, 29 and 35 d using SIFT-MS (Figure 6.2, Voice 200^{TM} SYFT technologies, Christchurch, New Zealand). A method containing 27 compounds, previously identified as key odorants in poultry litter (Murphy et al., 2014), was developed using the selected ion mode (SIM) and method development software (LabSyft) of the Voice 200^{TM}. The targeted compounds were: 2,3-butanedione (diacetyl), 3-methyl-1-butanol, total butanol (1-butanol + 2-butanol), 3-methylbutanal, 3-methylindole (skatole), 3-hydroxy-2-butanone (acetoin), acetic acid, benzene, butanoic acid, 2-butanone, dimethyl disulfide , dimethyl sulfide , dimethyl trisulfide , ethyl mercaptan , hexane, H_2S , indole, methylamine, methyl mercaptan , naphthalene, total cresol (p-cresol + m-cresol), phenol, propionic acid, trimethylamine, NH_3, methylamine, and dimethylamine . The scan duration was 130 s. The scans were repeated until the measured concentration of the compounds reached equilibrium. Prior to each analysis, the SIFT-MS was run with standard gases which included ethylbenzene, tetrafluorobenzene, toluene, hexafluorobenzene, ethylene, octafluorotoluene, benzene, and isobutene to ensure the instrument's mass-calibration and quantification for these compounds was consistent

for each measurement session. A reconfigured sample inlet system was designed to allow a bypass flow system to let the sample gas flow continuously through the inlet. This inlet design was to ensure a minimal sample loss and to retain sample integrity.

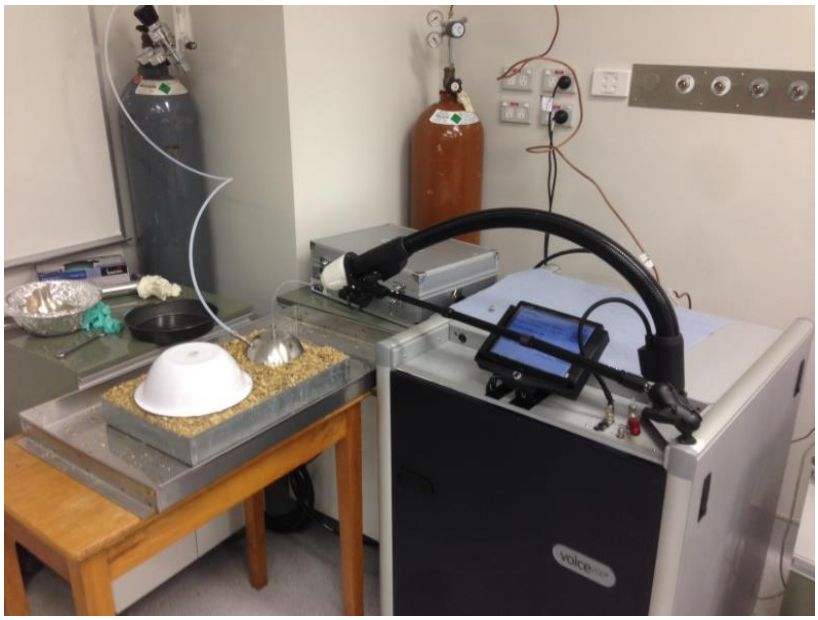

Figure 6.2 Measurement of odorants from litter headspace using flux hood and SIFT-MS.

6.2.5 Litter water activity and moisture content

At d 29, approximately 10 g of litter was sampled from the tray before odorant measurements to measure litter water activity using tuneable diode laser water activity meter (AquaLab-TDL, Decagon Devices Inc, USA). After A_w measurements, the sample was stored in an airtight container and refrigerated for 8 hours before measuring the moisture content. At d 35, immediately after odorant measurements, a sample of the same surface litter was placed in a tightly sealed plastic container and stored refrigerated for 8 hours before measuring the moisture content. Correlation between the moisture content of litter and the odorant concentration values was anlaysed. Litter moisture and pH were also measured separately at d 35 from the pens housing birds fed the high CP and low CP diets.

6.2.6 Chemical and gross energy analysis

Dry matter contents of diets and litter were determined as described in 4.2.6. Litter pH was determined by mixing litter and de-ionised water in a ratio of 1:5 with a pH meter (EcoScan 5/6 pH meter, Eutech Instrument Pte Ltd; Singapore). The nitrogen content of each diet was determined by the Dumas combustion technique as described in 4.2.6 and CP calculated by multiplying nitrogen by 6.25). Analysis of feed for crude fibre, amino acids and Cl was conducted at Experimental Research Station, University of Missouri, USA. Minerals in the feed were analysed using inductively coupled plasma optical emission spectrometer (**ICP-OES**, Model- 725 radial viewed). Gross energy

contents of feeds were determined on a 0.5 g sample using an adiabatic bomb calorimeter (IKA Werke, C7000, GMBH and Co., Staufen, Germany) with benzoic acid as standard.

6.2.7 Statistical analysis

Odorant concentrations were anlaysed following a 5 × 3 factorial arrangement using JMP statistical software version 8 (SAS Institute Inc, Cary, NC) to test the main effects of diet, age and their interactions. Odorant concentrations were not normally distributed and thus were transformed to a base 10 logarithm before analysis. Data were subjected to two-way ANOVA with repeated measures, and means were separated by Tukey's HSD test at a probability level of 0.05. Pearson correlation coefficients and associated significance were generated using JMP software to determine the relationship between litter moisture content and odorants. The relationship between litter moisture content and Aw was investigated by non-linear (exponential) regression analysis using JMP software.

6.3 Results

6.3.1 Feed analysis

The nutrient contents of finished feeds are presented in Tables 6.1 and 6.2. The anlaysed CP was 1-2% higher than the calculated values but the trend was similar across all the treatments. The anlaysed total amino acid contents in the high CP diets were higher than the low CP diets but the calculated digestible amino acid contents were nearly identical. The dietary electrolyte balance (dEB) calculated as $Na^{+}+K^{+}-Cl^{-}$ and dEB with Mg and S calculated as $Na^{+}+K^{+}+Mg^{2+}-Cl^{-}-S^{2-}$ were nearly identical within each diet but varied across diets. The high CP diets had both forms of dEB approximately 100 higher than the low CP diet in all starter, grower and finisher phases. The S content in the high CP diet was 0.03/0.04 percentage points higher in starter/grower phases but similar in the finisher phase.

6.3.2 Odorants from litter headspace

The odorants under the fluxhood in the litter headspace reached equilibrium concentration after 7-8 scans in 15-20 mins.

a) Sulfur compounds, phenolic compounds, amines and ammonia

The effect of diets on concentrations of odorants belonging to the group of sulfur compounds, phenolic compounds, amines and NH_3 are presented in Table 6.3. Dimethyl amine, trimethyl amine, H_2S, NH_3, and phenol were produced at lower levels in litter of the pens housing birds fed the low CP diet compared to those fed the high CP diet ($P < 0.05$). Birds fed the high CP diet with probiotic produced lower

concentration of H_2S ($P < 0.05$) in litter than those fed the high CP diet but the level was similar to those fed the low CP diet. Antibiotic addition to the high CP diet had no effect on the release of odorants compared to the high CP only diet ($P > 0.05$). Similarly, birds fed the high CP diet with saponin produced a lower concentration of trimethylamine and phenol in litter compared to those fed the high CP diet ($P < 0.05$) but the concentrations of these odorants were similar to those fed the low CP diet. The dietary treatments tended to have a significant effect on the concentration of methyl mercaptan ($P = 0.065$) and the highest value was observed in the litter of the birds fed the high CP diet. Dietary treatments had no effect on the concentrations of DMS, dimethyl disulfide, dimethyl trisulfide, methyl amine and total cresol in litter ($P > 0.05$). There was an effect of age on concentrations of sulfur compounds, phenolic compounds, amines and NH_3 in litter (Table 6.3). Among the amines, the concentrations of dimethyl amine and trimethyl amine increased ($P < 0.01$) whereas the methylamine concentration decreased ($P < 0.01$) with age. Among the phenolic compounds, the concentrations of phenol in litter decreased as the birds aged ($P < 0.01$) whereas total cresol remained constant throughout the study period ($P > 0.05$). The concentration of NH_3 in litter increased with age and the highest concentration was observed at d 35 ($P < 0.01$). The comparison of concentrations of sulfur odorants in litter at three different ages are presented in Figure 6.3. The concentration of DMS increased at d 29 and then remained constant at d 35. Hydrogen sulfide concentration remained similar at d 15 and 29 but increased at d 35. The concentration of methyl mercaptan increased in litter as the birds aged from 15 to 35 d. The concentrations of dimethyl trisulfide and ethyl mercaptan decreased with age ($P < 0.01$) and dimethyl disulfide did not change with age ($P > 0.05$).

b) Compounds belonging to the group of alcohols, aldehydes, ketones, indoles, SCFAs and benzene

There was no effect of diet on the emissions of total butanol, 2-butanone, indole, skatole, acetic acid and butanoic acid (Table 6.4). In contrast, birds fed the high CP diet with saponin produced a lower concentration of benzene in litter ($P < 0.05$) than those fed the high CP diet but the concentration was similar to that in the low CP diet group. The concentrations of total butanol and 2-butanone increased at d 29 and then remained constant until d 35. The concentrations of indole and skatole were similar at d 15 and 35 but their concentrations were lower at d 29 compared to d 15 ($P < 0.05$). Acetic acid and butanoic acid concentrations in litter decreased with age ($P < 0.01$) whereas the benzene concentration increased at d 35 compared to days 15 or 29 ($P < 0.05$).

Table 6.5 shows the interaction between diet and age on the concentrations of 2,3-butanedione, acetoin, 3-methyl-1-butanol, 3-methylbutanal, ethyl mercaptan, propionic acid and hexane ($P < 0.05$). Concentrations of 2,3-butanedione, acetoin, propionic acid and hexane in litter were higher from the birds fed the low CP diet compared to all other treatments on d 35 ($P < 0.05$) but not on days 15 and 29. The low CP diet and the high CP diet with saponin produced higher levels of 3-methyl-1-butanol and ethyl mercaptan in litter at d 35 compared to other diets ($P < 0.05$).

Table 6.3 Main effect of diet or age on the concentration of odorants belonging to the group of sulfur compounds, phenolic compounds, amines, and NH_3 (log_{10} μgm^{-3})[1]

Treatments	Odorants										
	Dimethyl sulfide	Dimethyl disulfide	Dimethyl trisulfide	Hydrogen Sulfide	Methyl mercaptan	Methyl amine	Dimethyl amine	Trimethyl amine	*NH3*	Phenol	Total cresol[5]
Diets											
Low CP	1.059	0.289	0.654	0.714[b]	0.757[b]	0.823	0.859[b]	1.536[b]	2.067[b]	0.683[b]	0.746
High CP	1.186	0.361	0.670	1.241[a]	0.932[a]	0.854	1.028[a]	1.819[a]	2.517[a]	0.757[a]	0.776
High CP+ antibiotic[2]	1.214	0.349	0.704	1.167[a]	0.879[ab]	0.845	1.047[a]	1.853[a]	2.421[a]	0.713[ab]	0.809
High CP+ probiotic[3]	1.150	0.306	0.635	0.870[b]	0.832[ab]	0.846	0.960[ab]	1.687[ab]	2.329[ab]	0.712[ab]	0.802
High CP+ saponin[4]	1.075	0.302	0.641	1.027[ab]	0.717[b]	0.826	0.892[ab]	1.569[b]	2.211[ab]	0.666[b]	0.732
SEM	0.063	0.025	0.020	0.137	0.051	0.013	0.050	0.065	0.111	0.021	0.027
Age											
15d	0.744[b]	0.302	0.811[a]	0.525[b]	0.526[c]	0.941[a]	0.781[c]	1.088[c]	1.373[c]	0.771[a]	0.800
29d	1.286[a]	0.327	0.602[b]	0.609[b]	0.872[b]	0.829[b]	0.972[b]	1.896[b]	2.412[b]	0.682[b]	0.762
35d	1.381[a]	0.336	0.571[b]	1.877[a]	1.133[a]	0.747[c]	1.119[a]	2.094[a]	3.142[a]	0.665[b]	0.757
SEM	0.049	0.019	0.016	0.106	0.040	0.023	0.039	0.051	0.086	0.017	0.021
P-value											
Diets	0.369	0.188	0.214	0.045	0.065	0.333	0.045	<0.01	0.047	0.041	0.265
Age	0.001	0.444	0.001	0.001	0.001	0.001	0.001	0.001	0.001	<0.01	0.311
Diet×Age	0.625	0.501	0.314	0.475	0.829	0.732	0.364	0.590	0.700	0.291	0.091

[a,b,c]within each treatment factor, means in the same column with a different superscript differ significantly ($P < 0.05$)

[1]concentrations were measured in a flux hood placed on meat chicken litter and flushed with 500 ml/min N_2

[2]zinc bacitracin, ALBAC 150 (Zoetis)

[3]a combination of three *Bacillus subtilis* strains, ENVIVAPRO (Dupont Animal Nutrition)

[4]a blend of *Yucca schidigera* and *Quillaja saponaria*, NUTRAFITO PLUS (Desert King International), [5]p-cresol+m-cresol

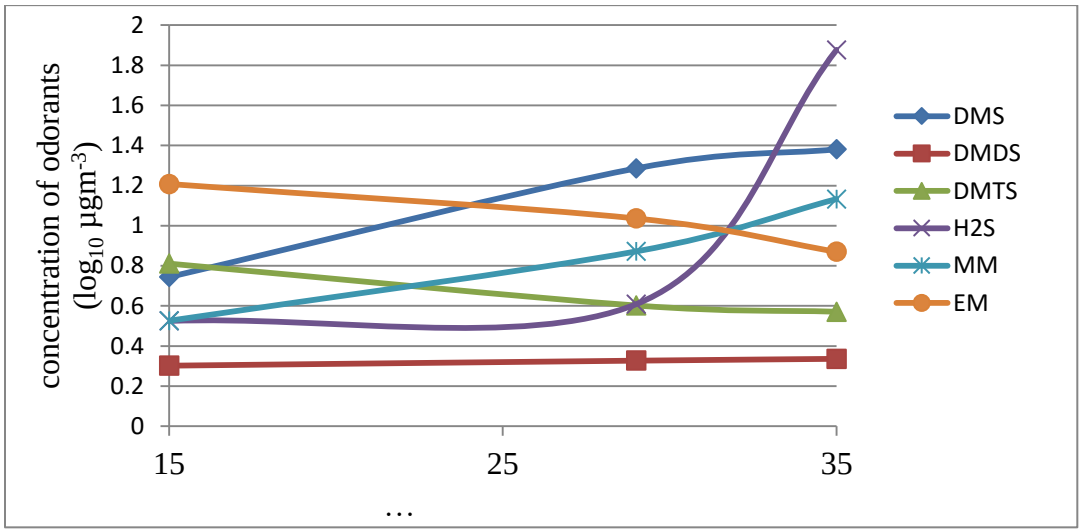

Figure 6.3 Comparison of litter headspace concentration of sulfur odorants at different ages

Table 6.4 Main effect of diet or age on the concentration of odorants belonging to the group of alcohols, ketones, indolic compounds, SCFAs and others (log_{10} µgm^{-3})[1]

Treatments	Odorants						
	Total butanol[5]	2-butanone	Indole	Skatole	Acetic acid	Butanoic acid	Benzene
Diets							
Low CP	1.398	1.529	0.308	0.361	2.260	1.709	0.531^{bc}
High CP	1.472	1.631	0.396	0.424	1.909	1.608	0.578^{ab}
High CP+ antibiotic[2]	1.250	1.421	0.372	0.406	2.002	1.708	0.552^{abc}
High CP+ probiotic[3]	1.409	1.502	0.376	0.359	1.948	1.663	0.633^{a}
High CP+ saponin[4]	1.199	1.375	0.325	0.351	2.122	1.681	0.468^{c}
SEM	0.103	0.110	0.037	0.037	0.121	0.061	0.034
Age							
15d	1.118^{b}	0.965^{b}	0.409^{a}	0.450^{a}	2.512^{a}	1.914^{a}	0.518^{b}
29d	1.500^{a}	1.781^{a}	0.288^{b}	0.324^{b}	2.164^{b}	1.698^{b}	0.524^{b}
35d	1.418^{a}	1.728^{a}	0.369^{ab}	0.365^{ab}	1.469^{c}	1.408^{c}	0.614^{a}
SEM	0.080	0.086	0.029	0.029	0.091	0.048	0.026
P-value							
Diets	0.319	0.523	0.361	0.525	0.207	0.749	0.020
Age	0.004	0.001	0.012	0.010	0.001	0.001	0.035
Diet×Age	0.356	0.466	0.244	0.366	0.444	0.176	0.305

[a,b,c]within each treatment factor, means in the same column with a different superscript differ significantly ($P < 0.05$)

[1]concentrations were measured in a flux hood placed on meat chicken litter and flushed with 500 ml/min N_2, [2]zinc bacitracin, ALBAC 150 (Zoetis)

[3]a combination of three *Bacillus subtilis* strains, ENVIVA PRO (Dupont Animal Nutrition)

[4]a blend of *Yucca schidigera* and *Quillaja saponaria*, NUTRAFITO PLUS (DesertKingInternational)

[5]1-butanol+2-butanol

Table 6.5 Interaction effect of diet and age on the concentration of odorants belonging to the group of alcohols, aldehydes, ketones, indolic compounds, SCFAs and others (log_{10} µgm^{-3})[1]

Treatments		Odorants						
		2-3, butanedione	Acetoin	3-methyl-1-butanol	3-methyl-butanal	Ethyl mercaptan	Propionic acid	Hexane
Diets								
Low CP		1.762	2.989	1.533	0.965	1.091	1.125	1.889
High CP		1.490	2.688	1.547	0.882	1.000	0.938	1.723
High CP+ antibiotic[2]		1.527	2.666	1.529	0.973	1.025	0.969	1.768
High CP+ probiotic[3]		1.578	2.750	1.578	0.918	1.032	0.995	1.717
High CP+ saponin[4]		1.703	2.836	1.525	0.857	1.042	1.016	1.831
SEM		0.049	0.061	0.041	0.052	0.039	0.069	0.051
Age								
15d		1.641	2.637	1.371	1.131	1.208	1.289	1.760
29d		1.643	3.072	1.739	0.879	1.036	1.074	1.782
35d		1.552	2.648	1.516	0.748	0.870	0.663	1.814
SEM		0.038	0.047	0.032	0.041	0.030	0.054	0.036
Diets	Age							
Low CP	15d	1.700^{bc}	2.688^{cde}	1.361^{g}	1.148^{ab}	1.155^{ab}	1.171^{ab}	1.813^{bcd}
	29d	1.611^{bcde}	3.056^{a}	1.603^{cdef}	0.744^{def}	1.050^{bcd}	1.121^{ab}	1.731^{bcd}
	35d	1.975^{a}	3.222^{a}	1.636^{bcd}	1.002^{abc}	1.068^{bcd}	1.082^{b}	2.124^{a}
High CP	15d	1.553^{cdef}	2.471^{def}	1.308^{g}	1.127^{ab}	1.167^{ab}	1.259^{ab}	1.641^{cd}
	29d	1.527^{bcdef}	2.962^{abc}	1.771^{abc}	0.847^{cde}	0.971^{cde}	0.981^{bc}	1.703^{bcd}
	35d	1.489^{cdef}	2.632^{def}	1.462^{ef}	0.671^{ef}	0.860^{ef}	0.573^{d}	1.823^{bc}
High CP+ Zn antibiotic[2]	15d	1.650^{bcd}	2.679^{cde}	1.440^{efg}	1.244^{a}	1.271^{a}	1.304^{ab}	1.849^{bc}
	29d	1.634^{bcd}	3.086^{a}	1.802^{ab}	0.866^{cde}	1.027^{bcde}	1.123^{ab}	1.767^{bcd}
	35d	1.297^{ef}	2.234^{f}	1.343^{fg}	0.809^{bcdef}	0.778^{ef}	0.480^{d}	1.686^{bcd}
High CP+ probiotic[3]	15d	1.652^{bcd}	2.617^{def}	1.437^{efg}	1.131^{ab}	1.262^{a}	1.429^{a}	1.664^{cd}
	29d	1.750^{ab}	3.231^{a}	1.875^{a}	0.870^{cde}	1.122^{abc}	1.109^{ab}	1.908^{ab}
	35d	1.332^{f}	2.402^{ef}	1.422^{fg}	0.554^{f}	0.713^{f}	0.448^{d}	1.578^{d}
High CP+ saponin[4]	15d	1.747^{ab}	2.732^{bcd}	1.310^{g}	1.002^{abcd}	1.185^{ab}	1.281^{ab}	1.833^{bcd}
	29d	1.694^{bc}	3.028^{ab}	1.645^{bcd}	0.867^{cde}	1.007^{bcde}	1.037^{bc}	1.800^{bcd}
	35d	1.667^{bcd}	2.747^{bcd}	1.618^{bcde}	0.703^{ef}	0.933^{de}	0.731^{cd}	1.859^{bc}
P-value								
Diets		0.001	0.005	0.880	0.482	0.506	0.350	0.076
Age		0.201	0.001	0.001	0.001	0.001	0.001	0.651
Diet×Age		0.003	0.001	0.019	0.017	0.030	0.047	0.017

^{a,b,c,d,e,f,g}within each treatment factor, means in the same column with a different superscript differ significantly ($P < 0.05$)

[1]concentrations were measured in a flux hood placed on meat chicken litter and flushed with 500 ml/min N_2

[2]zinc bacitracin, ALBAC 150 (Zoetis)

[3]a combination of three *Bacillus subtilis* strains, ENVIVAPRO (Dupont Animal Nutrition)

[4]a blend of *Yucca schidigera* and *Quillaja saponaria*, NUTRAFITO PLUS (Desert King International)

6.3.3 Litter moisture content and odorants

The correlation between litter moisture and odorants is presented in Table 6.6. Of the sulfur compounds, only methyl mercaptan (r = 0.453, $P < 0.01$), H_2S (r = 0.482, $P < 0.01$) and dimethyl sulfide (r = 0.621, $P < 0.01$) had a significant positive correlation with litter moisture content. Dimethyl disulfide tended to be positively correlated with litter moisture (r = 0.316, $P = 0.061$).

Table 6.6 Pearson correlation coefficient between litter moisture content and odorants

Items	Litter moisture (r)	*P*-value
Litter moisture	1.0	-
Sulfur compounds	0.534	0.001
Methyl mercaptan (methanethiol)	0.453	0.006
Ethyl mercaptan (ethanethiol)	0.108	0.531
Hydrogen sulfide	0.482	0.003
Dimethyl sulfide	0.621	0.001
Dimethyl disulfide	0.316	0.061
Dimethyl trisulfide	0.230	0.178
Amines	0.384	0.021
Methyl amine	-0.309	0.086
Dimethyl amine	0.287	0.089
Trimethyl amine	0.526	0.001
Ammonia	0.532	0.001
Phenolic compounds	0.376	0.024
Phenol	0.409	0.013
m-cresol+p-cresol	0.296	0.079
Indolic compounds	0.441	0.007
Indole	0.503	0.002
Skatole (3-methylindole)	0.344	0.041
Alcohols	0.271	0.111
1-butanol+2-butanol	0.286	0.104
3-methyl-1-butanol	0.180	0.292
Aldehydes	-0.012	0.943
3-methylbutanal	-0.012	0.943
Ketones	0.210	0.218
2,3-butanedione	0.188	0.274
3-hydroxy-2-butanone (acetoin)	-0.018	0.918
2-butanone	0.319	0.058
Short chain fatty acids	-0.249	0.184
Acetic acid	0.001	0.995
Propionic acid	-0.330	0.071
Butanoic acid	-0.318	0.072

Of the amines, only trimethyl amine had a positive correlation with litter moisture (r = 0.526, $P < 0.01$). Similarly, only phenol (r = 0.409, $P < 0.05$) of the phenolic group, indole (r = 0.503, $P < 0.01$) and skatole (r = 0.344, $P < 0.05$) had a significant positive correlation with litter moisture. Methyl amine (r = -0.309, $P = 0.086$), propionic acid (r = -0.318, $P = 0.072$) and butanoic acid (r = -0.318, $P = 0.072$) tended to be negatively correlated with the litter moisture content. No correlation was observed between the

litter moisture content and odorants belonging to the group of alcohols, aldehydes and ketones.

6.3.4 Litter moisture content and pH

The observations of litter moisture and pH with high and low CP diets are presented in Table 6.7. Reduction in dietary CP content lowered the moisture content in litter ($P < 0.01$). The low CP diet also tended to lower litter pH ($P = 0.05$).

Table 6.7 Effects of low protein and high protein diets on litter pH and moisture content

Diets	Litter pH	Litter moisture, %
High CP	7.5	38.3[a]
Low CP	6.9	31.8[b]
P-value	0.05	<0.01
SE	0.13	1.31

6.3.5 Litter moisture content and water activity

There was an exponential relationship between the litter moisture content and Aw values at d 29 ($r^2 = 0.938$, $P < 0.01$) which is presented in Figure 6.4. At d 29, Aw increased until it reached the value of 1.0 at a moisture content of approximately 50%.

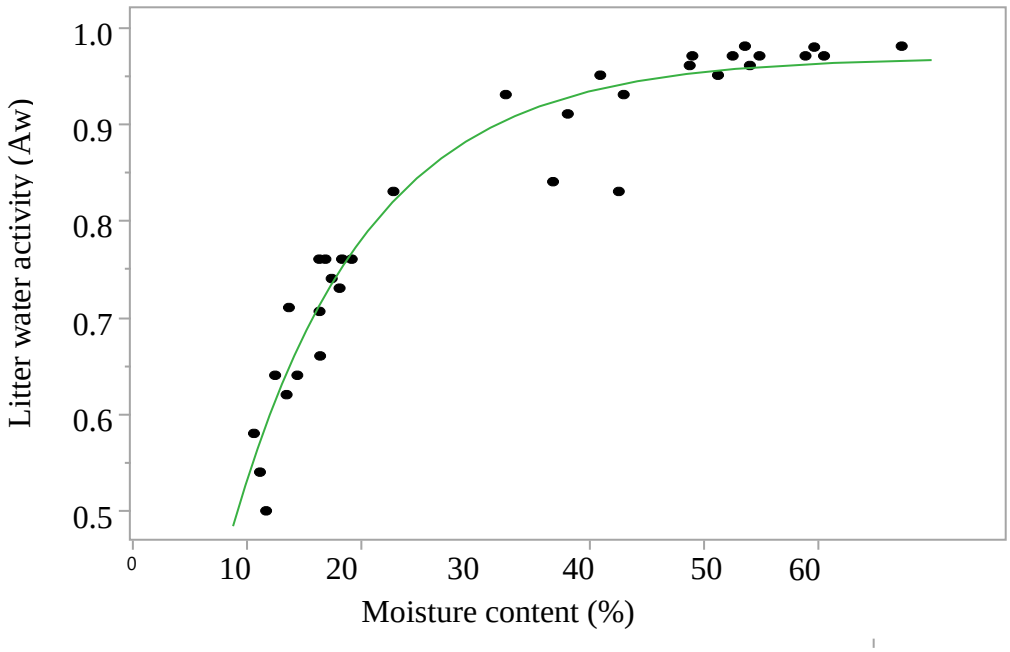

Figure 6.4 Relationship between litter moisture content and water activity

6.4 Discussion

Diet can play a significant role in controlling odour issues from meat chicken farms (McGahan et al., 2002). It has recently been reported that meat chicken diets

containing high levels of soybean meal produce a higher concentration of methyl mercaptan, a sulfur containing odorant (Sharma et al., 2015). In the same study, diets high in canola meal or soybean meal produced a high concentration of total elemental sulfur at the grower stage. Thus, meat chicken diets with different protein sources can affect the concentration of specific odorants. Similarly, manipulating dietary protein levels may alter the odorant concentrations in litter. Feeding low protein diets supplemented with a range of crystalline amino acids to provide required amino acids without excesses would be expected to reduce the excreted substrates for microbial fermentation to produce odorous metabolites.

In this study, reduction of dietary CP and simultaneous addition of synthetic amino acids lowered the litter headspace concentration of dimethyl amine, trimethyl amine, NH_3, H_2S and phenol. Studies in pigs have shown reduced methyl sulfide, carbon disulfide, ethanthiol, phenol, 4-ethyl phenol, indole and 3-methyl indole concentrations in manure by lowering CP in diet (Le et al., 2007). Similarly, NH_3 emissions have been reduced by feeding meat chickens with low CP diets supplemented with crystalline amino acids (Ferguson et al., 1998; Gates, 2000). Although lower emissions of NH_3 may not necessarily correlate with the reduced odour emission rates (Le et al., 2009; McGahan et al., 2002), the simultaneous reduction of some odorants along with NH_3 by feeding a low CP diet may reduce the overall odour intensity and offensiveness. Interestingly, the concentrations of 2,3-butanedione, 3-hydroxy-2-butanone (acetoin), propionic acid and hexane were higher in litter from the group fed the low CP diet compared to all other treatments on d 35 but not on d 15 and 29. Also, the group fed the low CP diet as well as the high CP diet with saponin produced higher levels of 3-methyl-1-butanol and ethyl mercaptan in litter at d 35 compared to other groups. These findings suggest that a low CP diet can reduce the production of some odorous metabolites in litter but may increase the production of some other odorants on d 35. The increase in some of the odorants on d 35 in the low CP group may be due to increased excretion and subsequently fermentation of carbohydrate-rich substrates relative to protein in litter. Birds fed the high CP diet, on the other hand, may have excreted more protein and amino acids relative to carbohydrates resulting into a higher amountof protein/AA fermentation products like phenol, H_2S and NH_3.

Litter moisture content and litter water activity may also influence microbial growth and odour emissions. Increased litter moisture content favoured the growth of *Atopostipes* and *Bacillus* species in meat chicken litter (Wadud et al., 2012) and these bacteria positively correlated with the levels of phenol, indole, iso-buyric acid and iso-valeric acid in pigs manure (Cho et al., 2015). In this study, birds fed the high CP diet had 6 percentage points higher litter moisture than those fed the low CP diet. High litter moisture was positively correlated to higher concentrations of trimethyl amine, H_2S, NH_3 and phenol. Thus, higher concentratons of these odorants from the birds fed the high CP diet may also be related to high litter moisture content. Further, there was an exponential relationship between litter moisture content and water activity at d 29. Water activity increased with litter moisture up to a level of 1.0 when the moisture level was nearly 50%. This finding was similar to the findings by Himathongkham et al. (1999). Increased litter water activity enhances the growth of micro-organisms (Payne et al., 2007), resulting in increased anaerobic degradation of litter. Thus, the difference in the flux of odorants from litter housing birds fed high and low protein

diets may be related to the difference in substrates available in litter, changes in litter moisture content, litter water activity and microbial ecosystem in litter.

Bacillus subtilis is a spore-forming bacterium that can be used as a probiotic in meat chicken diets (Dersjant-Li et al., 2014). In this study, birds fed the high CP diet with a probiotic based on three strains of *B. subtilis* produced a lower concentration of H_2S in litter than those fed the high CP diet. Our results agreed with the findings of Zhang et al. (2013) that there were lower concentrations of NH_3 and H_2S in excreta of meat chickens fed diets supplemented with *B. subtilits*. Addition of *B. subtilis* also showed improved nutrient retention, decreased caecal *Clostridium* and *Coliform* counts (Sen et al., 2012), reduced ileal, caecal and excreta *Salmonella* populations, increased *Lactobacillus* count in the ileum, caecum and excreta and reduced *E. coli* counts in the excreta of meat chickens (Jeong and Kim, 2014; Park and Kim, 2014). Thus, improved nutrient utilization and reduced counts of H_2S producing bacterial species such as *Salmonella* and *E. coli* in the gastrointestinal tract and excreta may have resulted in lower emissions of H_2S from litter in the probiotic fed group.

Saponins are natural detergents or surfactants found in a wide variety of plants. The major commercial saponin-containing products are those derived from *Yucca schidigera* and *Quillaja saponaria* (Cheeke, 2009). The group fed the high CP diet with saponin blend produced a lower concentration of trimethyl amine and phenol in litter compared to those fed the high CP diet alone. It has been reported that *Yucca* extract reduced faecal odours in dogs and cats and altered the chemical array of faecal volatiles (Lowe and Kershaw, 1997; Lowe et al., 1997). The researchers mentioned that some components of *Yucca* extracts may directly bind odorants. They also noted that the addition of *Yucca* extracts to dilute aqueous solutions of dimethyl sulfide, dimethyl disulfide, indole and skatole reduced the degree of offensiveness. Saponin is also reported to inhibit microbial fermentation of protein (Cheeke, 2000). All these properties of saponin may have resulted in a decreased concentration of some odorants in this study.

In conclusion, the results of this experiment showed that a low protein diet balanced with supplemental amino acids, a probiotic of three *Bacillus subtilis* strain and a blend of *Yucca* and *Quillaja* saponins were effective in reducing key odorants from meat chickens. However, none of the additives tested reduced the concentrations of odorants in litter below that produced from the low protein diet. Further research is warranted to investigate strategies to reduce odour emissions from birds fed low protein diets, which will have further commercial application in the future. There was a significant correlation between litter moisture content, litter water activity and odorant concentration in litter. A high litter moisture increased water activity and favored the emissions of sulfur containing odorants, trimethyl amine, phenol, indole and skatole over others. These results may have implications in controlling odour emissions from meat chicken facilities through dietary manipulation and effective control of litter moisture and water activity.

6.5 Supporting information

6.5.1 Performance of meat chickens

Feed intake (FI) and body weight (BW) were measured in each pen on d 10, 24 and 34, and mortality was recorded daily. The feed conversion ratio (FCR) was calculated by dividing total FI by BWG of live plus dead birds. The effect of dietary CP level and additives on performance of birds is presented in Tables 6.8 and 6.9.

The overall liveability during the entire study period was more than 98% but the birds fed the high CP diet had 2.19 % higher liveability during 0-34 d than the birds fed the low CP diet ($P < 0.05$). There was no effect of diets on liveability during 0-24 d ($P > 0.05$).

The birds fed the low CP diet had higher FI and BWG at all stages of growth than those fed the high CP diet ($P < 0.01$). Feed intakes were 6.36 % and 5.22 % higher in the low CP group compared to the high CP group at d 24 and 34 respectively. The addition of in-feed antibiotic, probiotic or saponin into the diets had no effect on FI at all the phases. Antibiotic improved BWG during the starter phase ($P < 0.01$) but had no effect during the finisher period ($P > 0.05$). During the grower period, there was an interaction between diet type and additive on BWG ($P = 0.05$). At this period, antibiotic tended to improve BWG by 5.52 % on the birds fed the low CP diet ($P = 0.05$) but had no effect on those fed the high CP diet. On d 34, antibiotic increased BWG by 4.86 % in the birds fed the low CP diet ($P < 0.05$) but had no effect on those fed the high CP diet. The addition of probiotic or saponin had no effect on BWG at any stages of growth but there was an interaction between these additives and diet on d 34. The high CP diet with probiotic produced better BWG in birds than the high CP diet with saponin ($P < 0.05$). However, there was no effect of probiotic on BWG when it was added to the low CP diet. The FCR was lower by 4 points in the birds fed the high CP diet compared to those fed the low CP diet during the starter period ($P < 0.05$).

Additives tended to lower FCR ($P = 0.056$) during the starter period with the lowest FCR in birds achieved by the addition of in-feed antibiotic. There was an effect of diet × additive on FCR on d 24 and 34. During these periods, the high CP diet without additive produced 3 points lower FCR in the birds compared to the low CP diet without additive ($P < 0.05$). Addition of antibiotic to the low CP diet decreased FCR in the birds on d 24 and 34 but antibiotic had no effect on FCR when used with the high CP diet ($P < 0.05$). Probiotic and saponin had no effect on FCR of birds when they were added to both the high CP and the low CP diets.

Table 6.8 Performance of meat chickens fed diets with different protein levels and additives at starter and grower stages

	0-10 d			10-24 d			0-24 d			
Diets	FI, g	WG, g	FCR	FI, g	WG, g	FCR	FI, g	WG, g	FCR	Liveability
Low CP	309[a]	250[a]	1.237[a]	1725[a]	1291[a]	1.324	2023[a]	1542[a]	1.313	98.62
High CP	275[b]	229[b]	1.202[b]	1640[b]	1239[b]	1.337	1902[b]	1468[b]	1.296	99.55
SEM	3.07	1.85	0.01	14.45	11.9	0.006	16.5	13.40	0.005	0.67
Additives										
None	288	232[b]	1.241	1668	1261	1.323	1947	1493	1.304	99.17
Zn bacitracin[1]	296	251[a]	1.182	1713	1296	1.322	1993	1547	1.289	99.17
Bacillus subtilis[2]	291	238[b]	1.224	1694	1269	1.335	1973	1507	1.310	99.08
Saponin blend[3]	294	238[b]	1.231	1655	1235	1.342	1938	1473	1.316	98.94
SEM	4.35	2.68	0.02	20.4	17.2	0.008	23.8	19.26	0.008	0.95
Treatments										
Low CP										
None	309	243	1.271	1723	1287[b]	1.339[ab]	2025	1530[b]	1.323[a]	100.0
Zn bacitracin[1]	308	266	1.161	1786	1358[a]	1.316[bc]	2076	1623[a]	1.279[c]	98.33
Bacillus subtilis[2]	305	245	1.247	1710	1262[bc]	1.355[a]	2003	1506[bc]	1.330[a]	98.08
Saponin blend[3]	315	248	1.268	1680	1259[bc]	1.336[abc]	1987	1507[bc]	1.320[ab]	98.08
High CP										
None	267	220	1.211	1612	1235[bc]	1.306[c]	1869	1455[c]	1.285[c]	98.33
Zn bacitracin[1]	284	236	1.202	1640	1235[bc]	1.329[abc]	1911	1471[bc]	1.299[abc]	100.0
Bacillus subtilis[2]	277	231	1.201	1677	1276[bc]	1.315[bc]	1942	1507[bc]	1.289[bc]	100.0
Saponin blend[3]	273	228	1.194	1631	1211[c]	1.347[ab]	1888	1439[c]	1.312[abc]	99.80
SEM	6.32	3.81	0.02	29.7	24.6	0.01	33.60	27.18	0.01	1.38
P-values										
Diet	<0.01	<0.01	0.045	<0.01	<0.01	0.14	<0.01	<0.01	0.054	0.33
Additive	0.54	<0.01	0.056	0.19	0.11	0.28	0.32	0.049	0.14	0.99
Diet × Additive	0.34	0.21	0.067	0.18	0.050	0.046	0.35	0.048	0.03	0.44

a,b Means in the same row with different superscripts differ ($P < 0.05$) or ($P < 0.01$), [1]Zinc bacitracin, Albac®150 (Zoetis), [2]a combination of three *Bacillus subtilis* strains, Enviva® Pro (Dupont Animal Nutrition), [3]a blend of *Yucca schidigera* and *Quillaja saponaria*, Nutrafito® plus (Desert King International)

Table 6.9 Performance of meat chickens fed diets with different protein levels and additives at finisher stage

	24-34 d			0-34 d			
Diets	FI, g	WG, g	FCR	FI, g	WG, g	FCR	Liveability
Low CP	1626[a]	1047[a]	1.554	3649[a]	2588	1.406	97.35[b]
High CP	1566[b]	1013[b]	1.546	3468[b]	2481	1.396	99.48[a]
SEM	14.85	10.14	0.005	25.5	18.9	0.004	0.69
Additives							
None	1597	1035	1.543	3544	2528	1.400	99.06
Zn bacitracin[1]	1612	1042	1.547	3605	2589	1.388	97.43
Bacillus subtilis[2]	1591	1030	1.545	3564	2537	1.402	98.40
Saponin blend[3]	1582	1011	1.564	3520	2485	1.414	98.77
SEM	21.61	14.75	0.007	35.23	27.61	0.006	0.97
Treatments							
Low CP							
None	1621	1042	1.556	3646	2573[b]	1.415[a]	98.96
Zn bacitracin[1]	1657	1075	1.541	3733	2698[a]	1.377[c]	95.89
Bacillus subtilis[2]	1584	1020	1.553	3587	2527[bc]	1.415[a]	96.65
Saponin blend[3]	1641	1048	1.566	3629	2556[b]	1.416[a]	97.90
High CP							
None	1574	1028	1.531	3443	2483[bc]	1.384[bc]	99.17
Zn bacitracin[1]	1567	1009	1.553	3478	2480[bc]	1.400[abc]	98.96
Bacillus subtilis[2]	1598	1040	1.537	3541	2547[b]	1.389[bc]	100.0
Saponin blend[3]	1523	975	1.562	3411	2414[c]	1.411[ab]	99.64
SEM	30.49	20.81	0.010	49.83	37.97	0.009	1.432
P-values							
Diet	<0.01	0.03	0.31	<0.01	<0.01	0.13	0.04
Additive	0.78	0.52	0.28	0.39	0.07	0.069	0.63
Diet × Additive	0.17	0.09	0.35	0.21	0.02	0.01	0.61

[a,b]Means in the same row with different superscripts differ ($P < 0.05$) or ($P < 0.01$), [1]Zinc bacitracin, Albac®150 (Zoetis), [2]a combination of three *Bacillus subtilis* strains, Enviva® Pro (Dupont Animal Nutrition), [3]a blend of *Yucca schidigera* and *Quillaja saponaria*, Nutrafito® plus (Desert King International)

6.5.2 Caecal SCFAs

On d 34 and 35 each, two birds were randomly selected from each pen and euthanized by cervical dislocation to collect the caecal contents. The caecal contents of d 34 birds were analysed for measurements of (SCFAs, lactic acid and succinic acid using the method described by Jensen et al. (1995) and were quantified using a Varian CP3800 gas chromatograph (Varian Analytical Instruments, Palo Alto, CA, USA).

The effect of dietary protein levels and additives on the concentration of SCFAs, lactic acid and succinic acid in the caecal contents on d 34 is presented in Table 6.10. Birds fed the low CP diet had lower concentration of isovaleric acid in the caecal contents compared to those fed the high CP diet ($P < 0.05$). There was diet × additive effect on concentrations of isobutyric acid and BCFA in the caecal contents ($P < 0.05$). Birds fed the high CP diet without additives had higher concentration of isobutyric acid and branched chain fatty acids (BCFA) in the caecal contents compared to all other treatments ($P < 0.05$). Addition of antibiotic or probiotic or saponin to the high CP diet reduced the concentrations of isobutyric acid and BCFAs ($P < 0.05$) but there was no effect when these additives were added to the low CP diet. The concentration of propionic acid in caecal contents tended ($P = 0.09$) to change by diet × additive interaction with highest concentration observed in the birds fed the low CP diet with antibiotic.

6.5.3 Quantification of caecal bacteria

The caecal contents of d 34 birds were also analysed for bacterial quantification. The extraction of bacterial DNA from caecal contents and the quantification of total bacteria, *Enterobacteriaceae*, *Lactobacillus spp.*, *Bifidobacteria* and *Clostridium spp.* were performed according to the procedures described by M'Sadeq et al. (2015). PCR was performed in a Rotorgene 6500 real-time PCR machine and the quantification of bacteria was conducted by the Rotorgene 6000 series software v.1.7 (Corbett, Sydney, Australia). The effects of diets and additives on the counts of *Enterobactericeae,* total bacteria, *Lactobacillus spp.*, *Bifidobacteria* and *Clostridium spp.* in caecal contents are presented in Table 6.11. Diets or additives had no effect on the caecal counts of *Enterobacteria* and *Clostridium spp.* but the birds fed the high CP diet had higher number of *Lactobacillus spp.* ($P < 0.01$) and total bacteria ($P < 0.05$) but lower number of *Bifidobacteria* ($P < 0.05$) compared to those fed the low CP diet. Additives had no effect on the total bacteria, *Enterobactericeae, Lactobacillus spp.* and *Bifidobacteria* counts in the caeca.

Table 6.10 Effect of in-feed additives in meat chicken diets with different levels of protein on caecal metabolites at d 34 (mmolkg^{-1})

Caecal metabolites	Dietary treatments											
	Low CP diet				High CP diet					P-value		
	None	Antibiotic[1]	Probiotic[2]	Saponins[3]	None	Antibiotic[1]	Probiotic[2]	Saponins[3]	SEM	Diet	Additive	Diet× Additive
Formic acid	0.953	1.074	1.315	0.856	1.094	0.605	0.972	1.067	0.21	0.46	0.52	0.32
Acetic acid	73.43	84.44	78.48	70.31	79.23	83.93	88.56	77.52	6.73	0.25	0.36	0.87
Propionic acid	3.882	5.106	2.972	4.069	4.088	3.842	3.998	3.250	0.49	0.55	0.20	0.09
Butyric acid	21.78	26.53	22.58	18.39	20.13	21.28	26.75	22.40	2.85	0.88	0.38	0.29
Isobutyric acid	0.451^{b}	0.546^{b}	0.414^{b}	0.488^{b}	0.769^{a}	0.487^{b}	0.493^{b}	0.482^{b}	0.065	0.08	0.11	0.04
Isovaleric acid	0.146	0.186	0.152	0.151	0.260	0.161	0.219	0.217	0.03	0.02	0.81	0.15
Valeric acid	1.109	1.264	0.983	0.953	1.322	1.135	1.417	1.110	0.14	0.11	0.62	0.25
Total VFA[4]	101.7	119.2	106.9	95.20	106.9	111.4	122.4	106.0	9.25	0.37	0.32	0.56
Lactic acid	0.328	0.478	0.498	0.316	0.245	0.421	0.302	0.417	0.16	0.62	0.78	0.86
Succinic acid	4.432	5.135	6.647	5.745	3.711	7.675	6.195	7.560	1.57	0.48	0.33	0.65
BCFA[5]	0.597^{b}	0.733^{b}	0.566^{b}	0.639^{b}	1.029^{a}	0.649^{b}	0.712^{b}	0.698^{b}	0.08	0.04	0.25	0.048

[a,b,c] Means in the same row with different superscripts differ ($P \leq 0.05$), [1]Zinc bacitracin, Albac®150 (Zoetis), [2]a combination of three *Bacillus subtilis* strains, Enviva® Pro (Dupont Animal Nutrition), [3]a blend of *Yucca schidigera* and *Quillaja saponaria*, Nutrafito® plus (Desert King International), [4]Total VFA= Total volatile fatty acids is the sum of formic acid, acetic acid, propionic acid, butyric acid, valeric acid, isobutyric acid and isovaleric acid, [5]BCFA= branched chain fatty acids (sum of isobutyric and isovaleric acids).

Table 6.11 Effect of in-feed additives in meat chicken diets with different levels of protein on caecal bacterial counts at d 34 (log_{10} CFU/g of digesta)

Treatments	Bacterial counts in caeca (log_{10} CFU/g of digesta)				
Diets	Enterobacteria	Total bacteria	*Lactobacillus spp.*	*Bifidobacteria*	*Clostridium spp.*
Low CP	7.72	10.71	8.460	8.318	6.007
High CP	7.91	10.78	8.748	8.231	5.953
SEM	0.13	0.027	0.058	0.028	0.048
Additives					
None	7.88	10.79	8.620	8.240	5.994
Zn bacitracin[1]	7.53	10.76	8.771	8.347	6.035
Bacillus subtilis[2]	7.86	10.67	8.532	8.277	6.034
Saponin blend[3]	8.00	10.73	8.493	8.234	5.856
SEM	0.18	0.041	0.081	0.039	0.065
P-values					
Diet	0.30	0.040	0.001	0.037	0.430
Additive	0.33	0.182	0.104	0.185	0.232
Diet × Additive	0.67	0.701	0.410	0.802	0.481

[1]Zinc bacitracin, Albac®150 (Zoetis),

[2]Combination of three *Bacillus subtilis* strains, Enviva® Pro (Dupont Animal Nutrition),

[3]A blend of *Yucca schidigera* and *Quillaja saponaria*, Nutrafito® plus (Desert King International).

7. Experiment 4

Title: Effect of necrotic enteritis challenge and high dietary salt (wet litter) on odour emissions from meat chickens

7.1 Introduction

Necrotic enteritis (NE) affected flocks have poor enteric health and nutrient digestibility leading to decreased performance and increased excretion of nutrients (Barekatain et al., 2013; Hofacre et al., 2003). Sub-clinical forms of NE can lead to sticky droppings and dark and moist litter (Kalshusdal and Hofshagen, 1992). Evidence suggests that there is an association between diarrhea, wet litter and NE (Williams, 2005) but it is unclear whether the occurrence of NE leads to wet litter or vice-versa (Hermans and Morgan, 2007). Acute forms of NE may lead to diarrhea (Helmboldt and Bryant, 1971) but this is not always the case (Nairn and Bamford, 1967). If NE affected birds produce sticky droppings, diarrhea and wet litter, then NE may exacerbate odour emissions. However, this has not been investigated to the best of our knowledge.

Wet litter in commercial poultry farms is an increasing problem around the world and litter quality has come under great scrutiny after new standards were set by animal welfare organizations in Australia (RSPCA, 2013). According to RSPCA (2013) meat chicken standard, "litter must be maintained in a dry and friable condition." Litter is also considered to be the primary source of odour because the majority of odorants are released during the decomposition of organic matter (Hobbs et al., 2004; Mackie et al., 1998). Litter with high moisture content has been shown to instigate the emission of highly odorous sulfur compounds (Sharma et al., 2016). Increasing dietary levels of sodium (Na) has been shown to increase water intake and litter moisture (Francesch and Brufau, 2004). WI:FI, litter moisture and emission of odorants that produce noxious smell from meat chicken farm may be heightened in birds that are challenged with NE or fed diets with high levels of Na. Understanding the emissions of odorants from NE affected flocks in wet litter conditions provides opportunity to target combating these specific odorants through the use of in-feed or litter additives. This study was conducted to investigate the effect of sub-clinical NE and high dietary Na level on litter headspace concentration of odorants in a meat chicken farm.

7.2 Materials and methods

7.2.1 Animal ethics

All the experimental procedures were approved by the Animal Ethics Committee at the University of New England, Australia (Authority No.: AEC16-019).

7.2.2 Bird husbandry, experimental design and diets

A total of 160 d-old Ross 308 male broiler chicks were assigned to four dietary treatments each with 4 replicates of 10 birds/pen (pen size: 1.2 m × 0.65 m up to d 10 and 0.84 m × 0.60 m thereafter) with fresh pine shavings (Hysorb wood shavings, ECW, Australia) as bedding material. A litter collection tray measuring 0.46 m × 0.29 m × 0.065 m was placed in each pen away from the feeder and drinker before litter was spread over the pens covering the tray. A 2 × 2 factorial arrangement of treatments was employed in a completely randomized design to study the effect of NE challenge (no, yes), dietary Na level (1.6 g/kg, 4.0 g/kg) and their interaction on litter headspace concentration of odorants. The high Na diet contained a high level of salt. The diets contained wheat, SBM, CM and MBM as basal ingredients and were formulated to meet the 2014 Ross 308 nutrient specifications (Aviagen, 2014) except for the high Na diet which contained 2.5 times more Na than the normal diet. Feed was provided in three phases: starter (0-10 d), grower (10-24 d) and finisher (24-33 d). The composition of the experimental diets and their calculated and anlaysed nutrients are presented in Table 7.1. Feed was mixed and cold pelleted at 65 °C at the University of New England, Australia. All diets were fed in crumble form to 10 d and as 3 mm diameter pellets thereafter until finishing the 33 d study period. Feed and water were provided *ad libitum* throughout the study. Lighting program was provided according to the Ross 308 breed management manual (Aviagen, 2014).

7.2.3 Necrotic enteritis challenge

Necrotic enteritis challenge was based on a previously conducted procedure (Rodgers et al., 2015; Wu et al., 2010). The challenged birds were given a one-off dose of 1 ml *per os Eimeria* species (*E. acervulina*, 5000 oocytes; *E. maxima*, 5000 oocytes; *E. brunetti*, 2500 oocytes) in PBS on d 9, and the same dose *per os* of sterile PBS was administered to the birds in the non-challenge group. On d 14, each bird in the challenge group was subjected to 2 ml *per os* the culture of *Cp* type A strain EHE-NE18 containing approximately 10^7 CFU/ml in thioglycollate (USP-alternative; Oxoid, UK) broth supplemented with peptone and starch. The non-challenge group received the same dose of sterilised broth. Aseptic technique was applied in the growth of broth culture and the inoculation processes to prevent the chance of contaminations.

7.2.4 Performance measurements

Birds and leftover feeds were weighed in all pens on d 16 and 33 to measure FI, BWG and FCR (corrected for mortality) during these periods. Water and FI were measured from d 13-20 to calculate WI:FI during this period. On d 16, two birds from each pen were randomly selected and euthanized by cervical dislocation to investigate the intestinal lesions caused by NE challenge.

Table 7.1 Ingredient composition, calculated and anlaysed nutrients of experimental diets (as-fed basis)

Ingredients, %	Normal Na diet			High Na diet		
	Starter	Grower	Finisher	Starter	Grower	Finisher
Wheat	55.8	60.4	64.9	54.6	59.2	65.2
Soybean meal	25.1	19.2	14.2	25.3	19.4	15.8
Canola meal	8.0	10.0	10.0	8.0	10.0	7.3
Meat meal	5.9	5.0	4.6	5.9	5.0	4.7
Canola oil	3.6	3.9	4.9	4.0	4.3	4.9
Limestone	0.31	0.32	0.33	0.31	0.32	0.33
Salt	0.11	0.12	0.12	0.72	0.73	0.73
Na bicarbonate	0.150	0.150	0.150	0.150	0.150	0.150
D,L-methionine	0.309	0.252	0.220	0.311	0.250	0.237
L-Lysine HCl	0.260	0.246	0.248	0.258	0.240	0.250
L-Threonine	0.181	0.146	0.128	0.181	0.150	0.134
Vitamin-mineral premix[1]	0.200	0.200	0.200	0.200	0.200	0.200
Choline Cl, 70%	0.070	0.070	0.060	0.070	0.070	0.060
Calculated nutrients						
ME, MJ/kg	12.55	12.76	13.18	12.55	12.76	13.18
Crude protein	24.5	22.5	20.5	24.5	22.5	20.3
Crude fibre	2.83	2.89	2.83	2.81	2.87	2.69
dig lysine	1.28	1.15	1.03	1.28	1.15	1.03
dig M+C	0.95	0.87	0.80	0.95	0.87	0.80
dig arginine	1.43	1.27	1.12	1.43	1.27	1.13
dig isoleucine	0.92	0.84	0.75	0.92	0.83	0.76
dig threonine	0.86	0.77	0.69	0.86	0.77	0.69
dig valine	1.05	0.97	0.87	1.05	0.96	0.87
Ca	0.96	0.86	0.80	0.96	0.86	0.80
Total P	0.79	0.74	0.70	0.79	0.74	0.69
Av. P	0.48	0.43	0.40	0.48	0.43	0.40
Na	0.16	0.16	0.16	0.40	0.40	0.40
K	0.94	0.85	0.76	0.94	0.85	0.76
Cl	0.20	0.20	0.20	0.57	0.57	0.57
Anlaysed nutrients						
Dry matter	90.7	91.2	91.1	91.3	91.4	91.2
Crude protein	25.6	23.7	21.2	25.4	23.5	20.9
Na	0.15	0.16	0.15	0.42	0.40	0.40

[1]Vitamin-Mineral concentrate supplied per kilogram of diet: retinol, 12000 IU; cholecalciferol, 5000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamine, 16 µg; biotin, 200 µg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg; Cu (sulfate), 16 mg; Fe (sulfate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulfate and oxide), 120 mg; Zn (sulfate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg

7.2.5 Flux hood

The flux hood used in this experiment was similar to the one used to measure odour flux from litter by Sharma et al. (2016). The features of the flux hood were similar to that of the U.S. EPA flux chamber (Kienbusch, 1986) but this flux hood was modified and miniaturised compared to the U.S. EPA design.

7.2.6 Litter collection trays

The collection trays were carefully removed from each pen without disturbing the litter surface. The trays were covered with aluminium foil and immediately transferred to the laboratory under controlled conditions of 21°C (±1°C) with continuous air ventilation. Immediately before odorant analysis, the aluminium foil was removed and the sample litter tray was covered with the fabricated flux hood. Ultra-high purity N_2 gas (Nitrogen 99.99%, BOC Limited, Australia) was used as the sweep air in the flux hood at a flow rate of 500 ml/min. The ultra-high purity N_2 gas entered the flux hood through the inlet tube and was uniformly distributed in the inside the flux hood using a 1/8" teflon tube, approximately 295 mm long, that was positioned around the inner circumference of the hood and had four evenly spaced holes drilled in it. Samples were extracted from the flux hood at a flow rate of 14 ml/min through a short outlet tube (1/8" stainless steel) that was connected to the SIFT-MS with 1/8" Teflon tube that was 260 mm long. After each measurement, the SIFT-MS was flushed with the N_2 gas for 8 min to prevent cross contamination within the sampling lines or instrument. The trays were put back to the respective pens immediately after odorant measurements without disturbing the litter surface.

7.2.7 SIFT-MS measurement of odorants

Emissions of odorants from sample litter headspace were measured on d 20 using SIFT-MS (Voice 200^{TM} SYFT technologies, Christchurch, New Zealand). A method containing 28 key odorants that constitute emissions from meat chicken farm was developed using the selected ion mode (SIM) and method development software (LabSyft) of the Voice 200^{TM}. The scan duration was 130 s. The scans were repeated until the measured concentration of the compounds reached equilibrium. Prior to each analysis, the SIFT-MS was run with standard gases which included ethylbenzene, tetrafluorobenzene, toluene, hexafluorobenzene, ethylene, octafluorotoluene, benzene, and isobutene to ensure the instrument's mass-calibration and quantification for these compounds was consistent for each measurement session. A reconfigured sample inlet system was designed to allow a bypass flow system to let the sample gas flow continuously through the inlet. This inlet design was to ensure a minimal sample loss and to retain sample integrity.

7.2.8 Litter moisture and litter pH

Litter samples were collected from each pen on d 20 to measure the moisture content. From d 25 to d 30, several litter samples were collected from the pens and anlaysed for pH and moisture contents. Litter pH was determined by the method described in 6.2.6.

7.2.9 Chemical and gross energy analysis

Gross energy, dry matter and nitrogen content of the diets and litter were determined by the method described in 4.2.6. Mineral contents in the feed were anlaysed by the method described in 6.2.6.

7.2.10 Statistical analysis

Data were anlaysed following a 2 × 2 factorial arrangement using JMP statistical software version 8 (SAS Institute Inc, Cary, NC) to test the main effects of diet, NE challenge and the interaction between them. Odorant concentrations were not normally distributed and thus were transformed to a base 10 logarithm before analysis. Data were subjected to two-way ANOVA and means were separated by Tukey's HSD test at a probability level of 0.05. The relationship between litter moisture content and pH was investigated by linear regression analysis using JMP software.

7.3 Results

7.3.1 Feed analysis

The calculated and anlaysed nutrient contents of the diets are presented in Table 7.1. The anlaysed Na contents in the diets were similar to the calculated values.

7.3.2 Growth performance, water to feed intake ratio and litter moisture

The overall mortality during the entire study period was less than 3% and there was no diet or challenge related mortality ($P > 0.05$, data not shown). The effects of NE challenge and high dietary Na level on performance of birds are shown in Table 7.2. On d 16, the birds in the challenged group had 15.14% lower FI ($P < 0.01$) and 18 points poorer FCR ($P < 0.01$) than those in the unchallenged group. The birds fed the high Na diet had 4 points better FCR ($P = 0.05$) than those fed the normal Na diet. On d 16, NE challenge reduced BWG by 31% in the birds fed the normal Na diet but by only 22% in those fed the high Na diet ($P < 0.05$). During the period of 0-33 d, NE challenge reduced FI by 5.48% ($P < 0.05$), BWG by 9.02% ($P < 0.01$) and worsened FCR by 5 points ($P < 0.01$). There was diet × challenge effect on WI:FI of birds during the 13-20 d period.

During this period, NE challenge had no effect on WI:FI in the birds fed the normal Na diet but increased it by 14% ($P < 0.05$) in the birds fed the high Na diet.

Table 7.2 Effect of *Cp* challenge and high dietary sodium level on performance, WI:FI and litter moisture content of meat chickens

Treatments	FI, g	BWG, g	FCR	FI, g	BWG, g	FCR	FI, g	BWG, g	FCR	WI:FI	Litter moisture, %
		0-16 d			16-33 d			0-33 d		13-20 d	d 20
Diet											
Normal Na[1]	737	605	1.238[a]	2544[a]	1786	1.424	3281	2391	1.373	2.24	32
High Na[2]	739	619	1.203[b]	2408[b]	1709	1.409	3147	2328	1.353	2.81	51
SEM	12.3	9.3	0.01	38.3	22.5	0.01	47.5	28.1	0.01	0.06	0.75
CP challenge											
No	799[a]	706	1.131[b]	2506	1766	1.419	3304[a]	2471[a]	1.337[b]	2.43	35
Yes	678[b]	518	1.310[a]	2446	1730	1.414	3123[b]	2248[b]	1.389[a]	2.62	47
SEM	12.3	9.3	0.01	38.3	22.5	0.01	47.5	28.1	0.01	0.06	0.75
Treatments											
Normal Na+ No challenge	814	716[a]	1.138	2535	1765[ab]	1.437	3349	2481	1.350	2.23[c]	23[d]
Normal Na + *Cp* challenge	661	494[b]	1.339	2552	1808[a]	1.411	3212	2301	1.396	2.25[c]	40[c]
High Na + No challenge	783	696[a]	1.125	2476	1766[ab]	1.402	3259	2462	1.324	2.62[b]	48[b]
High Na + *Cp* challenge	695	542[b]	1.282	2339	1652[b]	1.416	3034	2195	1.383	2.99[a]	54[a]
SEM	17.4	13.1	0.02	54.2	31.8	0.01	67.1	39.6	0.01	0.06	1.07
P-value											
Diet	0.92	0.29	0.05	<0.05	<0.05	0.31	0.07	0.14	0.15	<0.001	<0.01
Cp challenge	<0.01	<0.01	<0.01	0.29	0.28	0.69	0.02	<0.01	<0.01	<0.05	<0.01
Diet × *Cp* challenge	0.08	<0.05	0.20	0.18	<0.05	0.18	0.52	0.29	0.60	<0.05	<0.01

[a,b,c,d]within each treatment factor, means in the same column with a different superscript differ significantly ($P \leq 0.05$)

[1]Na- 1.6 g/kg, [2]Na- 4.0 g/kg

Birds fed the high Na diet had higher WI:FI compared to those fed the normal Na diet ($P < 0.05$) irrespective of presence or absence of the NE challenge. Necrotic enteritis challenge increased ($P < 0.01$) litter moisture irrespective of dietary Na level with that of challenged being 1.7 times of unchallenged (from 23 to 40%) in the group fed the normal Na diet and that of challenged being only 1.1 times of unchallenged (from 48 to 54%) in those fed the high Na diet. Similarly, the birds fed the high Na diet had higher litter moisture than those fed the normal Na diet irrespective of presence or absence of the NE challenge ($P < 0.01$) with high Na diet giving 2.1 times of the moisture of normal Na diet in unchallenged birds but only 1.4 times in challenged birds.

7.3.3 Litter headspace concentration of odorants

The odorants under the flux hood in the litter headspace reached equilibrium concentration after 7-8 scans in 15-20 mins.

a) Odorants belonging to the group of sulfur and phenolic compounds

The effect of diet and NE challenge on concentration of odorants belonging to the group of sulfur and phenolic compounds is presented in Table 7.3. The birds fed the high Na diet produced higher concentrations of dimethyl sulfide ($P < 0.01$), dimethyl disulfide ($P < 0.01$), H_2S ($P < 0.05$), ethyl mercaptan ($P < 0.01$), propyl mercaptan ($P < 0.05$), phenol ($P < 0.01$) and tended to produce higher concentrations of dimethyl trisulfide ($P = 0.08$) and methyl mercaptan ($P = 0.10$) in litter headspace compared to those fed the normal Na diet. Necrotic enteritis challenged birds produced higher concentrations of dimethyl sulfide ($P < 0.05$), propyl mercaptan ($P < 0.05$) and tended to produce higher concentrations of ethyl mercaptan ($P = 0.07$) and carbon disulfide ($P = 0.09$) in the litter headspace compared to the unchallenged birds. No interactions between Na level in diet and necrotic enteritis challenge were observed ($P > 0.05$).

b) Odorants belonging to the group of alcohols, aldehydes, ketones, amines and carboxylic acids

As shown in Table 7.4, the birds fed the high Na diet produced lower concentration of 3-methyl butanal ($P < 0.05$) and tended to produce higher concentrations of skatole ($P = 0.06$) and indole ($P = 0.10$) in the litter headspace compared to those fed the normal Na diet. Necrotic enteritis challenged birds produced higher concentrations of total butanols ($P < 0.05$), acetoin ($P < 0.01$), skatole ($P = 0.05$), butyric acid ($P < 0.05$), methyl amine ($P < 0.05$) and tended to produce higher concentrations of indole ($P = 0.10$) and formic acid ($P = 0.10$) in the litter headspace compared to the unchallenged group. Significant interactions between diet and NE challenge were observed on the concentrations of odorants belonging to the group of alcohols, aldehydes, ketones, amines and carboxylic acids (Table 7.5).

Table 7.3 Main effect of diet or *Cp* challenge on the litter headspace concentration of odorants belonging to the group of sulfur compounds, phenols and cresols on d 20 ($log_{10}\mu gm^{-3}$)[1]

Treatments	Odorants									
	Dimethyl sulfide	Dimethyl disulfide	Dimethyl trisulfide	Hydrogen Sulfide	Methyl mercaptan	Ethyl mercaptan	Propyl mercaptan	Carbon disulfide	Phenol	Total cresols[2]
Diet										
Normal Na[3]	1.15[b]	0.50[b]	0.73	0.78[b]	0.94	1.23[b]	0.97[b]	1.27	0.58[b]	0.87
High Na[4]	1.75[a]	1.38[a]	0.86	1.38[a]	1.30	1.77[a]	1.14[a]	1.46	1.44[a]	0.93
SEM	0.07	0.16	0.05	0.13	0.14	0.07	0.05	0.08	0.15	0.04
Cp challenge										
No	1.32[b]	1.04	0.80	1.20	1.08	1.40	0.94[b]	1.26	1.10	0.90
Yes	1.57[a]	0.85	0.79	0.96	1.15	1.61	1.17[a]	1.47	0.91	0.90
SEM	0.07	0.16	0.05	0.13	0.14	0.07	0.05	0.08	0.15	0.04
P-value										
Diet	<0.01	<0.01	0.08	<0.05	0.10	<0.01	<0.05	0.13	<0.01	0.27
Cp challenge	<0.05	0.42	0.92	0.23	0.76	0.07	<0.05	0.09	0.41	0.99
Diet×*Cp* challenge	0.83	0.55	0.72	0.10	0.66	0.35	0.10	0.08	0.54	0.24

[a,b]within each treatment factor, means in the same column with a different superscript differ significantly ($P < 0.05$)

[1]concentrations were measured in litter headspace using flux hood placed on meat chicken litter and flushed with 500 ml/min N_2

[2]m-cresol+p-cresol

[3]1.6 g/kg Na, [4]4.0 g/kg Na

Table 7.4 Main effect of diet or *Cp* challenge on the litter headspace concentration of odorants belonging to the group of alcohols, aldehydes, ketones, amines and carboxylic acids on d 20 (log_{10} μgm^{-3})[1]

Treatments	Odorants							
	Total butanol[2]	Acetoin	Indole	skatole	3-methyl butanal	Formic acid	Butanoic acid	Methyl amine
Diet								
Normal Na[3]	1.85	2.01	0.34	0.34	1.49[a]	2.95	3.12	1.66
High Na[4]	1.94	1.93	0.55	0.60	1.23[b]	2.91	3.34	1.66
SEM	0.08	0.11	0.08	0.08	0.06	0.09	0.14	0.01
Cp challenge								
No	1.74[b]	1.73[b]	0.34	0.33[b]	1.30	2.81	2.98[b]	1.64[b]
Yes	2.05[a]	2.21[a]	0.56	0.61[a]	1.43	3.06	3.48[a]	1.68[a]
SEM	0.08	0.11	0.08	0.08	0.06	0.09	0.14	0.01
P-value								
Diet	0.40	0.65	0.10	0.06	<0.05	0.77	0.30	0.76
Cp challenge	<0.05	<0.01	0.10	0.05	0.16	0.10	<0.05	<0.05
Diet×*Cp* challenge	0.13	0.06	0.33	0.58	0.29	0.26	0.14	0.56

[a,b]within each treatment factor, means in the same column with a different superscript differ significantly ($P \leq 0.05$)

[1]concentrations were measured in litter headspace using flux hood placed on meat chicken litter and flushed with 500 ml/min N_2

[2]1-butanol+2-butanol

[3]1.6 g/kg Na, [4]4.0 g/kg Na

In the birds fed the high Na diet, NE challenge increased the litter headspace concentrations of 2,3-butanedione ($P < 0.05$), acetic acid ($P < 0.01$), propionic acid ($P < 0.01$), isobutyric acid ($P < 0.01$), isovaleric acid ($P < 0.01$), pentanoic acid ($P < 0.05$), 2-butanone ($P < 0.05$) and 3-methyl-1-butanol ($P < 0.05$) and decreased the concentrations of dimethyl amine ($P < 0.05$) and trimethyl amine ($P < 0.05$) but NE challenge had no effect on these odorants in the birds fed the normal Na diet. Similarly, the birds fed the high Na diet produced lower litter headspace concentrations of acetic acid ($P < 0.01$), propionic acid ($P < 0.01$), isobutyric acid ($P < 0.01$), isovaleric acid ($P < 0.01$), pentanoic acid ($P < 0.05$), and higher concentrations of dimethyl amine ($P < 0.05$) and trimethyl amine ($P < 0.05$) compared to those fed the normal Na diet in unchallenged birds but not in challenged birds. Moreover, the birds fed the high Na diet produced higher litter headspace concentrations of 2-butanone ($P < 0.05$) and 3-methyl-1-1butanol ($P < 0.05$) only in challenged birds but not in unchallenged birds.

Table 7.5 Interaction effect of diet and Cp challenge on the litter headspace concentration of odorants belonging to the group of alcohols, aldehydes, ketones, amines and carboxylic acids on d 20 ($log_{10}\mu gm^{-3}$)[1]

Treatments	Odorants									
	2,3-butanedione	Acetic acid	Propionic acid	Isobutyric acid	Isovaleric acid	Pentanoic acid	Dimethyl amine	Trimethyl amine	2-butanone	3-methyl-1- butanol
Diet										
Normal Na[2]	1.57	2.77	1.98	2.13	1.62	1.85	1.06	1.44	2.16	1.61
High Na[3]	1.57	2.01	1.50	1.68	1.35	1.48	1.19	2.19	2.83	2.16
SEM	0.08	0.10	0.07	0.06	0.06	0.05	0.02	0.09	0.16	0.08
No	1.36	2.00	1.44	1.63	1.29	1.51	1.20	2.05	2.11	1.73
Yes	1.78	2.79	2.04	2.18	1.68	1.82	1.05	1.58	2.88	2.04
SEM	0.08	0.10	0.07	0.06	0.06	0.05	0.02	0.09	0.16	0.08
Treatments										
Normal Na + No challenge	1.52^{ab}	2.68^{a}	1.97^{a}	2.07^{a}	1.59^{a}	1.78^{a}	1.10^{b}	1.47^{b}	2.07^{b}	1.60^{b}
Normal Na + *Cp* challenge	1.62^{ab}	2.85^{a}	1.98^{a}	2.20^{a}	1.65^{a}	1.91^{a}	1.02^{b}	1.41^{b}	2.26^{b}	1.62^{b}
High Na + No challenge	1.19^{b}	1.31^{b}	0.91^{b}	1.20^{b}	0.99^{b}	1.23^{b}	1.30^{a}	2.63^{a}	2.15^{b}	1.87^{b}
High Na + *Cp* challenge	1.94^{a}	2.72^{a}	2.10^{a}	2.16^{a}	1.70^{a}	1.74^{a}	1.08^{b}	1.75^{b}	3.50^{a}	2.46^{a}
SEM	0.11	0.14	0.10	0.08	0.08	0.07	0.02	0.13	0.23	0.12
P-value										
Diet	0.98	<0.01	<0.01	<0.01	<0.05	<0.01	<0.01	<0.01	<0.05	<0.01
Cp challenge	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05
Diet × *Cp* challenge	<0.05	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	<0.05	<0.05	<0.05

[a,b]within each treatment factor, means in the same column with a different superscript differ significantly ($P < 0.05$)

[1]concentrations were measured in litter headspace using flux hood placed on meat chicken litter and flushed with 500 ml/min N_2

[2]1.6 g/kg Na, [3]4.0 g/kg N

7.3.4 Litter moisture content and pH

There was a negative linear relationship between litter moisture and litter pH (r^2 = 0.54, $P < 0.01$) as shown in Figure 7.1. As litter moisture increased, litter pH decreased and vice-versa.

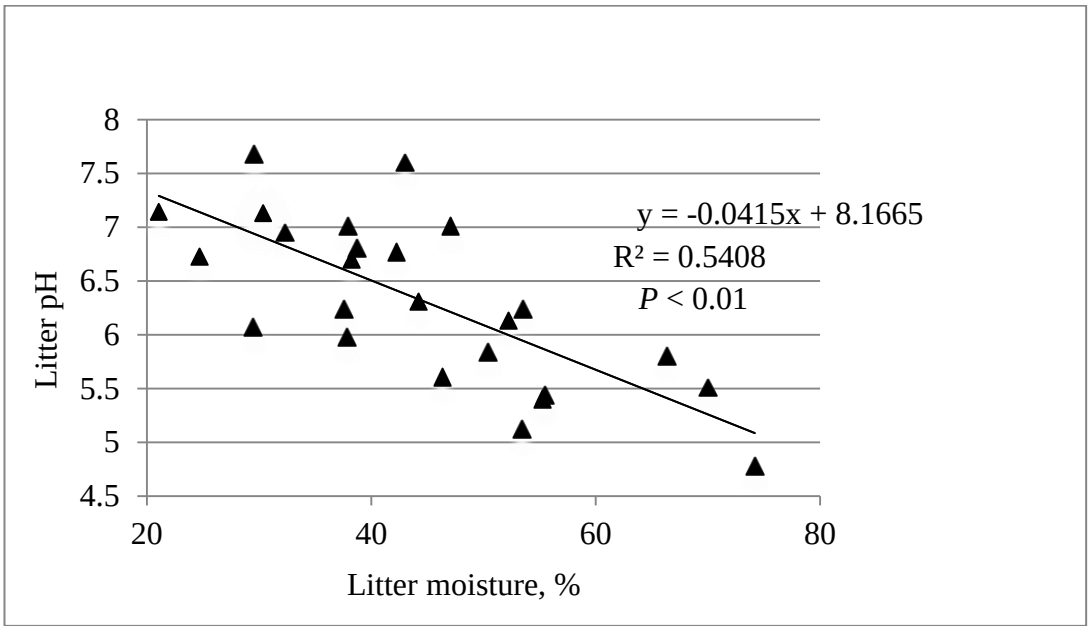

Figure 7.1 Relationship between litter moisture content and litter pH

7.4 Discussion

Dry and friable litter conditions are desirable in commercial poultry farms (Dunlop et al., 2016). When the litter is wet or birds are infected with enteric disease or both, there are more complaints on odour (Dr. Ferry Poernama, Technical Director, Japfa Comfeed, Indonesia, personal communication, 2015). We have recently shown that it may be possible to reduce the key odorants from meat chickens by dietary manipulation (Sharma et al., 2015; 2016). However, the understanding of the odorants that are emitted from wet litter or diseased flock has not been shown while this is important to allow nutritionists to target combating their negative effects through using feed or litter additives in odour abatement studies. This study aimed to investigate the effect of NE challenge and high dietary Na level on litter moisture and litter headspace concentration of odorants in meat chicken farm.

The challenge model used in this study was successful at inducing sub-clinical NE in meat chickens. The lower FI, lower BWG and poorer FCR in the challenged group as compared to the unchallenged group are in agreement with previous findings on sub-clinical NE affected meat chickens (Kalshusdal and Hofshagen, 1992; Lovland and Kaldhusdal, 2001). The performance losses may be associated with chronic intestinal mucosal damage caused by *Cp* resulting in poor nutrient digestion and absorption, and

higher excretion of nutrients (Van Immerseel et al., 2004; Timbermont et al., 2011). The high WI:FI in NE affected meat chickens observed in this study and the possible higher excretion of undigested nutrients may have resulted in increased litter moisture in NE affected group. High WI:FI in NE affected birds has been reported previously (van der Sluis, 2000) which supports our findings. Together with high litter moisture, sub-clinical NE also resulted in higher litter flux of odorants belonging to the group of sulfur compounds (dimethyl sulfide, propyl mercaptan and ethyl mercaptan), alcohols (total butanols), ketones (acetoin), carboxylic acids (butyric acid) and amines (methyl amine) regardless of Na level in the diet. Thus, sub-clinical NE in meat chickens resulted in wet litter with higher emissions of key odorants from litter. In addition, sub-clinical NE increased the litter flux of additional odorants in the group fed the high Na diet but not in those fed the normal Na diet. These included 2,3-butanedione, acetic acid, propionic acid, isobutyric acid, isovaleric acid, pentanoic acid, 2-butanone and 3-methyl-1-butanol. These odorants are carbohydrate and protein fermentation products (Mackie et al., 1998; Wadud, 2011), and were produced at concentrations higher than minimum ODT. These findings suggest that sub-clinical NE affected meat chickens may further increase odour emissions if the disease comes together with wet litter condition. This may be due to high litter water activity associated with wet litter condition. Data collected by Carr et al. (1995), van der Hoeven Hangoor (2014) and Sharma et al. (2016) showed that water activity in litter increased to 0.98-0.99 when litter moisture content reached 38-55%. It has been reported that *Clostridium spp.* grow well at high litter water activity of 0.90-0.97 (Dunlop et al., 2016). Thus, when litter is wet, *Clostridium spp*. may grow rapidly resulting into higher production of odorous metabolites.

Most of the sulfur compounds were produced at higher levels in the litter headspace of the pens housing birds fed the high Na diet with greater litter moisture compared with those fed the normal Na diet possibly due to the anaerobic biodegradation of litter in wet litter condition (McGahan et al., 2002; Hobbs et al., 2004). Sulfur compounds have very low ODT and higher emission of these odorants may affect the surrounding environment when the ventilation carries these odorants downwind the meat chicken farms. Most of these sulfur compounds were positively correlated with litter moisture, as illustrated in a previous study (Sharma et al., 2016). Litter moisture was negatively correlated with litter pH in this study which implies that a low litter pH may favor the emissions of sulfur containing odorants. Thus feeding a high Na diet results in wet litter condition which has a reduced pH that increases the litter flux of sulfur containing odorants. Interestingly, in the pens housing the unchallenged birds, the litter headspace concentrations of acetic acid, propionic acid, isobutyric acid, isovaleric acid and pentanoic acid was lower in the birds fed the high Na diet but this was not the case in the NE challenged birds. It may be possible that in the absence of NE, high Na diet can increase water intake and produce wet litter but water activity in litter may still be lower resulting in lower microbial activity for some specific microbes and hence lower emission of some microbial metabolites.

In conclusion, both high Na diet (wet litter) and sub-clinical necrotic enteritis may increase the odour nuisance potential of meat chicken farms. Therefore, control of litter conditions of farms and prevention of enteric diseases such as NE in meat chickens can alleviate the possible odour problems around the chicken farms.

8. Experiment 5

Title: Emissions of volatile odorous metabolites by *Cp- in vitro* study using two broth cultures

8.1 Introduction

Cp is a gram-positive anaerobic spore-forming rod-shaped bacterium that was first described to cause necrotic enteritis (NE) in poultry by Parish (1961). To date, the disease represents a devastating cost of approx. US$6 billion per annum to poultry industries around the world primarily from NE related production loss as well as subsequent counter measures (Wade and Keyburn, 2015).

One of the major gross pathological changes in the NE affected meat chickens is the presence of odorous brown fluid content in the intestine (Helmboldt and Bryant, 1971). The volatile metabolites produced by *Cp* causing the foul odour during NE infections have not been investigated. Research has shown that *Clostridium spp* produce various SCFAs, dimethyl disulfide, 2,3-butanediol, isopentanol and 3-hydroxy-2-butanone (Stotzky and Schenck, 1976). Among *Clostridium spp, Cp* has been reported to produce ethanol, propanol and n-butanol *in vitro* using gas GC-MS (Larsson et al., 1978). There has been a tremendous improvement in the field of mass spectrometry and it is now possible to anlayse most of the VOC produced by bacteria in real time (Thorn et al., 2011; Allardyce et al., 2006). There is hardly any information in the literature about volatile odorous metabolites produced by *Cp in vitro*. This study was an *in vitro* measurement of odorants produced by the growth of NE inducing *Cp* type A field strain (EHE-NE18) in two commonly used culture media, thioglycollate broth (USP-alternative) with peptone and starch supplementation and brain heart infusion broth.

8.2 Materials and methods

8.2.1 Experimental design

A total of six treatments each with three replicates were arranged in a completely randomized design with a 2 × 3 factorial arrangement of treatments to study the effect of two broth media (thioglycollate broth, brain heart infusion broth), three *Cp* levels (0, 10^3 cfu/ml, 10^6 cfu/ml), and their interaction on the concentration of odorants measured in media/bacterial culture headspace. The culture media with and without *Cp* were freshly prepared on the day of odorant measurements.

8.2.2 Media preparations, culturing and bacterial counts

Two types of broth media were used in this study, namely thioglycollate (USP-alternative) broth CM0173 (Oxoid, UK) and brain heart infusion broth CM1135 (Oxoid, UK). Desired amounts of broth media were dissolved in deionised water

according to the manufacturers` recommendation. Thioglycollate broth had additional supplementation with bacterial peptone (5 g/l) and soluble starch (10 g/l). Each broth was prepared in 2 litre conical glass bottles and mixed thoroughly to ensure the uniformity. The composition of broths is presented in Table 8.1.

Table 8.1 Composition of thioglycollate broth and brain heart infusion broth

Thioglycollate broth		Brain heart infusion broth	
Composition	Amount (g/litre)	Composition	Amount (g/litre)
l-cystine	0.5	Brain infusion solids	12.5
Sodium chloride	2.5	Beef heart infusion solids	5.0
Glucose	5.5	Protease peptone	10.0
Yeast extract	5.0	Glucose	2.0
Pancreatic digest of casein	15.0	Sodium chloride	5.0
Sodium thioglycollate	0.5	Disodium phosphate	2.5
pH 7.1 ± 0.2 @ 25°C		pH 7.4 ± 0.2 @ 25°C	

The media was divided into the 75 ml portion to Wheaton bottles (100 ml) before being autoclaved at 121^{o}C for 15 mins to eliminate possible biological contaminants. The subject bacteria used in this study was *Cp* type A field strain (EHE-NE18, CSIRO, Australia)(Rodgers et al., 2015) which has been confirmed to produce NetB toxin, an essential toxin to cause NE (Keyburn et al., 2008). *Cp* were stored in -80^{o}C freezer and revived in a cooked meat media (CMM) (Oxoid, UK) from overnight growth till the culture became viable (approx. 10^5 cfu/ml) before use in the following procedures. Using a sterile medical grade 1 ml syringe (23 gauge needle), 0.1 ml of *Cp* culture was transferred to each of prepared thioglycollate broth and brain heart infusion media in Wheaton bottles. Broth media were placed in 39^{o}C incubator for 4 h and 15 h periods to reach bacteria growth of approximately 10^3-10^4 cfu/ml, and 10^6-10^7 cfu/ml, while the non-growth control media were included for incubation for the respective incubation periods. To evaluate the *Cp* concentration, bacterial growth on selective agar was used with factors of serial dilution. Specifically, when the incubation time was reached, each culture was gently shaken, and 1 ml sample broth was aseptically put into a prepared 9 ml of sterile brain heart infusion broth in a Hungate tube. Serial dilutions were prepared by putting 1 ml of broth from the vortexed Hungate tube into next Hungate tube filled with fresh 9 ml of brain heart infusion until the factor of dilution reached 10^7 (7^{th} tube). Using a syringe and 23 gauge needle, 0.1 ml of each dilution factor was aseptically spread onto an individual *Cp* (TSC) selective agar base (Oxoid, UK). Duplicate plates were made and the agar plates were briefly dried before being incubated at 39^{o}C anaerobically overnight. Bacterial counts were recorded the next day. Mean bacterial colony counts of replicates were calculated and colony forming units (cfu) in the original broth were calculated according to the serial dilution factors, i.e. the concentration of *Cp* in the broth was the log conversion to the bacterial colonies on the agar plates with respective dilutions. Incubated non-growth control medium was also subjected to plating onto the selective agar base.

8.2.3 SIFT-MS measurement of odorants

Around 25±1 ml of culture media was transferred to a clean aluminium tray (diameter-90 mm, height- 20 mm) and immediately covered and sealed with a flux hood that was used to measure litter headspace concentration of odorants in previous studies (Sharma et al., 2016 a, b). The flux hood was purged with ultra-high purity N_2 gas at 500 ml/min from one end and connected to a SIFT-MS instrument (Voice 200^{TM} SYFT technologies, Christchurch, New Zealand) at the other end. SIFT-MS drew the gas sample at 14 ml/min from the headspace of the liquid broth media. The measurements were taken every two min for a duration of 16 min and the average of the last 5 readings were reported. After each measurement, the SIFT-MS was flushed with the ultra-high purity N_2 gas for 4 min to prevent cross contamination within the sampling lines or instrument. The measurement was taken using selected ion mode (SIM) of SIFT-MS that consisted of 28 selected odorants as described in a previous study (Sharma et al., 2016b). Prior to each analysis, the SIFT-MS was run with standard gases which included ethylbenzene, tetrafluorobenzene, toluene, hexafluorobenzene, ethylene, octafluorotoluene, benzene, and isobutene to ensure the instrument's mass-calibration and quantification for these compounds were consistent for each measurement session. In all the measurements, the headspace of the medium alone (also held at 37 °C overnight in a sealed bottle) was anlaysed, immediately followed by the analysis of medium/bacterial culture headspace.

8.2.4 Statistical analysis

Odorant concentrations were anlaysed following a 2 × 3 factorial arrangement of treatments using JMP statistical software version 8 (SAS Institute Inc, Cary, NC) to test the main effects of *Cp*, broth media, and their interaction. Data were subjected to two-way ANOVA and means were separated by Tukey's HSD test at a probability level of 0.05.

8.3 Results

8.3.1 Measured *Cp* concentration

Measured concentrations of *Cp* in each broth medium of different incubation period are summarized in Table 8.2. The non-growth control broth did not show any evidence of *Cp* growth on the selective agar.

Table 8.2 Estimated and actual growth of *Cp* at different incubation times

Broth medium	Incubation time (h)	Estimated bacterial concentration (cfu/ml)	Actual bacterial concentration (cfu/ml)
Thioglycollate broth USP	0	0	0
	4	10^3-10^4	10^4
	15	10^6-10^7	10^6
Brain heart infusion broth	0	0	0
	4	10^3-10^4	10^4
	15	10^6-10^7	10^6

8.3.2 Odorants from media/bacteria culture headspace

8.3.2.1 Concentration of odorants produced by *Cp* as affected by two broth media

a) Odorants belonging to the group of sulfur compounds

As shown in Figure 8.1, there was a *Cp* × media interaction on the culture headspace concentrations of carbon disulfide, carbonyl sulfide, ethyl mercaptan, H_2S and methyl mercaptan ($P < 0.05$). There was no effect of *Cp* level on the headspace concentrations of carbonyl sulfide (Fig. 8.1b), ethyl mercaptan (Fig. 8.1c) and H_2S (Fig. 8.1d) in BHI medium but such effects were present in TPS medium where 10^6 cfu/ml of *Cp* increased the headspace concentrations of carbonyl sulfide by 90 fold ($P < 0.01$), ethyl mercaptan by 2.5 fold ($P < 0.01$) and H_2S by 19 fold ($P < 0.01$) compared to the medium with no *Cp*. *Cp* increased the headspace concentration of methyl mercaptan in both media. The concentration of methyl mercaptan was lower in BHI than TPS at zero *Cp* or the start of the culture (Fig. 8.1e). In TPS medium, the headspace concentration of methyl mercaptan increased by only 1.8 fold but in BHI medium, it increased by 42 fold ($P < 0.01$) when *Cp* concentration in culture increased from 0 to 10^6 cfu/ml. The headspace concentrations of methyl mercaptan in both media were same at 10^6 cfu/ml of *Cp*.

b) Odorants belonging to the group of alcohols and ketones

There was an interaction between *Cp* and media on the concentrations of 2,3-butanedione, 3-hydroxy-2-butanone (acetoin), 3-methyl-1-butanol and total butanols (1-butanol+2-butanol) as shown in Figure 8.2. There was no effect of *Cp* on the concentrations of 2,3-butanedione (Fig. 8.2a) and 3-hydroxy-2-butanone (Fig. 8.2b) in BHI medium but in TPS medium when *Cp* concentration increased from 0 to 10^6 cfu/ml, the headspace concentration of 2,3-butanedione increased by 3.2 fold ($P < 0.01$) and 3-hydroxy-2-butanone increased by 35.4 fold ($P < 0.01$). Compared to the culture media without *Cp*, 10^6 cfu/ml of *Cp* increased the headspace concentration of 3-methyl-1-butanol (Fig. 8.2c) and total butanols (Fig. 8.2d) in both media ($P < 0.01$) but the concentration of these odorants in TPS medium was higher than that of BHI medium at this *Cp* level.

c) Odorants belonging to the group of amines

There was a *Cp* × media interaction on the culture headspace concentrations of methylamine, dimethylamine and trimethylamine as shown in Figure 8.3. There was no effect of *Cp* on the concentrations of dimethylamine (Fig. 8.3b) and trimethylamine (Fig. 8.3c) in BHI medium but in TPS medium, 10^6 cfu/ml of *Cp* increased the concentration of dimethylamine by 5.3 fold ($P < 0.01$) and trimethylamine by 3.8 fold ($P < 0.01$) compared to the medium with no *Cp*. In TPS medium at 10^3 cfu/ml, *Cp* increased the culture headspace concentration of methylamine by 1.2 fold and further by 1.8 fold at 10^6 cfu/ml as compared to the medium with no *Cp*.

d) Odorants belonging to the group of carboxylic acids

There was a *Cp* × media interaction on the culture headspace concentrations of acetic acid, propionic acid, butyric acid, and isobutyric acid as shown in Figure 8.4. fold ($P < 0.01$), propionic acid by 13.5 fold ($P < 0.01$), butyric acid by 100 fold ($P < 0.01$), and isobutyric acid by 23 fold ($P < 0.01$) compared to the medium with no *Cp*. There was no effect of *Cp* on the concentrations of acetic acid (Fig. 8.4a), propionic acid (Fig. 8.4b), butyric acid (Fig. 8.4c), and isobutyric acid (Fig. 8.4d) in BHI medium but in TPS medium, 10^6 cfu/ml of *Cp* increased the concentration of acetic acid by 13.7.

e) Other odorants

There was a *Cp* × media interaction on the culture headspace concentrations of hexane, indole and 1,4-diaminobutane as shown in Figure 8.5. There was no effect of *Cp* on the concentrations of hexane (Fig. 8.5a), indole (Fig. 8.5b) and 1,4-diaminobutane (Fig. 8.5c) in BHI medium but in TPS medium, 10^6 cfu/ml of *Cp* increased the concentration of hexane by 2.6 fold ($P < 0.01$), indole by 3.9 fold ($P < 0.01$) and 1,4-diaminobutane by 2.3 fold ($P < 0.01$) compared to the medium with no *Cp*.

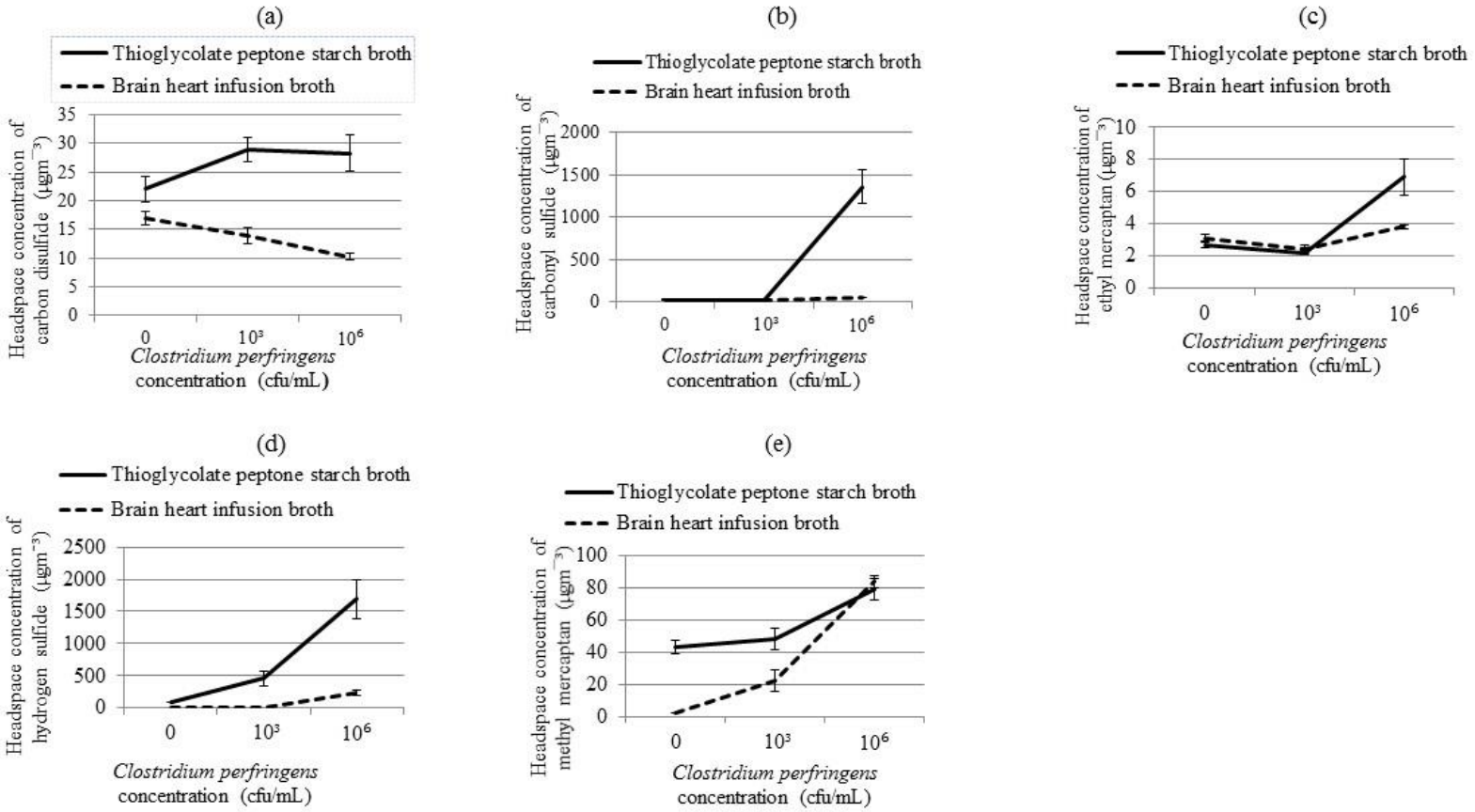

Figure 8.1 Concentration of sulfur compounds: (a) carbon disulfide, (b) carbonyl sulfide, (c) ethyl mercaptan, (d) hydrogen sulfide and (e) methyl mercaptan produced by three levels of *Clostridium perfringens* (0, 10^3 cfu/mL, 10^6 cfu/mL) in two broth media (thioglycollate peptone starch broth and brain heart infusion broth) as measured by SIFT-MS.

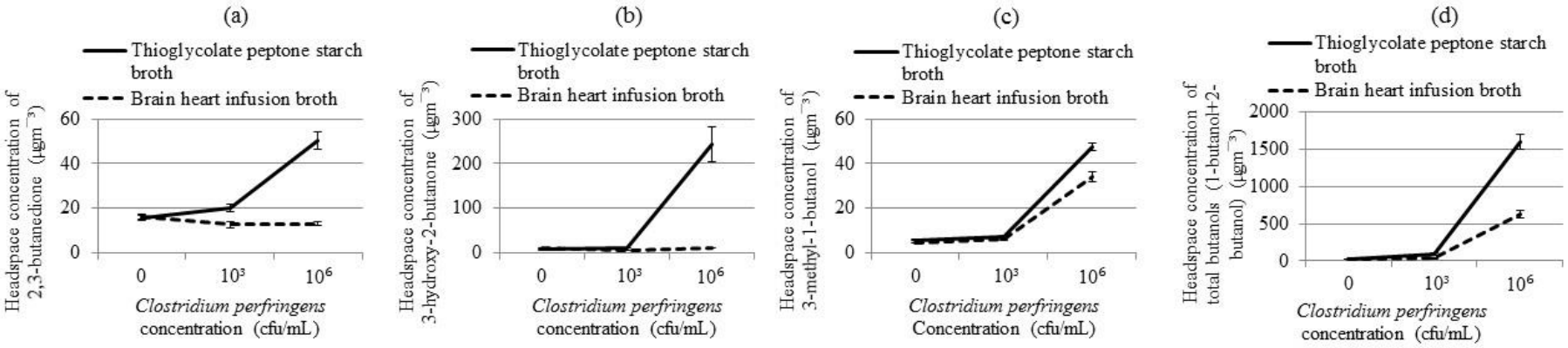

Figure 8.2 Concentration of ketones: (a) 2,3-butanedione, (b) 3-hydroxy-2-butanone; alcohols: (c) 3-methyl-1-butanol and (d) total butanols (1-butanol and 2-butanol) produced by three levels of *Clostridium perfringens* (0, 10^3 cfu/mL, 10^6 cfu/mL) in two broth media (thioglycollate peptone starch broth and brain heart infusion broth) as measured by SIFT-MS.

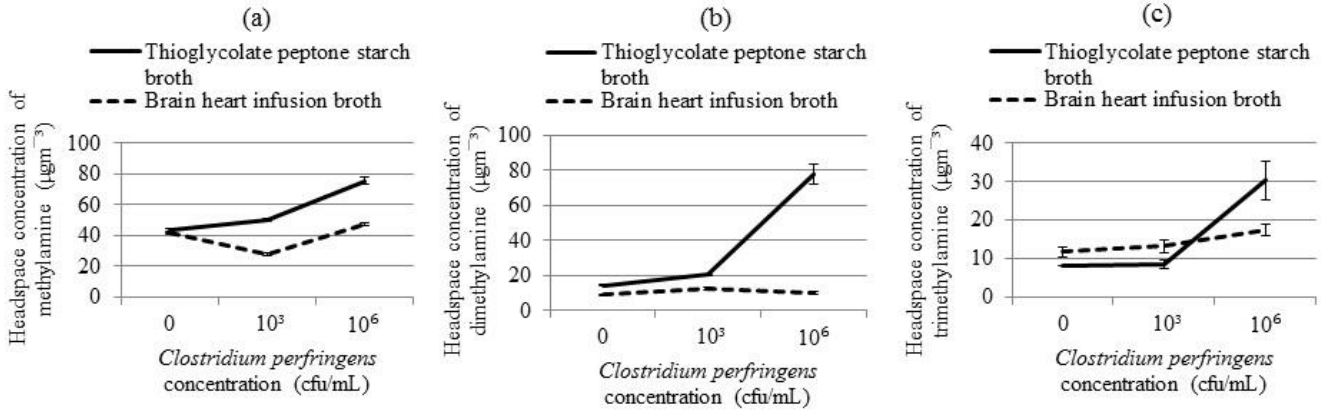

Figure 8.3 Concentration of amines: (a) methylamine, (b) dimethylamine and (c) trimethylamine produced by three levels of *Clostridium perfringens* (0, 10^3 cfu/mL, 10^6 cfu/mL) in two broth media (thioglycollate peptone starch broth and brain heart infusion broth) as measured by SIFT-MS.

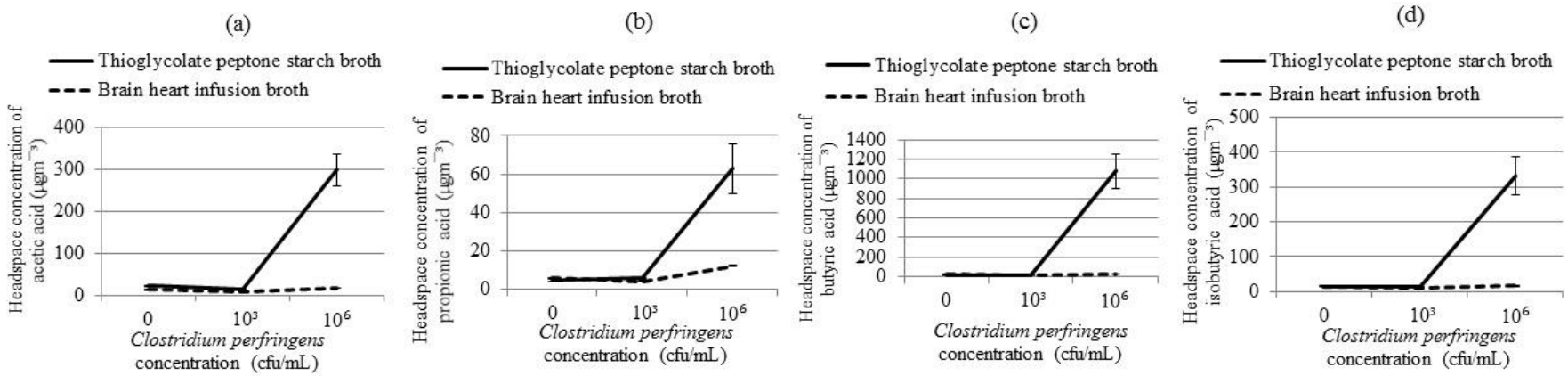

Figure 8.4 Concentration of carboxylic acids: (a) acetic acid, (b) propionic acid, (c) butyric acid and (d) isobutyric acid produced by three levels of *Clostridium perfringens* (0, 10^3 cfu/mL, 10^6 cfu/mL) in two broth media (thioglycollate peptone starch broth and brain heart infusion broth) as measured by SIFT-MS.

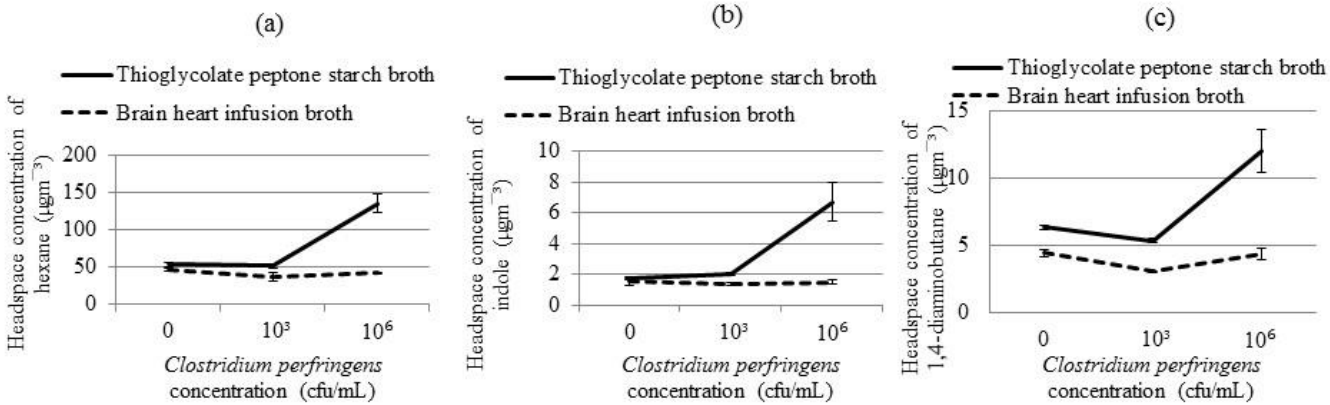

Figure 8.5 Concentration of: (a) hexane, (b) indole and (c) 1,4-diaminobutane produced by three levels of *Clostridium perfringens* (0, 10^3 cfu/mL, 10^6 cfu/mL) in two broth media (thioglycollate peptone starch broth and brain heart infusion broth) as measured by SIFT-MS.

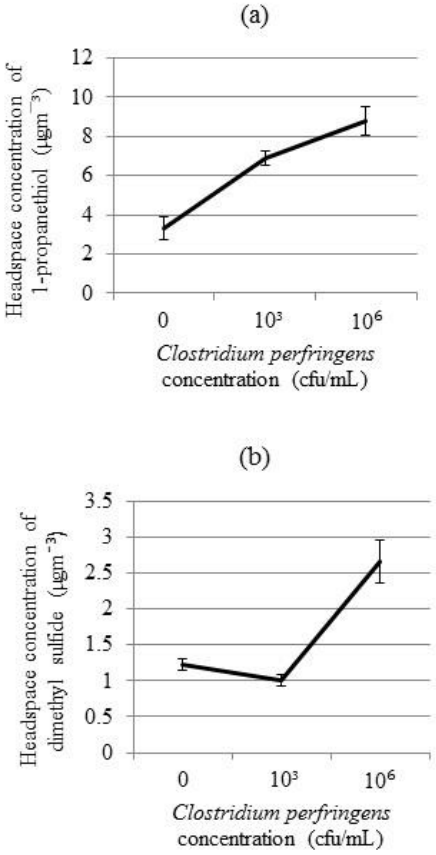

Figure 8.6 Concentration of: (a) 1-propanethiol and (b) dimethyl sulfide produced by three levels of *Clostridium perfringens* (0, 10^3 cfu/mL, 10^6 cfu/mL) as measured by SIFT-MS.

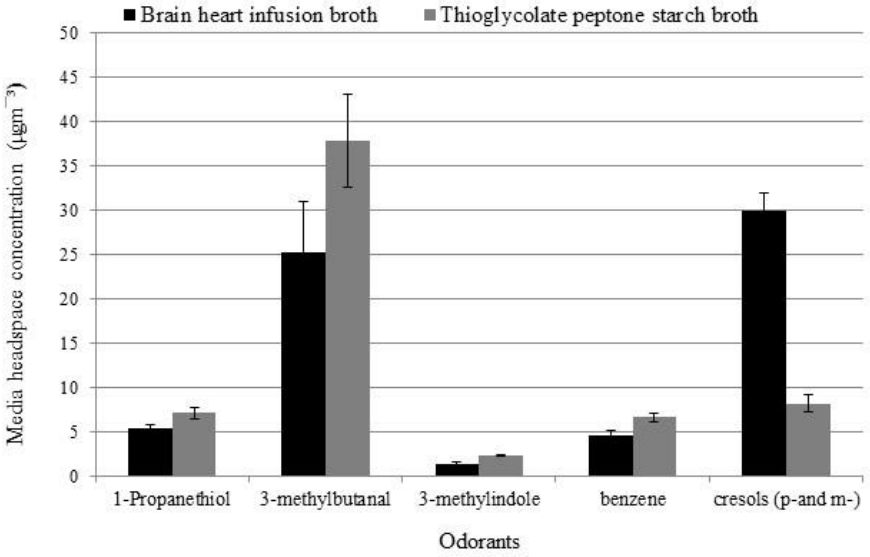

Figure 8.7 Concentration of odorants produced by brain heart infusion broth and thioglycollate peptone starch broth as measured by SIFT-MS.

8.3.2.2 Concentration of odorants produced by *Cp* not affected by media type (main effect of *Cp*)

The effect of *Cp* on the culture headspace concentrations of 1-propanethiol and dimethyl sulfide is presented in Figure 8.6. *Cp* increased the concentration of 1-propanethiol by 2 fold at 10^3 cfu/ml and by 2.6 fold at 10^6 cfu/ml ($P < 0.01$) compared to zero concentration (Fig. 8.6a). Similarly, at 10^6 cfu/ml, *Cp* increased the concentration of dimethyl sulfide by 2.2 fold ($P < 0.01$) compared to zero concentration (Fig. 8.6b).

8.3.2.3 Changes in concentration of odorants produced by broth media (main effect of broth media)

As shown in Figure 8.7, TPS medium produced higher headspace concentrations of 1-propanethiol ($P < 0.01$), 3-methylbutanal ($P < 0.05$), 3-methyl indole ($P < 0.05$) and benzene ($P < 0.01$) but lower concentration of cresols ($P < 0.01$) compared to BHI medium.

8.4 Discussion

Clostridium spp. have been found to correlate with the production of 1,3-butanediol and 3-hydroxy-2-butanone from wet litter in meat chicken farms and butyric acid from fermentation of sugars (Wadud, 2011). In a recent study (Sharma et al., 2016b), *Cp* infection producing sub-clinical NE in meat chickens resulted in high litter flux of odorants belonging to the group of sulfur compounds (dimethyl sulfide, propyl mercaptan and ethyl mercaptan), alcohols (total butanols), ketones (acetoin), carboxylic acids (butyric acid) and amines (methyl amine). In the same study, some more odorous metabolites such as 2,3-butanedione, acetic acid, propionic acid, isobutyric acid, isovaleric acid, pentanoic acid, 2-butanone, and 3-methyl-1-butanol were increased in litter headspace when NE came together with wet litter. Most of these odorants are similar to those observed in this in-vitro study. However, the conditions in the litter are different to those of media as there can be many bacteria other than *Clostridium spp.* that can contribute to odour emissions from litter (Wadud, 2011). However, it is possible to identify the odorants specific for *Cp* with culture headspace as shown in the current study. There is a wide range of VOC produced by bacteria such as fatty acids, aliphatic alcohols, ketones, hydrocarbons, aromatic compounds, nitrogen containing compounds and volatile sulfur compounds as part of their normal metabolism (Schulz and Dickschat, 2007). Some of these VOC are odorous and can produce a characteristic smell in tissue infection such as the one observed in gas gangrene by cytotoxic *Clostridia* (Boland, 1929 cited in Thorn et al., 2011). *Cp* infected meat chickens generally have an odorous gas-filled intestine (Helmboldt and Bryant, 1971) but these odiferous VOC have not been investigated yet. We measured the odiferous VOC produced by *Cp* cultured in two broth media using SIFT-MS and found that *Cp* produced a wide range of odorants that belonged to the group of sulfur compounds, alcohols, aldehydes, ketones, amines and carboxylic acids.

The concentration of odorants increased greatly at high *Cp* concentration (i.e. 10^6 cfu/ml). There was a clear difference between the odorants produced by *Cp* type A field strain as cultured in two different media. Most of the odorants were produced at higher levels when *Cp* was cultured in TPS medium compared to BHI medium. The VOC produced by bacteria may differ according to the media type (Wadud, 2011). For example, H_2S was the major compound formed in Dulbecco's modified Eagle's medium by *E. coli* whereas NH_3 was highly produced

when this bacterium was cultured in lysogeny broth (Chippendale et al., 2011). In a study by Scotter et al. (2005), six different fungi were cultured in five different media that produced odorants such as ethanol, acetaldehyde, acetone, methyl mercaptan and 2-butanal the concentrations of which varied according to the fungus type and media used to culture them. These differences are to be expected as the bacterium feeds on the constituents present in the medium to release the odorants as metabolic end products. The TPS medium contained yeast extract and a sulfur-containing amino acid both of which may have been used by *Cp* to release a large number of odorants. In this study, *Cp* produced a wide range of volatile odorous metabolites belonging to the group of sulfur compounds, alcohols, ketones, amines and carboxylic acids the concentrations of which varied according to the media type and *Cp* concentration.

Thus, *Cp* contributes to the production of a wide range of odorous metabolites that can impart noxious smell during infection. This work supports previous findings (experiment 4) that odour produced from *Cp* infected chickens is related to diet composition.

9. Discussion

Odour emission from chicken meat production is inevitable but the results herein showed that the concentration of odorants that are emitted from litter may be reduced by dietary means. It is important to measure the concentration of odorants accurately in odour abatement studies as there are chances of sample loss during collection and storage. Analytical instruments such as SIFT-MS and PTR-MS can measure odorants in real time and at very low concentrations and may be useful for odour abatement studies in future. In the experiments described herein it was found that diet composition affected water intake, WI:FI, litter moisture, litter pH and odour emissions. A high litter moisture increased water activity and favoured the emissions of sulfur containing odorants, trimethylamine, phenol, indole and skatole over others. As litter moisture increased, litter pH decreased and vice-versa which implies that a low litter pH associated with high litter moisture may favor the emissions of sulfur containing odorants. In experiment 1, phytase enzyme decreased WI:FI in meat chickens and improved litter quality when formulated using nutrient matrix values as compared to adding it over the top in an already nutrient sufficient diet. However, SPME-GC-MS analysis of gaseous emissions indicated no difference in the emission of odorants that belonged to the group of alcohols, aldehydes, ketones, volatile fatty acids and phenols between phytase supplemented and non-supplemented groups.

In experiment 2, it was hypothesized that the use of different protein sources in meat chicken diets can affect odour emissions partly due to the difference in digestibilities of protein meals that may affect the post excretion decomposition pattern. Meat chickens that were fed the diet containing a high level of SBM produced a higher concentration of methyl mercaptan, a sulfur containing odorant compared to those that were fed diets containing high levels of CM or MBM. In the same study, the diets that contained high levels of SBM and CM produced high concentrations of total elemental sulfur at the grower stage compared to the diet that contained a high level of MBM. These results suggest that both SBM and CM may increase sulfurous odour if they are included in the diets at higher levels and thus it may be desirable to replace some portions of these protein meals with MBM in the diet. This speculation coincides with the general observation being made in commercial meat chicken farms where wet litter problem was significantly reduced after some portion of SBM was replaced with MBM in the diet (Dr. Tim Walker, Nutritionist, personal communication, 2015). Drying the litter are likely to reduce the emission of odorants from litter (experiment 3).

Ammonia emissions can be reduced by feeding meat chickens with a low CP diet supplemented with crystalline amino acids. Although lower emissions of NH_3 may not necessarily correlate with the reduced odour emission rates, the simultaneous reduction of phenol, nitrogen and sulfur containing odorants such as dimethyl amine, trimethyl amine, H_2S, methyl mercaptan and NH_3 by feeding a low CP diet may reduce the overall intensity and offensiveness of broiler litter odour (experiment 3). There will be challenges if the poultry industry moves towards antibiotic free poultry production and decreases reliance on animal origin feedstuffs. There will be increased enteric disease, decreased performance, increased mortality, increased nutrient excretion, welfare issues and likely increased odour problems. One way to address these challenges is to find alternative feed additives to in-feed antibiotics. In meat chickens, it may concluded with confidence that *Bacillus subtilis* based probiotics can reduce NH_3 and H_2S emissions from litter (experiment 3). Similarly, the study showed that a blend of *Yucca* and *Quillaja* saponin reduced the emissions of trimethyl amine and phenol in litter (experiment 3). These findings suggest that a combination of probiotic and saponin may be ideal alternatives to in-feed antibiotics in reducing odour issues from chicken meat production.

Odour complaints may be higher when the litter is wet or the birds are infected with enteric disease. One of the common enteric diseases in meat chickens is *Cp* induced NE. In experiment 4, the effect of NE challenge and high dietary Na level (wet litter) on odour emissions was investigated. Challenge increased litter moisture and litter headspace concentrations of dimethyl sulfide, propyl mercaptan, total butanols, acetoin, skatole, butyric acid, methyl amine and tended to increase concentrations of ethyl mercaptan, carbon disulfide, indole and formic acid compared to the unchallenged group. The birds fed a high Na diet produced a higher litter moisture and a higher litter headspace concentration of sulfur compounds and phenol compared to those fed a normal Na diet. In the birds fed a high Na diet, challenge increased the litter flux of some additional odorants which included 2,3-butanedione, acetic acid, propionic acid, isobutyric acid, isovaleric acid, pentanoic acid, 2-butanone and 3-methyl-1-butanol. These findings suggest that both high Na diet and sub-clinical NE increase the odour nuisance potential of meat chicken farms. In addition, sub-clinical NE affected meat chickens may further increase odour emissions if the disease comes together with wet litter condition. An in-vitro study (experiment 5) showed that *Cp* can produce a wide range of volatile odorous metabolites belonging to the group of sulfur compounds, alcohols, ketones, amines and carboxylic acids the concentrations of which varied according to the media type and *Cp* concentration. This suggests that *Cp* contributes to the production of a wide range of odorous metabolites that can impart noxious odour during infection.

Thus, diet may affect odour emissions directly by changing the amount of undigested substrates and microorganisms in the gut and in excreta and indirectly by changing litter moisture content, litter water activity and litter pH. Nutritional strategies such as partial replacement of soybean meal with meat meal in the diet, use of low protein diet, *Bacillus subtilis* based probiotic and saponin blend may lower odour emission from chicken meat production. Additionally, drying the litter and prevention of wet litter condition and NE in meat chickens may alleviate possible odour problems around the meat chicken farms.

10. Implications

There was a dearth of knowledge on dietary roles on odour emissions from chicken meat production. The results of the five experiments showed that odour emissions may be reduced by dietary manipulation and effective control of litter moisture, litter pH and water activity. Diet affects WI:FI, litter moisture, litter pH all of which may affect odour emissions. WI:FI can be decreased and litter quality may be improved when phytase is formulated in the diets using appropriate nutrient matrix values. Thus, the practice of adding phytase over the top of the nutrient sufficient diets may not be desirable. The inclusion of higher levels of soybean or canola meals in the diets may increase litter moisture and sulfurous odour and it may be desirable to replace some portions of these protein meals with animal protein meal in the diet.

Similarly, the reduction in phenol, nitrogen and sulfur containing odorants along with NH_3 by feeding a low protein diet may add to the future commercial potential of feeding a low protein diet to meat chickens. A combination of probiotic and saponin may represent a useful viable alternative to in-feed antibiotics in reducing odour issues from meat chicken production. Wet litter and necrotic enteritis affected flocks may increase odour emissions and thus control of litter conditions by lowering litter moisture content and water activity, and prevention of necrotic enteritis may alleviate possible odour problems around the meat chicken farms.

11. Recommendations

- In odour abatement studies, odour measurements should be done using analytical instruments that can measure odorants in real time and at a very low concentration. Instruments such as SIFT-MS may be used for this purpose. Along with analytical measurements, olfactometry measurements of odour is suggested in future.
- Further research is warranted to investigate odour reduction strategies from meat chickens fed low protein diets. Such work will have future commercial application.
- As alternatives to in-feed antibiotics, a combination of probiotic and saponin is suggested in meat chicken diets to reduce odour emissions.
- Efforts should be directed to prevent wet litter conditions and occurrence of enteric diseases in meat chickens to reduce odour emissions.

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14. Appendix

14.1 Pilot study

Title: Effect of feeding high protein and low protein diets on odour emissions from meat chickens

An initial study was conducted in collaboration with University of New South Wales (UNSW) using thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS).

Fifty six Ross 308 (29 d old) male meat chickens were randomly allocated to two treatment diets with eight replications of four birds each in 16 equal sized metabolic chambers. The chambers that were used in this experiment were the same as the ones used to measure net energy in meat chickens at University of New England, Australia. Two wheat-soybean-meat meal based grower diets were prepared as shown in Table 14.1. Treatment diets varied in protein content (246 g/kg vs 180 g/kg) but had similar metabolisable energy levels (12.98 MJ/kg). Diets were supplemented with crystalline amino acids to achieve amino acid levels similar to or slightly higher than Ross 308 guidelines. Rice hull was used as a bedding material @ 2 kg per chamber to match closely with the commercial conditions. Birds were reared on litter with chamber lids open and fresh air maintained in excess at all times through the use of stirring fans and an exhaust fan. Birds were provided *ad libitum* feed and water inside the chamber. The birds were adapted to the diets for 72 hours, weighed and transferred to the chambers at d 29. Daily feed and water intake were recorded. Odour was collected from the litter accumulated over four days in presence of birds.

14.1.1 Odour collection and measurements using gas chromatography-mass spectrometry

Gaseous samples were collected at d 33 from litter collected over four days (day 29-32) period in presence of birds. Chamber lids were closed for approximately 30 min before sample gas collection. At that time there was zero air exchange and odorants were allowed to concentrate prior to sampling. Carbon dioxide and oxygen levels had been determined to be within acceptable limits during the period of closure (< 2% and > 18% respectively). Litter and bird emissions from chambers were captured on a conditioned Tenax TA sorbent tubes and nalophene gas bags by connecting each of them to one of the outlets of these chambers.

Volatiles were concentrated on sorbent tubes at a flow rate of 100ml/min for 20 mins by an AirChek 2000 air sampling pump (SKC). However, flow rate was not maintained for gas bags and emissions were trapped directly inside gas bags within 4 mins after connecting to the chamber outlet. After sample collection, sorbent tubes were wrapped in aluminium foils, labelled and sent to University of New South Wales (UNSW), Australia odour laboratory for further analysis. Gas bags were put in an airtight container and immediately sent to UNSW odour lab for analysis within 24 hours after collection to prevent degradation of sulfur compounds.

Table 14.1 Composition of experimental diets as-fed basis (g/kg)

Ingredients	High CP diet	Low CP diet

Wheat	537	766
Soybean meal	365	149
Meat meal	34.6	20.0
Canola oil	43.1	19.0
Limestone	7.63	11.8
Dicalcium phosphate	-	4.16
Xylanase powder	0.05	0.05
Phyzyme XP	0.10	0.10
Salt	1.91	2.01
Na bicarbonate	2.00	2.00
TiO2	5.00	5.00
Vitamin mix[1]	0.50	0.50
Mineral mix[2]	0.75	0.75
Choline Cl, 70%	0.01	0.54
L-lysine HCl	-	5.37
D,L-methionine	1.93	3.42
L-threonine	-	2.53
L-isoleucine	-	3.85
L-arginine FB	-	2.28
L-valine	-	1.29
Calculated nutrients (g/kg)		
ME, MJ/kg	12.98	12.98
Crude protein	246	180
Crude fat	62.4	36.8
Crude fiber	25.3	23.6
d Arg	15.49	11.4
d Lys	11.84	11.0
d M+C	8.40	8.40
d Trp	3.14	2.08
d Ile	7.98	7.50
d Thr	7.30	7.30
d Val	10.98	8.40
Calcium	8.00	8.85
Av. Phosphorus	4.00	4.00
Sodium	1.84	1.84
Analysed nutrients (g/kg)		
Dry matter	901	916
Gross energy, MJ/kg	17.8	17.3
Crude protein	247	183

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

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

Figure 14.1 VOCs trapped in Tenax tubes and gas bags (Gavin Parcsi, UNSW)

14.1.2 Results

UNSW results were received after nearly 8 months due to technical problems and the results were highly variable as shown in Appendix (Tables 14.2 and 14.3). The procedures and methods required revision but there were issues with collaboration with UNSW and time factor. The TD-GC-MS method was not effective as there was potential for sample loss during collection, storage and long distance transport with long delays in reporting of analytical results.

Table 14.2 UNSW odour results (sulfur compounds)

			Concentration of sulfur compounds (ng/lit)			
Chambers	Diets	Code	Carbonyl sulfide	Dimethyl sulfide	Carbon disulfide	Dimethyl disulfide
3	1	High CP	57.74	147.17	3.35	23.63
5	1	High CP	45.39	29.38	1.96	2.71
6	1	High CP	52.35	27.94	2.49	2.76
9	1	High CP	27.30	3.86	0.98	0.72
11	1	High CP	21.22	2.78	1.21	0.00
12	1	High CP	19.30	9.98	1.35	1.46
14	1	High CP	18.17	5.94	1.25	0.79
15	1	High CP	19.65	40.13	1.83	1.81
Mean			32.64	33.40	1.80	4.23
STD DEV			16.46	48.03	0.80	7.90
CV			50.42	143.8	44.3	186.5
1	2	Low CP	163.39	27.98	3.01	2.54
2	2	Low CP	57.22	82.92	3.01	6.20
4	2	Low CP	52.96	72.02	3.11	12.5
7	2	Low CP	36.00	14.91	1.64	1.54
8	2	Low CP	35.65	47.47	1.86	3.12
10	2	Low CP	20.70	9.14	1.07	0.99
13	2	Low CP	19.74	7.99	1.42	1.93
16	2	Low CP	12.61	41.76	2.59	1.87
Mean			49.78	38.03	2.22	3.83
STD DEV			48.54	28.40	0.81	3.84
CV			97.50	74.7	36.6	100.2

Table 14.3 UNSW VOC result measured at 16 chambers (raw data)

RT	Compound	CMB-1	CMB-2	CMB-3	CMB-4	CMB-5	CMB-6	CMB-7	CMB-8
3.93	Isopropyl alcohol								365876
3.98	Acetone	2804461	2036049	3202617	3462790	3941413	2651825	2566167	3273356
4.34	Dimethyl sulfide			269121	446502	824871			
4.82	Trimethyl silanol	555801							
5.35	2,3-butanedione	3673431	2976281	4141695	2813597	10030318			11559751
5.49	2-butanone	952636	1260445		935484	1715032			2012140
6.66	Cyclohexane	276383		464367	460631				276474
7.23	Heptane	506580	698005	697489	787025	358467	308178	338334	347466
7.48	3-hydroxy-2-butanone					13925109	9751561		
7.81	Methyl cyclohexane				296787				
8.12	Methyl isobutyl ketone	385290			674059		438073	416538	447796
8.23	Dimethyl disulfide								
8.78	Toluene	277281	426433	472601	380911	255306			
9.85	2,4-dimethyl-heptane	334570		322084	379348				
10.09	1,2,4-trimethyl-cyclohexane		274741						
10.34	4-methyl-octane		326231	307839	279357	316708			
11.19	Cyclohexanone	505136				252581		502780	
11.72	(1-methylethyl)-benzene		537730	520017	381730	443675	496801	250277	276206
11.82	4,5-dipropyl-octane					300515			
12.41	1,1,2,3-tetramethyl-cyclohexane								316139
12.41	Phenol								
12.76	Decane				629630				
13.14	1,2,3-trimethyl-benzene		502724	499715	397819	565466	544638	328371	398555
13.2	2,6,7-trimethyl-decane						489679		
13.24	2-ethyl-1-hexanol		1426836	2302837	718978	2439791	1753599	550267	363570
13.86	2,5,9-trimethyl-decane					1387472		655306	733911

RT	Compound	CMB-9	CMB-10	CMB-11	CMB-12	CMB-13	CMB-14	CMB-15	CMB-16
3.93	Isopropyl alcohol								
3.98	Acetone	3978916	3514923	3826010	2453732	3808761	3400321	2402001	2217197
4.34	Dimethyl sulfide				523447		9650625		
4.82	Trimethyl silanol					541598			
5.35	2,3-butanedione	13432704	9550465	7688281	12058117				8002409
5.49	2-butanone		1814318		1658017	1251238	1416124		
6.66	Cyclohexane								
7.23	Heptane								
7.48	3-hydroxy-2-butanone					12319859			
7.81	Methyl cyclohexane								
8.12	Methyl isobutyl ketone	350049		548713	596143				
8.23	Dimethyl disulfide				254255				
8.78	Toluene								
9.85	2,4-dimethyl-heptane								
10.09	1,2,4-trimethyl-cyclohexane								
10.34	4-methyl-octane								
11.19	Cyclohexanone				513895	408868			
11.72	(1-methylethyl)-benzene		436837	279739	317282	295424			
11.82	4,5-dipropyl-octane								
12.41	1,1,2,3-tetramethyl-cyclohexane								
12.41	Phenol								1579146
12.76	Decane								
13.14	1,2,3-trimethyl-benzene	312921	341837	458576	439470	453439	387542	392670	449721
13.2	2,6,7-trimethyl-decane								
13.24	2-ethyl-1-hexanol	1035498	1010324	2557251	2300284	2239241	1753285	1759997	3038158
3.86	2,5,9-trimethyl-decane				705424	810000			

13.92	1-methyl-2-pyrrolidinone	275068		3866555	4340263	4804947	4583059	23032943
14.43	Undecane		578705		960457		803711	1182754

15. Plain English Compendium Summary

Sub-Project Title:	Pilot study on nutritional manipulation of odour emissions from poultry
Poultry CRC Sub-Project No.:	2.2.8
Researcher:	Robert A. Swick
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Sub-Project Overview	This project investigated the role of diets on odour emission from chicken meat production. In the introduction part of this study, a comprehensive review has been done on the key odorants from chicken meat production, their origin, analytical techniques for odour measurements and the nutritional effects on odour emissions. The five experiments that follows the introduction part investigate the role of phytase enzyme (experiment 1), protein sources (experiment 2), protein levels (experiment 3), probiotic and saponin (experiment 3), litter condition (experiments 3 and 4), necrotic enteritis and high sodium diet (experiment 4) and *Clostridium perfringens* culture (experiment 5) on odour emissions. Nutritional strategies such as the use of meat meal in the diet, use of low protein diet, *Bacillus subtilis* based probiotic and saponin blend may lower odour emission from chicken meat production. Control of litter conditions by lowering litter moisture content and water activity, and prevention of necrotic enteritis in meat chickens may alleviate possible odour problems around the meat chicken farms.
Background	Odour emissions have been identified as a potential threat for the sustainable development of the chicken meat industry. Of the several methods examined so far to reduce odour emissions, none has proved to be effective. The reasons are that these methods are either impractical to apply or too costly to use in commercial farms. Diets can be formulated to more closely meet the bird's nutritional requirements to avoid overfeeding and to reduce the excretion of undigested components. This will decrease the amount of substrates that the microbes metabolize to odorous compounds. Diet can also affect gut microflora, faecal microflora, litter moisture content, litter pH and litter water activity all of which may affect the emission of odorants from litter. There are no published literature that shows the interrelationship between diet and odour in chicken meat production. This study has been undertaken to understand the role of feed ingredients, enzymes, feed additives, minerals, dietary protein levels, necrotic enteritis and litter conditions on odour emissions.

	This study will help to understand the possible link between diet and odour, and potentially lead to the development of some nutritional strategies to mitigate emissions of key odorants that have previously been identified from meat chicken facilities.
Research	**Experiment 1: Performance, litter quality and gaseous odour emissions of meat chickens fed phytase supplemented diets** The effect of graded levels of phytase on performance, bone characteristics, excreta/litter quality and odorant emissions was examined using Ross 308 male meat chickens. The results showed that phytase had greater performance benefits when formulated using nutrient matrix values as compared to adding it over the top in an already nutrient sufficient diet. The later method would be expected to increase feed costs without concomitant performance benefits. Phytase enzyme also decreased water to feed intake ratio and improved litter quality when formulated using nutrient matrix values as compared to adding it over the top in an already nutrient sufficient diet. A reduction in odour from birds and litter could not be detected with phytase supplemented diets using the SPME-GC-MS technique. Further work is needed to study the effect of phytase enzyme on odour emissions using instrumentation designed to detect sulfurous compounds more highly associated with smells from meat chicken farms. **Experiment 2: Effect of ingredient composition in diets on odour emissions in chicken meat production** The effect of feeding high levels of soybean, canola and meat and bone meals on odour emissions was studied using Ross 308 male meat chickens. Meat chickens that were fed the diet containing a high level of soybean meal produced a higher concentration of methyl mercaptan, a sulfur containing odorant compared to those that were fed diets containing high levels of canola or meat and bone meals. Also, the diets that contained high levels of soybean and canola meals produced higher concentrations of total elemental sulfur at the grower stage compared to the diet that contained a high level of meat and bone meal. These results suggests that both soybean and canola meals may increase sulfurous smell if they are included in the diets at higher levels and thus it may be desirable to replace some portions of these protein meals with meat and bone meals in the diet.

	Experiment 3: Effect of dietary protein levels and feed additives on odour emissions in chicken meat production The effect of feeding a high and a low protein diet with antibiotic, probiotic and saponin on odour emissions was investigated using Ross 308 male meat chickens. The results showed that reduction of dietary CP and simultaneous addition of synthetic amino acids decreased litter moisture, litter pH and lowered the emission of dimethyl amine, trimethyl amine, ammonia, hydrogen and phenol. This suggests that a low CP diet may reduce the overall odour intensity and offensiveness in chicken meat production. *Bacillus subtilis* based probiotics reduced ammonia and hydrogen sulfide emissions from litter. A blend of *Yucca* and *Quillaja* saponin reduced the emissions of trimethyl amine and phenol in litter. These findings suggest that a combination of probiotic and saponin may be ideal alternatives to in-feed antibiotics in reducing odour issues from chicken meat production. A high litter moisture increased water activity and favoured the emissions of sulfur containing odorants, trimethylamine, phenol, indole and skatole over others. As litter moisture increased, litter pH decreased and vice-versa which implies that a low litter pH associated with high litter moisture may favor the emissions of sulfur containing odorants. **Experiment 4: Effect of necrotic enteritis challenge and high dietary salt (wet litter) on odour emissions from meat chickens** The effect of necrotic enteritis and high dietary Na level (wet litter) on odour emissions was investigated in Ross 308 male meat chickens. Necrotic enteritis challenge increased litter moisture and litter headspace concentrations of dimethyl sulfide, propyl mercaptan, total butanols, acetoin, skatole, butyric acid, methyl amine and tended to increase concentrations of ethyl mercaptan, carbon disulfide, indole and formic acid compared to the unchallenged group. The birds fed a high Na diet produced a higher litter moisture and a higher litter headspace concentration of sulfur compounds and phenol compared to those fed a normal Na diet. In the birds fed a high Na diet, challenge increased the litter flux of some additional odorants which included 2,3-butanedione, acetic acid, propionic acid, isobutyric acid, isovaleric acid, pentanoic acid, 2-butanone and 3-methyl-1-butanol. These findings suggest that both high Na diet and sub-clinical necrotic enteritis increase the odour nuisance potential of meat chicken farms. In addition, sub-clinical necrotic enteritis affected meat chickens may further

	increase odour emissions if the disease comes together with wet litter condition. **Experiment 5: Emissions of volatile odorous metabolites by *Clostridium perfringens- in vitro* study using two broth cultures** Necrotic enteritis inducing *Clostridium perfringens (Cp)* bacteria produced a wide range of volatile odorous metabolites belonging to the group of sulfur compounds, alcohols, ketones, amines and carboxylic acids the concentrations of which varied according to the media type and *Cp* concentration. This suggests that *Cp* contributes to the production of a wide range of odorous metabolites that can impart noxious smell during infection.
Sub-Project Progress	All the milestones have been completed.
Implications	There was a dearth of knowledge on the role of diet on odour emission from chicken meat production. The results of the five experiments showed that odour emissions can be controlled by dietary manipulation and effective control of litter moisture and water activity. Diet affects water to feed intake ratio, litter moisture and litter pH. All of these affect odour emission. Water to feed intake ratio can be decreased and litter quality may be improved when phytase is formulated in the diets using nutrient matrix values. Thus, the practice of adding phytase over the top of the nutrient sufficient diets may not be desirable. The inclusion of higher levels of soybean or canola meals in the diets may increase litter moisture and sulfurous smell and it may be desirable to replace some portions of these protein meals with meat meal in the diet. Similarly, the reduction in phenol, nitrogen and sulfur containing odorants along with ammonia by feeding a low protein diets may be commercially beneficial. A combination of probiotic and saponin may be ideal alternatives to in-feed antibiotics in reducing odour issues from chicken meat production. Wet litter and necrotic enteritis affected flocks may increase odour emissions. Thus, lowering litter moisture content and water activity, and prevention of necrotic enteritis would be expected to alleviate odour problems around the meat chicken farms.

Publications	**Journal papers** **Sharma, N. K.,** M. Choct, S.-B Wu, R. Smillie, N. Morgan, A. S. Omar, N. Sharma, and R. A. Swick. Performance, litter quality and gaseous odour emissions of broilers fed phytase supplemented diets. *Animal Nutrition* 2016; http://dx.doi.org/10.1016/j.aninu.2016.10.003. **Sharma, N. K.,** M. Choct, S.-B Wu, R. Smillie, and R. A. Swick. Dietary composition affects odour emissions from meat chickens. *Animal Nutrition* 2015;1:24-29. **Sharma N. K,** M. Choct, M. W. Dunlop, S.-B Wu, H. Z. Castada, and R. A. Swick. Characterisation and quantification of changes in odorants from litter headspace of meat chickens fed diets varying in protein levels and additives. *Poultry Science* 2016; doi 10.3382/ps/pew309. **Sharma, N. K.,** M. Choct, C. Keerqin, N. Morgan, S.-B Wu, and R. A. Swick. Necrotic enteritis challenge and high dietary sodium level increase litter headspace concentration of odorants in meat chickens. *Poultry Science, submitted in August 2016.* **Sharma, N. K.,** M. Choct, C. Keerqin, S.-B Wu, and R. A. Swick. Emissions of volatile odorous metabolites by *Clostridium perfringens- in vitro* study using two broth cultures. *Poultry Science, submitted in October, 2016.* **Sharma N. K.,** M. Choct, S.-B Wu, and R. A. Swick. Nutritional effects on odour emissions in broiler production. *World's Poultry Science Journal, submitted in November, 2016* **Conference papers with oral presentations** **Sharma, N. K.,** M. Choct, S.-B Wu, R. Smillie, I. Ruhnke, and R. A. Swick. Role of diets on odour emissions from meat chickens. *Proceedings of the 26th Australian Poultry Science Symposium 2014, 189.* **Sharma, N. K.,** R. A. Swick, M. W. Dunlop, S.-B Wu, and M. Choct. Feeding low protein diets to meat chickens: effects on emissions of toxic and odorous metabolites. *Proceedings of the 27th Australian Poultry Science Symposium, Sydney 2015, 46.* **Sharma, N. K.,** R. A. Swick, M. W. Dunlop, S.-B Wu, and M. Choct. Odour flux from litter of meat chickens fed diets differing in protein levels and additives. *Proceedings of the* 25th *World Poultry Congress, Beijing 2016, 14.*

Sharma, N. K., C. Keerqin, N. Morgan, S.-B Wu, T. Walker, M. Choct and R. A. Swick. Necrotic enteritis, wet litter and odour: their interrelationship. *Proceedings of the 28th Australian Poultry Science Symposium Feb. 2017 (accepted for oral presentation).*

Sharma, N. K., S.-B Wu, M. Choct, and R. A. Swick. Litter quality: investigating the interrelationship between litter moisture content, pH, water activity and odour emissions. *Proceedings of the 28th Australian Poultry Science Symposium Feb. 2017 (accepted for oral presentation).*

Conference papers with poster presentations

Sharma, N. K., M. Choct, S.-B Wu, N. Sharma, A. S. Omar, and R. A. Swick. Impacts of graded levels of phytase in nutritionally adequate and downspec wheat based broiler diets on performance, carcass yield, water:feed intake ratio, excreta and litter quality. *Recent Advances in Animal Nutrition, Australia, 2015, 77-78.*

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Others (invited for oral presentation)

Sharma, N. K., M. Choct, S. –B Wu, and R. A. Swick. Diet and odour: dissecting the complicated web of interrelationship. *Australian poultry CRC Ideas Exchange conference, Gold Coast, Australia, 22-24 September 2015.*