Chapter 1

Introduction

Meat is a valuable food source rich in many of the essential nutrients including protein (essential amino acids), minerals (e.g. iron, zinc, selenium), vitamins (e.g. vitamin E) and fat (essential fatty acids such as Omega 3 fatty acids) (Schonfeldt and Gibson, 2008). Beef, lamb, poultry and fish are considered the major sources of meat protein for humans. Apart from the nutritional attributes of meat, it provides several eating attributes and fulfilling experiences that normally are not achieved by other protein sources. However, several epidemiological studies linked health problems such as obesity and high saturated fat intake to increased consumption of animal products (Biesalski, 2005; Chao et al., 2005). This has led to a concern that total dietary fat intake should be restricted by consuming smaller portions less frequently (Schonfeldt and Gibson, 2008) or replace red meat by consumption of white meat. Fowler (2004) and Williamson et al. (2005) reported that red meat consumption in developed countries has decreased due to health concerns and the demand for high-value alternate protein is increasing.

Birds have always been a source of meat protein for human with a recent increasing trend in the consumption of poultry (Magdelaine et al., 2007). At present, many birds are domesticated and farmed commercially for food (e.g. turkey chicken) while others are still gathered annually from the wilderness for the same purpose (e.g. quail, Mutton bird). As a result of their feeding habits, the meat of some birds is suspected to have additional nutritional benefits. Mutton birds are traditional food for native people of New Zealand and Australia (Richdale et al., 1946). Mutton birds eat krill and other small seafood organisms for food. Thus,
there may be some nutritional advantages of eating Mutton bird since their diet is perceived to contain a high percentage of polyunsaturated fatty acids (PUFA). Several publications have demonstrated effects of diet on manipulating the quality and the nutritional value of the meat in poultry (Rodriguez, 2008; Zelenka et al., 2008; Cortinas et al., 2004; Crespo and Esteve-Garcia, 2001). For example, the protein profile of chicken breast was modified as a result of diet enriched with different digestible lysine/crude protein ratios (Rodrigues, 2008). Similarly, the fatty acid profile of chicken meat was modified when linseed oils were incorporated in their diet, leading to increased concentrations of n-3 polyunsaturated fatty acids in the muscles (Zelenka et al., 2008; Cortinas et al., 2004). Thus the unique diet of Mutton birds may play an important role in increasing nutritionally important nutrients such as n-3 PUFA.

Mutton bird refers to a member of a group of 60 medium to large seabirds in the family Procellaridae. Mutton bird is a member of the subdivision called shearwaters (Richdale, 1946). The most abundant New Zealand Mutton bird is the sooty shearwater (Puffinus griseus) and is known to Maori as 'titi' (Anderson, 1996). It has the greatest total biomass of any seabird in New Zealand (Warham and Wilson, 1982). Historically in southern New Zealand, the annual Mutton bird harvest was a major event for many South Island Maori. Preserved Mutton birds are an important source of winter food and a major commodity for Maori (Anderson, 1996). Currently, Mutton birds are of commercial value to the iwis that have regulated control over the harvesting of these birds. The birds are mainly consumed by certain groups (e.g. Maori, Polynesian) who are familiar with them. Therefore, it is of great interest to have more information on the contribution of Mutton birds to the diet of these groups.
The majority of the published studies on sea birds, in particularly Mutton birds, were concerned on environmental pollution and the use of sea birds as a biomarker of diverse effects of chemical pollutants in environmental marine (Furness, 1993; Furness and Camphuysen, 1997). Information on the composition, nutritive value and toxicological aspects of Mutton bird meat is very scarce. Therefore, the current study was designed to evaluate the nutritional and toxicological aspects of New Zealand Mutton birds collected in 2 consecutive years (2007 and 2008). The emphasis of nutritional studies would be on the macro-chemical composition, fatty acid profile, amino acid profile and cholesterol content. The concentrations of heavy metals and pesticide (DDTs) will be determined in the Mutton bird breast muscle to assess the toxicological impact. All parameters will be compared with common meat especially, chicken breast meat.
Chapter 2

Literature Review

2.1. Overview of Mutton bird

2.1.1 Procellaridae family

Mutton birds are members of a group of 60 medium to large sized seabirds in the family *Procellaridae* that contains 145 species that are divided into petrels and prions (Richdale, 1976). Mutton bird belongs to the petrel group, and to a subdivision called shearwaters (Richdale, 1976). The most abundant procellariforms in the South Pacific Ocean is the sooty shearwater (*Puffinus griseus*) and the short-tailed shearwater, known as the Tasmanian Mutton-bird (*Puffinus tenuirostris*, ‘yolla’) (Anderson, 1996). Sooty and short-tailed shearwaters chicks are annually harvested from their breeding grounds in Australia and New Zealand (Skira 1990; Lyver and Moller, 1999).

2.1.2 Sooty shearwater

The sooty shearwater (*Puffinus griseus*), is a medium-sized member of the petrel family of seabirds (*Procellariidae*) that breeds in burrows on islands in southern South America, Australia and New Zealand (Cooper et al. 1991; Spear and Ainley, 1999). The sooty shearwater (*Puffinus griseus*), known to Maori as 'titi', is the main species known in New Zealand as Mutton bird. It is probably the most ecologically important and abundant of the Procellariiform in the New Zealand region (Warham 1990; Cooper *et al.* 1991; Anderson, 1996).
Sooty shearwater comprises about 20 to 50% of seabirds in Pacific Ocean and about 30 to 70% of seabird biomass in the subarctic North Pacific in summer (Sanger and Ainley 1988). Historically, the annual Mutton bird harvest was a major event involving Maori from most of the South Island. Preserved Mutton-birds were an important source of winter food and a major commodity in Maori exchange networks for food and trade (Waitangi Tribunal, 1991; Evison, 1993; Beattie, 1994). The sooty shearwater is culturally very important for Rakiura (Stewart Island) Maori (the indigenous people from the southernmost part of New Zealand) and their harvest is one of the last large scale customary uses of native wildlife in New Zealand.

The relationship between Rakiura Maori and titi is a good example of the linkage between social and ecological systems (Berkes and Folke, 1998). Rakiura Maori have harvested sooty shearwater since prehistoric time from islands adjacent to Stewart Island (Figure 2.1) and customary use rights allow only Rakiura Maori to harvest chicks in that area from 1 April to 31 May each year (Anderson, 1997; Wilson, 1979; Beattie, 1994, Kitson et al. 2000).

Figure 2.1: Main islands for the traditional harvest of Mutton bird in the south of New Zealand (Kitson et al., 2000).
2.1.3 Location and distribution of Mutton bird in New Zealand

Generally, sooty shearwater breeds during the southern hemisphere summer and migrates to the northern Atlantic and northern Pacific during the southern winter (Jones, 2000). They breed mainly around New Zealand, south-eastern Australia, and southern South America from September to April (Marchant and Higgins 1990; Hamilton et al. 1997). It is estimated that there are a total of 20-30 million pairs in the New Zealand region (Robertson and Bell, 1984; Woodward et al. 1995). Breeding colonies of sooty shearwater have been widespread around the coast of the South Island (Hamilton et al. 1997) with the islands adjacent to Rakiura (Stewart Island) being the main breeding grounds (Kitson, 2003).

Although, the sooty shearwater is mainly captured on islands around Stewart Island, it breeds on several other islands near the north of New Zealand and the Bay of Plenty; Kapiti and Mana Islands, near Wellington and the Otago coast and further south on the Snares Islands (about 100 kilometres south of Stewart Island) (Richdale, 1976; Moller et al. 1999; Moller et al. 2003; Warham and Wilson, 1982; Marchant and Higgins 1990). Today, Mutton bird is harvested from 36 different islands around Stewart Island (Lyver et al., 1999; Woodward et al. 1995). As shown in Figure 2.2, it has been estimated that around 200,000 to 300,000 Mutton bird chicks are harvested annually.
2.1.4 Characteristics and life cycle of Mutton bird

Sooty shearwaters (Figure 2.3) are medium-sized, burrow-nesting seabirds, brown in colour, although appear grey. Its length is approximately 42cm and its wings span is approximately 1metre (Kunisue et al. 2003). Richdale, (1976) described some characteristics of the bird as show in Table 2.1.

Table 2.1: Main characteristics of sooty shearwater (Richdale, 1976)
They are migratory seabirds which return to the breeding colonies on the coastal islands and shorelines of New Zealand around September each year (Marchant and Higgins, 1990). Egg-laying occurs between November and early December and hatching between late January and early February (Warham, 1990; Hunter et al. 2000). Fledging is from around 20 April until mid-late May and chicks come to the surface after dark for a few days before leaving (Warham, 1990). In the non-breeding season, most of migratory birds migrate to the North Pacific, but some birds migrate to the North Atlantic or remain in the Southern Hemisphere (Marchant and Higgins, 1990).

The titi harvest season, from 1 April to 31 May, is divided into two periods. The first period, the ‘nanao’ in Maori language, starts on 1 April (Wilson, 1979). During this period chicks are extracted from their burrows using a wire probe. The second period, the ‘rama’, begins when chicks start emerging from their burrows at night in preparation for fledging (usually about 20 April) allowing harvesters to pick them up off the ground. The harvest ends when chicks become scarce, usually around mid-May (Hunter et al. 2000; Lyver, 2000). Marine birds are sensitive to perturbations in climate, prey abundance and food-web structure (Walford and Shelton, 1978; Cairns, 1987; Furness, 1987; Aebischer et al. 1990, Montevecchi, 1993; Ainley et al. 1996; Montevecchi and Myers, 1997).

### 2.1.5 The industry and Maori methods

Mutton-birding as a systematic industry is known historically from Australia, New Zealand and several smaller islands in the pacific region (Carter and Malcolm, 1927). Mutton-birding covers the body of techniques whereby the sooty shearwater chicks and fledgling are caught, processed and preserved for food (Anderson, 1997). The modern industry began with the subsistence use of Mutton-birds by
European sealers and Aboriginal partners at the end of the eighteenth century. By the early twentieth century substantial businesses had been established, with up to one million birds harvested annually (Hunter and Caswell, 2005).

Traditional methods used for Mutton bird handling and storage relied on natural local materials (Punui’s leaves, kelp, flax leaves and totara bark). Baduini (2001) compared traditional and modern processing (using wax and plucking machines) methods to determine if new technology increases the efficiency, size, and cost effectiveness of harvest. He found that chicks were plucked 6 seconds quicker with the new technology, which could potentially increase the catch by up to 4%. However, both wax and plucking machines increased costs, which led to a net loss with the suggested modern processing compared to the traditional methods.

2.1.6 The relationships between Mutton-birds and krill

Maori people extracted Mutton bird oil from young Mutton birds (Bishop et al., 1983). The oil comes from the Mutton bird diet which mainly consists of krill (Carter and Malcolm, 1927). "Krill" is a term applied to describe over 80 species of open-ocean crustaceans known as Euphausiids, most of which are planktonic (Everson, 2000). Antarctic krill is one of the most abundant and successful animal species on earth (Gascón and Werner, 2005). The biomass of Antarctic krill may be the largest of any multi-cellular animal species on the planet (Nicol, 2004). *Euphausiids superba* is the species commonly referred to as “Antarctic krill” and it is widespread and subjected to significant commercial fishing (Everson, 2000). The ecological dependence of sea creatures on krill as diet is shown in Fig 2.4 which demonstrates the Southern Ocean food web linkages that are centred on krill (Everson, 2000).
An important characteristic of krill is its high concentration of omega 3 fatty acids, which increases the natural omega-3 content of organisms and fed diets containing krill (Sclabos and Toro, 2003). Therefore, Mutton birds may potentially contain significant amounts of these valuable fatty acids since krill is their main feed.

2.2. Nutrition of Avian meat

2.2.1 Mutton bird

There is lack of information in the literature regarding the nutritional value of New Zealand Mutton birds. Although traditionally eaten as a protein source, they could also be considered a good energy source because of their fat content. Mutton birds rely on krill and other marine organisms as food for their entire life. So, from a nutritional and toxicological view, the Mutton bird meat may be beneficial / detrimental to the consumer. These birds are consumed at relatively high intakes mostly by a limited number of social groups who have the customary rights to harvest them. The fatty acids profile was characterised (Table 2.2) but only one bird was used in this report (Quigley et al., 1995). Therefore, information regarding the biological variation and differences in fatty acid profile related to the season...
cannot be established. This research will fill this gap by profiling the nutritional profile of Mutton bird for two consecutive years. There is also a lack of information on proximate analysis and cholesterol content, and also the amino acid profile of Mutton bird meat.

Table 2.2: Fatty Acid Profile of NZ Mutton bird by Quigley et al., 1995

Woodward et al. (1995) investigated the nutritional value (minerals, vitamins, fatty acid profile and cholesterol) of cooked skinless Mutton bird (*Puffinus tenuirostris*). While the fat content of the cooked skinless Mutton bird was high (18.7%), the polyunsaturated: monounsaturated: saturated ratio (0.47: 2.30: 1.0) was more favourable than most other meats. Summary of nutritional values of cooked skinless Mutton bird is presented in the Tables 2.3 and 2.4.
Several publications demonstrated the effect of diet on manipulating the quality and the nutritional value of poultry meat (Rodrigues et al. 2008; Cortinas et al. 2004; Crespo and Esteve-Garcia, 2001). For example, the protein profile of chicken breast was modified by the diet enriching with different digestible lysine/crude protein ratios (Rodrigues et al. 2008).

Similarly, the fatty acid profile of chicken meat was modified when linseed oils were incorporated in their diet, leading to increased concentrations of n-3 polysaturated fatty acids (PUFA) in the muscles (Cortinas et al. 2004). Thus, the
unique diet of the Mutton birds may play an important role in increasing nutritionally important nutrients such as n-3 PUFA. This research will fill this gap by profiling the nutritional profile of Mutton bird from two consecutive years to determine availability and the annual variation of these important fatty acids. However, to highlight the potential value of Mutton bird as a unique source of valuable nutrients (e.g. n-3 PUFA) a comparison with a common source of land-based bird meat such as chicken is required. In the following section, a brief review for the nutrition value of chicken is presented.

2.2.2 Chicken meat quality

The poultry production has increased steadily in the past 20 years due to safety issues with red meat and consumer perception of poultry meat as a healthy option (Pereira et al., 2000). Chicken meat is a very important source of protein for human nutrition with high nutritive value and relatively low production costs. It is considered to be low in fat, particularly low in saturated fat, and is a good source of high biological value protein (20-22%) (Stanishevskaya et al., 2005). Chicken meat contains all the essential amino acids in the proper proportion and is a rich source of taurine, which is a conditional essential amino acid in infants (Sotelo and Perez, 2003).

Meat and meat products are one of the main sources of cholesterol and they have been implicated in the development of coronary heart disease. The cholesterol content of chicken meat varies between 57-78 mg/100g breast meat (Sharma 1984). In comparison to other meats, chicken has been reported to be relatively abundant in polyunsaturated fatty acids (PUFA), including the key n-3 fatty acids, as diets of fast growing broilers are generally rich in PUFA (Asghar et al. 1990). Beynen (1984) observed that eating poultry meat instead of red meat and pork can
lead to a slight decrease in serum cholesterol. The advantageous influence of poultry meat is related to its relatively high content of PUFA.

2.2.2.1 Proximate composition of chicken meat

The proximate composition of chicken meat varies with the muscle/part of interest. For example, the fat content of chicken breast meat was 3.46, which was lower than in skin (37.07%) > thigh (11.02%) > drum (6.80%) while, the protein contents (28.78%) was higher in the drum (28.26%) > thigh (23.90%) and skin (17.56%). The ash and carbohydrate content in different parts were not significant (Mueller, 2006). Thus, it is considered that the breast meat is best for human consumption and this study will focus on the Mutton bird breast meat. Also, the monounsaturated and polyunsaturated fatty acid contents varied widely among different chicken muscles/parts as shown in Table 2.5.

Table 2.5: Proximate composition mg/100g and lipids in various parts of chicken (Mueller, 2006)
2.2.2.2 Fatty acids in chicken

White (breast) chicken meat is the most popular poultry meat. Although chicken breast is rich in phospholipids, and so relatively rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the dark (leg) meat and skin are the richest sources of all n-3 FA because of the higher lipid content of these tissues (Rymer and Givens, 2003). PUFA contents in chicken meat (2.1–6.3%) is higher compared with beef (1.03%), lamb (1.97%) and pork (1.41%) (Higgs, 2000).

Many consumers believe that red meat is unhealthful compared with chicken meat, because of the higher levels of SFA and cholesterol (Kee et al., 2000). There is growing evidence that health benefit can be gained by replacing red meat with chicken meat as since chicken meat was associated with a significant decrease in apolipoprotein B and total cholesterol levels in microalbuminuric type 2 diabetic patients (Gross et al. 2002). This effect is probably related to the higher PUFA content of chicken meat in comparison to beef. The beneficial effects of PUFA depend on the ratio of the fatty acid omega 6 (n-6) to omega 3 (n-3); it is generally accepted that the ideal proportion of n-6 to n-3 is about 4:1. However, the current ratio in the usual Western diet ranges from 20:1 to 30:1, which may favour a prothrombotic and proaggregatory state (Schaefer et al. 2002). Therefore, knowledge concerning the exact fatty acid composition of the meat consumed by different populations is extremely important (De Almeida et al. 2006).
2.2.2.3 Nutritional benefits of fatty acids

The effects of fatty acids on the health of human can be beneficial or harmful, depending on the specific fatty acids and the mix of fatty acids in the diet and the body (Bayır et al. 2006). High consumption of saturated fatty acids causes high
serum cholesterol concentration, which is associated with an increased risk for coronary heart disease (Conference, 1985; Grundy et al. 1982). The consumption of fish has been linked to health benefits such as a reduced risk of coronary heart disease, arterial hypertension, human breast cancer growth, inflammatory diseases, asthma and disorders of the immune system (Uauy and Valenzuela, 2000), which are largely attributed to the polyunsaturated fatty acids (PUFA) in fish oils. Deficiency of n-3 PUFA may be linked with hypertension, inflammatory disorders, immune disorders, depression and neurological dysfunction (Williams, 2000).

2.2.2.4 The relationship between fatty acids and cholesterol with lipid oxidation

Food oxidation affects the quality and the safety of the human diet by generating compounds with biological activities that can adversely affect health (De Souza et al., 2006). Fish products are rich in cholesterol, and it is well recognized that oxidation of the lipid fraction of fish muscle is a major cause of deterioration in fatty fish (Brannan and Erickson, 1996) due to the high degree of unsaturated fatty acids (May et al., 1978; Saldanha and Bragagnolo, 2008). PUFA are very susceptible to oxidation even under mild ambient conditions and are easily incorporated into the chain mechanism of lipid peroxidation, to yield free and peroxy radicals, which may accelerate cholesterol oxidation (Hsieh and Kinsella, 1989). Cholesterol oxidation may proceed by the same mechanisms described for lipid oxidation or can be initiated by free radicals generated during the lipid oxidation (Finocchiaro and Richardson, 1983). The susceptibility of muscle to lipid oxidation can be reduced by naturally available antioxidants such as α-tocopherol which is a highly effective chain-breaking antioxidant (Machlin and Bendich, 1987; Wilson, 1987).
2.2.2.5 The correlation between fatty acids and cholesterol

Meat and meat products are one of the main sources of cholesterol and they have been implicated in the development of coronary heart disease and atherosclerosis. The cholesterol content of chicken meat varies between 57 and 78 mg / 100g (Sharma 1984; Krzynowek, 1985). Chicken meat cholesterol content at 245–627 mg/ kg is similar to those reported by Al-Najdawi and Abdullah (2002) and Rule et al. (2002), but lower than for beef (860 mg /kg), pork (850 mg kg) and lamb (920 mg/ kg) (USDA Human Nutrition Information Service, 1989). The relationship between increased concentration of plasma total cholesterol and coronary heart disease is established. So, research has been directed towards reducing the cholesterol levels in meat and meat products (Kumari et al. 2006).

The American Heart Association has recommended 300mg cholesterol per day for men and 225mg for women (Krzynowek, 1985). Breast muscle had a lower cholesterol value than thigh muscle, because of its lower fat content. Cholesterol content is significantly influenced by the dietary fatty acid profile in broiler chicken (Crespo and Esteve-Garcia, 2001). Cholesterol values were 88.9, 130.1, 140.6, and 159.9 mg/100 g for breast meat, thigh meat, skin, and drum meat, respectively, and were all significantly higher than values reported in the nutrient database (Ravindran et al., 2006).

2.2.2.6 The role of Vitamin E in meat quality

Vitamin E was discovered in 1922 but it was not until 40 years later that was established as essential to for human nutrition. Vitamin E consists of two families of compounds, the tocopherols and tocotrienols, characterised by a 6-chromanol ring and an isoprenoid side chain (Figure 2.5 Kramer et al. 1997). The members of
each family are designated alpha (α), beta (β), gamma (γ), or delta (δ) according to
the position of methyl groups attached to the chroman nucleus. They are
differentiated by their phenyl “tails” as these are saturated in the tocopherols but
unsaturated in the tocotrienols (Combs, 1992).

Figure 2.6: Structure of tocopherol and tocotrienol (Kramer et al., 1997).

Foods rich in vitamin E are those from plant origin like seed oils, vegetables and
whole grains. Most animal products are poor sources of this vitamin (Meydani,
1995). Poultry meat has a significant content of vitamins, however, the quantity of
vitamin E of poultry meat is low (Barroeta, 2006). The main function of vitamin E
(α-tocopherol) is to protect susceptible cellular structures, especially PUFA in
membrane phospholipids and in plasma lipoproteins against damage from oxygen
free radicals (Leonhardt et al. 1997). Vitamin E functions primarily as an
antioxidant in biological systems by trapping peroxyl free radicals (Combs, 1992).
There is continued interest in the role of vitamin E in cellular metabolism with its
antioxidant properties and preventing free radical mediated diseases. Since α-
tocopherol is a fat-soluble compound, vitamin E has been reported to have many
beneficial health functions, such as preventing cardiovascular diseases, cancers, cataracts, etc. (Diplock, 1996; Knekt, 1994; Morrissey and Sheehy, 1999; Muller, 1994; Richard and Roussel, 1999).

2.2.2.7 Amino acids in chicken meat

Poultry meat and other meats are good sources of high biological value protein vary between 17-22% (Barroeta, 2006). Steinhauser et al. (2000) reported that proteins are the most important components of meat from both the nutritional and technological point of view. The highest content of protein is in chicken breast muscles. According to Simeonovová (1999) breast muscles contain approximately 22.00 % proteins while thigh muscles, which contain more fat, consist of approximately 17.20 % protein. Petkov (1988) reported the average of protein content in breast and thigh muscles are 20.73 % and 19.10 % approximately.

Table 2.6: Amino acid composition of red (pigs) and white meats (chicken) (Mavromichalis et al. 2000).
2.3 Toxicological aspects

2.3.1 Trace elements in sea birds

Trace elements are divided into two categories, essential metals and toxic elements. Essential trace elements are chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), molybdenum (Mo), nickel (Ni), magnesium (Mg) and selenium (Se) (Manahan, 2002). These are biologically essential nutrients and some aquatic species can regulate the body levels of them at constant levels, but the regulation is broken down at higher metal exposures and metal accumulation occurs (Elliott et al. 1992; Papagiannis, 2004).

Trace elements such as arsenic (As), cadmium (Cd), lead (Pb), strontium (Sr) and mercury (Hg) are found in the environment throughout the world and have long been recognized as a serious concern (Dural et al. 2007). There are biologically nonessential with no known biological function and demonstrably toxic even at relatively low concentrations. They are potentially harmful to most organisms even relatively in low exposure level (Pérez-López et al. 2003). Mercury particularly, the major organic form methylmercury biomagnifies through aquatic food chain (Gardner et al. 1978). The in vivo demethylation of methylmercury has been demonstrated or suggested for a number of mammalian and avian species (Komsta-Szumska et al. 1983; Norheim and Froslie 1978; Norseth and Clarkson 1970; Thompson and Furness 1989).

Toxic elements are continually released into the aquatic environment from natural sources (geological activities) such as volcanic activity and weathering of rocks (Mailman, 1980; Fan, 1996; Campbell et al. 2005). However, the main sources of toxic metal pollution are from garages, motor workshops, car washing stations,
silver crafting from urban, industrial, and agricultural runoff (Mailman, 1980; Papagiannis et al. 2004). In addition to, the global transport, deposition that is an important source of Hg for many ecosystems, including the ocean (Fitzgerald, 1989). The contamination chain of heavy metals almost always follows the cyclic order: industry, atmosphere, soil, water, fish, and humans (Stewart et al. 1996; Schon et al. 2006). Hall (2002) has shown that certain chemicals have the ability to bioconcentrate in organisms directly from the water, bioaccumulate and biomagnify in the food chain, causing higher trophic organisms to become contaminated with high concentrations of chemical contaminants than their prey.

Because of the elevated concentrations of some essential and nonessential toxic elements (Kim et al. 1999) as the result of biomagnification in the food chain, accumulation pathways of metals in oceanic seabirds has been increasingly studied during the last years, Once a metal enters the body of an organism it can be stored or excreted (Braune and Gaskin, 1987a; Lewis and Furness, 1991). In birds, metals circulate through the body and are deposited in a variety of tissues (Gochfeld et al. 1998). As the substances bioaccumulate over time, they can be directly related to harmful effects on bird populations (Thompson et al. 1992), which are at risk from both lethal and sub-lethal effects, as their body burdens increase (Gochfeld, 1997).

2.3.2 The use of seabirds as biomonitors for environmental pollution

Aquatic organisms have been widely used to assess environmental pollution because of their ecological and economic importance and their morphological, physiological and ecological diversity in aquatic habitats (Kemal et al. 1999). Seabirds have been used in ecotoxicology as monitors of ocean pollution for a great variety of xenobiotics and heavy metals (Burger, 1993). Migratory seabirds can be exposed to trace elements on wintering grounds or on their breeding sites or
along the migratory route (Burger et al. 2007). It must be emphasised that the levels of trace elements may vary widely among different seabird species depending on the feeding ecology, intensity, and timing of exposure in foraging areas, as well as their physiology and biochemical characteristics (Savinov et al. 2003).

Seabirds are useful as biomarkers of pollution because they are exposed to a wide range of chemicals, are often at the top of their food chain, and are susceptible to bioaccumulation (Walsh, 1990; VanStraalen and Ernst, 1991; Furness, 1993; Furness and Camphuysen, 1997; Burger and Gochfeld, 2002). In addition, seabirds are excellent subjects for examination of heavy metals because they are long-lived, feed at different distances from land, and exhibit different trophic levels (Honda et al. 1990; Elliott et al. 1992; Lock et al. 1992; Burger and Gochfeld, 2000). Furthermore, Gilbertson et al. (1987) suggested that contaminant levels in seabirds have a lower coefficient of variation than fish or marine mammals, making them important bioindicators of marine pollution. As top predators and consumers in the marine food chains, seabirds accumulate environmental contaminants that can be measured easily in eggs, feathers, internal organs, offering opportunities to detect and assess the biological effects of changes in physical and chemical parameters of the marine ecosystem (Diamond and Devlin, 2003).

There are three processes that occur in marine environments when marine animals are exposed to heavy metals; bioconcentration, bioaccumulation and biomagnification. These processes are defined as follows:

1-Bioconcentration is the intake of chemical contaminant through the organism’s epithelial tissues or gills, and the subsequent concentration of that chemical
contaminant within the tissues to a level that far exceeds ambient environmental concentrations.

2- Bioaccumulation is the process by which chemical contamination in organisms increases with each step in the food chain.

3- Biomagnification is the process by which chemical contaminants are concentrated at levels that exceed chemical equilibrium from dietary absorption of the chemical (Gobas et al. 1999). Therefore, biomagnification is when an increase in trace metal concentration occur at least two trophic levels in a food chain. If biomagnification of trace metals is happening, then elevated trace metal concentrations in higher trophic group organisms could pose a threat to the organisms or to human consumers (Barwick and Maher, 2003).

Mercury is one of the most toxic metals for seabirds. It has been established that concentrations between 1 and 2 ppm (wet weight) may be associated with high embryo/duckling mortality and brain lesions (Zillioux et al. 1993). Mercury accumulates and is stored in internal tissues throughout the year and is mobilized and excreted into the plumage during moult (Furness et al. 1986). The levels of Hg and Cd that accumulate in internal tissues of seabirds are frequently higher than those which cause toxic effects in terrestrial bird species (Scheuhammer, 1987). As a consequence of this, it is thought that seabirds have evolved several protective mechanisms to alleviate toxic effects. However, if seabirds were to be consumed by humans or terrestrial animals who do not have such protection, toxicity may occur.
2.3.3 Pesticides in sea birds

Pesticides are defined as any substance or mixture of substances that are used to control pests and fauna in many areas of primary production, including agricultural farming, forestry and horticultural production (Dich et al. 1997). Pesticides include insecticides (to kill insects), fungicides (to control fungal diseases), herbicides (to kill weeds), plant growth regulators and other vermin control agents (to kill rats and other vertebrate pests) (Manktelow et al. 2004).

Pesticides are used in many as tests of environmental management, in agriculture and forestry, environmental conservation, in urban and industrial areas, public spaces, homes and gardens, schools, hospitals, and food outlets. Their application is still the most effective and accepted means for the protection of plants from pests, and has contributed significantly to enhanced agricultural productivity and high crop yields (Bolognesi, 2003). However, the use of plant treatment products represents toxicological and eco-toxicological risks like exposure of non-target organisms, undesirable side-effects on some species, communities or on ecosystems as a whole (Juraske et al. 2007).

Pesticides with a high acute toxicity may not be easily metabolized and eliminated from the body. But following long-term low exposure, they may be less toxic but still may cause carcinogenic or mutagenic properties. Chemicals with low acute toxicity such as organic mercury compounds and some organochlorine compounds can accumulate in the body and cause chronic toxicity after long-term exposure even in comparatively low doses. For example, the solvents carbon tetrachloride and chloroform are both toxic to the liver and central nervous system (Dich et al. 1997).
The extensive use of persistent and lipophilic organochlorines (OCs) such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), during the past few decades has led to a number of negative effects on terrestrial and aquatic organisms (Sakellarides et al. 2006). OCPs include several compounds such as dieldrin, 4,4c-dichlorodiphenyltrichloroethane (4,4c-DDT), 2,4c dichlorodiphenyltrichloroethylene (2,4c-DDE), 4,4c dichlorodiphenyldichloroethane (4,4c-DDD), c-hexachlorocyclohexane (lindane), aldrin, endrin, heptachlor epoxide, permethrin, atrazine, and simazine (Salvado et al. 2005). Among these, DDT has been the major environmental contaminant.

DDT is a pesticide that was first synthesized in 1874 and used as an effective and cheap insecticide since 1939 (Beard, 2006). It was used worldwide, including New Zealand, to combat insect pests in agriculture. DDT has also been the most effective tool against malarial vector mosquitoes (Ross, 2005) until it was banned in some countries in the 1960s. DDT is extremely persistent in both the environment and biological systems. Therefore it is considered a major environmental contaminant and a risk for human and animal health upon exposure (Beard, 2006). Exposure to DDT has caused a decline in animal reproduction and cancer in humans (Beard, 2006). Fish-eating birds have been monitored regularly as a species potentially at risk of secondary poisoning by persistent organic pollutants (POPs) (Sakellarides et al. 2006). There is evidence that POPs affect the development and reproductive capacity of these birds (Giesy et al. 1994; Blus, 1996; Hoshi et al. 1998).

High levels of contaminants, including POPs such as DDTs, dieldrin and PCBs have also been associated with breeding failures in some Ardeid species (De Luca-Abbott et al. 2001; Hothem and Hatch, 2004). The adverse effects of PCBs in a
wide variety of birds have also been reported with the most prominent effects being reproductive failures, embryonic deformities, growth retardation and immunosuppression (Yamashita et al. 1993; Barron et al. 1995; Grasman et al. 1998). The above effects have resulted in population decline of several birds around the world (Harris et al. 2003; Murata et al. 2003).

2.3.4 Distribution and occurrence of toxic compounds in seabirds.

2.3.4.1 Heavy metals

Kim et al. (1998) determined the accumulated profile of trace elements (lithium (Li), vanadium (V), manganese (Mn), cobalt (Co), copper (Cu), zinc (Zn), selenium (Se), rubidium (Rb), strontium (Sr), silver (Ag), cadmium (Cd), caesium (Cs), lead (Pb), and mercury (Hg)) in the various tissues and organs of three species of seabirds. The distribution of trace elements in the various tissues and organs of seabirds showed a preferential disposition of certain elements in certain organs (Figure 2.7). A relatively high burden of Cu, Se, Rb, and Cs was found in the muscle, whereas high Pb and Cd concentrations were found in the feather and kidney, respectively. More than 50% of Li, V, Mn, Co, and Zn burdens in the seabird body was in the bone. While, 97% of Sr burden in the body was found in the bone. Silver burdens in the liver and fat exceed more than 35% of that found in the whole body (Kim et al. 1998). Lead burdens in the feather were rather higher than those in other tissues. This seems to may be similar to Hg which transfers from the soft tissue to the feathers during the moulting process (Honda et al. 1986; Braune and Gaskin, 1987).
Recent studies have elucidated a negative correlation between organic Hg and total Hg concentrations in the liver, muscle, and kidney of several seabird species (Thompson and Furness, 1989; Kim et al. 1996b). This may be a result of the association between inorganic Hg and Se in certain tissues, primarily liver, leading to increase the accumulation of inorganic total Hg and producing a positive correlation between Hg and Se concentrations (Kim et al. 1996b). Polonium (Po) accumulates in the marine biota and for this reason it is an important source of radiation dose in the body of marine animals (Skwarzec, 1995). Seabirds are very important element of the trophic chain of marine ecosystems. Particularly the ‘bird feathers’ are often used as a bioindicator of metal contamination of the marine and air environment (Burger and Gochfeld, 1995). But, Knowledge on the Po distribution in birds is very poor. Skwarzec and Fabisiak, (2007) determined the Po content in 11 species of sea birds which permanently or temporally live in the polish part of the Baltic Sea. They found a considerable differentiation of $^{210}$Po concentration in tissues and organs of marine birds. The biggest quantities of polonium accumulate in feathers and liver, While, smallest amounts in skeleton, muscles and skin.
Fabris et al. (2006) determined Cd, Cu, Hg, Pb and Zn and the metalloids (As and Se) concentrations in the edible tissue in various marine animals (snapper, flathead, lobster and abalone) and compared them with the Australia and New Zealand Food Standards code. Schon et al. (2006) determined the concentrations of (Hg), (Pb), and (Cd) and (As) in muscles, liver, kidneys, gills and gonads of five species by atomic absorption spectrophotometry. From their results, it is evident that fish tends to accumulate Hg, Pb, and Cd. Although total Hg, As and Pb levels are still lower than MAC in most countries, they suggested paying attention to their finding that highest concentrations were found in muscles and are already higher than MAC in some countries. Therefore, they may be potentially harmful as the constituent of human food. Papagiannis et al. (2004) studied the level of contamination of two essential heavy metals, Zn and Cu in lake water in four fish species in three different tissues (muscle, liver, gonads) in order to assess the fish contamination. Their finding observed that the concentrations of Cu and Zn in the muscle were lower than other organs.

Mercury and Cd are the important heavy metals which are found in the tissues of sea bird, Monteiro et al., (1998) showed that there is a high correlation between the levels of Hg in the diet of seabirds, and the concentration in feathers, so that seabirds can be used as indicators of food chain effects. Mercury especially in the major organic form, methylmercury, and biomagnifies through aquatic food chains (Gardner et al. 1978). The in vivo demethylation of methylmercury has been demonstrated or suggested for a number of mammalian and avian species (Komsta-Szumska et al. 1983; Norheim and Froslie, 1978; Norseth and Clarkson, 1970; Thompson and Furness 1989). Scheuhammer (1987) was noted that the levels of Hg and Cd accumulated by seabirds in internal tissues are frequently higher than those which would cause toxic effects in terrestrial bird species.
2.3.4.2 Organochlorine

Adult short-tailed shearwaters from Tasmania, Australia, have been shown to accumulate higher PCB and DDE concentrations (a major metabolite of DDT) compared with chicks (Tanaka et al. 1986). Bogan and Newton (1977) reported that the percentage of DDE located in the brain of sparrow hawks *Accipiter nisus* slowly increased with progressively less body fat and was markedly higher in birds which contained less than 1.5% body fat in their bodies. It was suggested that starving birds were more at risk from toxic shock because DDE was released into the bloodstream and tissues particularly in the adipose reserves were mobilized. A similar effect could be occurring in sooty shearwaters during their migration, especially if the birds are stressed by food shortages caused by climatic perturbations (Baduini1, 2001).

The thick-billed murre, *Uria lomvia*, is the most common of seabirds in the Canadian Arctic and feeds on an array of prey items including annelid, mollusc, crustacean, and fish species, with the latter often forming a significant proportion of the diet (Gaston and Noble, 1985; Gaston and Bradstreet, 1993). Donaldson et al. (1997) analyzed the breast muscle of thick-billed murres (*Uria lomvia*) for organochlorines (chlorobenzenes, hexachlorocyclohexanes, DDTs, chlordanes, mirex, dieldrin, and PCBs) and metals (Se, Cd, Hg, and Pb). They found that both organochlorine and metal residues levels were sufficiently low so that toxic effects were unlikely. Of the metals, only Cd was detected at higher levels in older birds.

Breast muscle metal concentrations found in the above study were similar to levels described for thick-billed murres collected in West Greenland (Nielsen and Dietz, 1989), in Arctic mainland Norway (Wenzel and Gabrielsen, 1995), and in Svalbard (Savinova and Gabrielsen, 1994) during approximately the same time period.
Breast muscle metal concentration was varied, but it did not usually contain a high metal burden (Donaldson et al. 1997). Although muscle is not the usual tissue analyzed for organochlorine and metal contamination, a significant relationships between the contaminant concentration and age in thick-billed murres from the Canadian Arctic was observed.

Correlations between different aged birds and organochlorine and metal contamination reflect the kinetics of the contaminants and the migratory behaviour of these birds (Donaldson et al. 1997). As indicated above, a majority of research that has been published point to the fact that seabirds may be used as bioindicators of marine environmental pollution. But, there is a shortage of information in nutritional and toxicological aspects of seabirds as human food and particularly Mutton bird.

### 2.3.4.3 Residual organochlorine pesticides in Mutton bird

The presence of residues of organochlorine pesticides in both land and sea birds throughout the world is well documented (Holmes et al. 1967; Risebrough et al. 1967, 1968; Prestt et al. 1970; Bogan and Bourne1, 1972). Risebrough et al. (1967, 1968) reported that three Mutton birds (P. griseus) migrating in the Monterey Bay area of California during 1966 had 8.4 ppm of total DDT residues (DDE (1,1-dichloro-2,2-his (p-chlorophenyl) ethylene) was 88%, DDD (i,i-dichloro-2,2-bis(p-chlorophenyl)ethane) was 3.8%, DDT was 5.6%, and 1.1 ppm PCB in the lipid extract. Risebrough et al. (1968) also found 32 ppm of total DDT of which pp'-DDE 92% and 2.1 ppm PCBs in the fat of one slender-billed shearwater from Australia.
In view of such relatively high levels of DDT and PCBs, and since it is also well known that residues of these organochlorine compounds in birds produce effects such as eggshell thinning and consequent population declines. It was considered to be of interest to further identify and measure the levels of these pesticides in the Mutton bird. For the comparison sake, the status of trace elements in chicken which is used as a reference material in the proposed research will be briefly reviewed.

2.4 Heavy metals in chicken

Meat and meat products are important for human diet because they provide a great part of nutrients, including the necessary trace elements. In recent years, much attention has been focused on the levels of heavy metals in fish and other sea foods with little attention on the levels in chicken meats. Iwegbue et al. (2008) determined the concentrations of Fe, Cu, Zn, Ni, Mn, Cd, Pb and Cr in chicken breast meat consumed in seven regions of southern Nigeria (Table 2.8).

Table 2.7: Concentrations mg.kg\(^{-1}\) of eight trace elements in chicken breast meat” in seven regions in Nigeria (Iwegbue et al., 2008) (mean±SD)
2.5 Hypotheses and objectives

2.5.1. Hypothesis

- The meat of Mutton bird will have a higher concentration of PUFA than other common meats particularly chicken meat.
- The meat of Mutton bird will have a lower cholesterol concentration than other common meats particularly in chicken meat.
- The meat of Mutton bird will have a higher concentration of essential trace minerals than in other common meats particularly chicken meat.
- The meat of Mutton bird will have a lower concentration of toxic metals than in chicken meat and other common meats.
- The meat of Mutton bird will have the same of concentration of essential amino acids with other common meats particularly chicken meat.
- The meat of Mutton bird will have a lower concentration of DDT than chicken meat and other common meats.

2.5.2 Objectives

2.5.2.1 Broad Objectives

To evaluate some nutritional and toxicological aspects of New Zealand Mutton birds collected in 2 consecutive years (2007 and 2008) and to compare with published information on chicken meat and other meats.

2.5.2.2 Specific Objectives

- Determine the macro-chemical composition, fatty acid profile, amino acid profile, and cholesterol content in Mutton bird breast muscle.
- Determine the concentration of heavy metals in Mutton bird breast muscle.
- Determine the concentration of DDT in Mutton bird muscle.
Chapter 3

Nutritional studies on Mutton bird meat (*Puffinus griseus*)

3.1 Introduction

Meat is a valuable food source that is rich in many of the essential nutrients including protein (especially essential amino acids), minerals (e.g. Fe, Zn, Se), vitamins (Vitamin E) and Fat (essential fatty acids such as Omega 3 fatty acids) (Schonfeldt and Gibson, 2008). Red meat include beef, veal, pork and lamb (Linseisen et al., 2002) and is a primary dietary component and forms an important part of a balanced and varied diet (Whitney and Rolfés, 2008). Many consumers believe that red meat is unhealthy, because it has a high concentration of saturated fatty acids (SFA) and cholesterol (Kee, 2000). The replacement of red meat with chicken was associated with a significant decrease in apolipoprotein B and total cholesterol levels in micro-albumin uric type 2 diabetic patients (Gross *et al.*, 2002). This effect was probably related to the higher PUFA content of chicken compared to beef (De Almeida *et al.*, 2006). Therefore, the nutritional quality of compounds in meat plays an important role in human health and it is important for scientists and nutritionists to monitor the quality of food products containing meat from different sources (Omole *et al.*, 2008).

Seabirds are abundant upper-trophic level predators in marine ecosystems, where they forage on zooplankton, fish and squid. By virtue of their ecological position high on the marine food web and their wide foraging range, seabirds provide an ideal vehicle for monitoring pollution in the marine ecosystems (Ryan and Fraser 1988, Furness and Camphuysen 1997, Van Franeker 2004). Moreover, since seabirds eat the same zooplankton, fish krill and squid prey consumed by commercially-valuable fish species; they may provide valuable information on the
pollutant loads of marine resources consumed by humans (Montevecchi 1993, Burger and Gochfeld 2004, Blais et al. 2005). As described previously, *Sooty shearwaters* (Mutton birds) are wild migratory seabird, traditionally consumed by New Zealanders and especially by the native Maori (Marchant and Higgins, 1990; Berkes and Folke, 1998). Their fledglings are annually harvested by Maori according to the customary rights agreement set by Treaty of Waitangi. This bird is interesting since its sole diet is based on krill and other seafood that are potentially rich in n-3 fatty acids and other marine bioactive compounds (Falla et al. 1978, Warham and Wilson 1982). Although, Mutton birds are sea birds and have been used as human food for centuries, the data on their nutritional value is limited (Miletic et al., 1990).

One of the main health aspects of meat for nutrition is its fatty acid composition since it is known that PUFA- and MUFA- rich diets decrease cholesterol levels in blood and are related to a low incidence of cardiovascular diseases (Mattson and Grundy, 1985). Marine animal foods are rich in polyunsaturated fatty acids which are considered anti-cholesterolemic (Krzeczkowski, 1970; Bottino et al., 1979; Krzynowek and Panunzio, 1989; Kotb et al., 1991). The PU FAs, EPA and DHA, found in fish and shellfish can help reduce high blood pressure, cholesterol levels and decrease the risk of heart attack and/or stroke (Kinsella et al., 1990). However, increasing the unsaturated fatty acids in the muscle cell membranes results in increased oxidative deterioration (Lin et al., 1989; Monahan, 1992), and oxidized lipids in food may have adverse effects on health (Benamira et al., 1995; Boyd and McGuire, 1991; Draper et al. 1986). Furthermore, an increase in the percentage of unsaturated fatty acids cause a decrease in the firmness and an increase in the oiliness of meat (Miller et al., 1990). This may cause some marketing issues since
increasing the hardness of the tissue fat can be advantageous in the marketing of broiler meat (Valencia et al., 1993).

Cholesterol belongs to the sterol group of fats. It is present in egg yolk, dairy products, fatty meats and organs meat (Khan, 1988). It is useful to identify and limit foods that are rich in cholesterol. Normally the intake of cholesterol in diet is not very high. It is necessary to limit the intake to less than 300 mg/ day (Sharma, 2000). About 1g of cholesterol is daily synthesized in body and all the 27 carbon atoms of cholesterol are synthesized from acetyl-CoA (Mehta et al., 1983). Cholesterol content in breast muscle in chicken is lower than thigh muscle, because of its lower fat content. Cholesterol performs a number of vital functions in the body, such as providing an essential component of membranes and serving as a precursor of bile acids, steroid hormones and vitamin D (Harvey and Champe, 1994). However, circulating cholesterol may injure arteries especially coronary arteries that supply blood to the heart (Lippy, 1998) leading to heart diseases. Moreover, cholesterol is prone to oxidation, which leads to the production of wide variety of cholesterol oxides, termed oxysterols. Cholesterol oxidation may proceed by the same mechanisms ascribed for lipid oxidation or can be initiated by free radicals generated during the lipid oxidation (Finocchiaro and Richardson, 1983). Both oxidized lipids and cholesterol oxides are implicated in the development of atherosclerosis (Kumar and Singhal, 1991).

Protein is used to build muscle and tissues, and to aid in metabolic processes (Salvatori et al., 2004). Thus providing the appropriate amount of each amino acid is important to avoid any problems associated with amino acid deficiency (Boylan and Borrelli, 2006). The nutritive value of proteins is primarily defined by the amount and composition of essential amino acids, and thus nutritional evaluation
of meat proteins may be obtained by the analysis of their amino acids (Dvorak and Vognarova, 1969). Individual amino acids are categorized as essential amino acids, semi-essential amino acids, and non-essential amino acids (Krishna et al., 1980). A number of studies have been published on the utilization of new meat protein resources as an alternative for common protein sources (Sotelo and Perez, 2003; Barroeta, 2007). Chicken meat is high in protein when compared to other nutrients (Salvatori et al., 2004). Birds have always been a source of meat protein for humans.

Mutton birds are traditional food for native people of New Zealand and Australia (Richdale et al., 1946). Mutton birds eat krill and other small seafood organisms for food. Thus, there may be some nutritional advantages of eating Mutton bird since their diet is perceived to contain a high percentage of PUFA and this may reflect in their body fat. The available information regarding the nutritional status of New Zealand Mutton Bird is scarce. Most of the available studies, as stated in the literature review, were focused on the use of these seabirds as bio-monitors or biomarkers of the marine environment (Furness, 1993; Furness and Camphuysen, 1997). The present study was designed to assess the nutritional aspects of New Zealand Mutton Bird from samples that were collected in 2 consecutive years 2007 and 2008. The emphasis of nutritional studies was on the macro-chemical composition, fatty acid profile, amino acid profile, and cholesterol content.
3.2 Material and Methods

3.2.1 Sample collection

Forty carcasses of Mutton birds (Figure 3.1) were purchased from a fish monger in 2007 and 2008 (20 carcasses for each year). The samples were vacuum packed and frozen upon arrival to the laboratory and kept at -30 C° until analysis.

![Figure 3.1: A representative sample of Mutton bird](image)

3.2.2 Size and weight measurements:

The samples were thawed overnight in a chiller at 4 °C. Breast muscles without skin were separated from carcasses after thawing. Breast meat was then weighed and their percentages of body weight were calculated. Each sample was minced, divided into seven containers (each one more than 5g) which were kept frozen at -30 °C till analysis.

3.2.3 Sample preparation for Analysis:

Weighed portions of samples were freeze dried, re-weighted to measure the moisture loss, stored in centrifuge tubes, sealed and kept in a refrigerator pending proximate and amino acid analysis. Weight loss was recorded and converted to Dry Matter Content (DM %).
3.2.4 Proximate composition

Proximate composition was carried out as described in standard published methods of the Association of Official Analytical Chemists (AOAC, 2000).

3.2.4.1 Moisture analysis

Duplicate sub-samples (1.0 g each) for each sample were dried in the oven at 103°C (±5 °C) for 48 hours. Weight loss was determined by the difference in the sample weight before and after drying and was converted to residual Dry Matter (rDM%). Moisture content was calculated by difference of total dry matter content (DM% × (rDM%/100)) and moisture content was expressed as % of the sample wet weight.

3.2.4.2 Crude fat analysis

Crude fat contents were determined using Soxhlet extraction method according to standard procedures (AOAC, 2000). Duplicate sub-samples (1.0 g each) for each sample were used and the fat content was expressed as % of the sample wet weight.

3.2.4.3 Crude protein (total nitrogen) analysis

Crude protein measurements were carried out using Kjeldhl digestion method according to standard procedures (AOAC, 2000). Duplicate samples (0.5 g each) for each sample were used and the crude protein content was expressed as % of the sample wet weight.
3.2.4.4 Ash analysis

Ash contents were determined according to AOAC (2000). Duplicate samples (0.3 g each) for each sample were used and the ash content was expressed as % of the sample wet weight.

3.2.4.5 Carbohydrate analysis

Carbohydrate content was calculated by difference of the sum of moisture, fat, protein and ash contents from 100%. (AOAC, 2000).

3.2.5 Fatty acid analysis

3.2.5.1 Extraction and preparation of samples

Various methods of lipid extraction have been established, the most common being soxhlet extraction, the Folch method (Folch et al., 1956) and the Bligh and Dyer method (Bligh and Dyer, 1959). A new modified Bligh and Dyer’ method for total lipid was introduced (Smedes, 1999) and this method used readily available non-chlorinated solvents (cyclohexane and isopropanol) with low toxicity, non-olfactory, non-explosive properties and low cost.

3.2.5.2 Extraction of oil

Breast muscle tissues from individual vacuum packed samples were used for fatty acid analysis. Samples were thawed at 4°C overnight and extraction of the lipids was carried out described by Smedes (1999) with some modifications. A 10g muscle sample was blended gradually with 32 mL of Isopropanol: cyclohexane mixture (4:5, v/v) for 2 min using Ultraturax homogeniser at 9500 rpm. Deionised water was added to the mixture to give the water: isopropanol: cyclohexane ratio of 11: 8: 10 (v/v/v) and the mixture were further homogenized for another 2 min on
ice. The mixture was centrifuged for 5 min at 9500 rpm (4000g) and the top organic phase (lipid containing layer) was transferred to a pre-weighed pear shaped flask by a Pasteur pipette.

The remaining mixture (Mutton bird tissue and solvents) was blended with cyclohexane/isopropanol (20 ml, 83:13 v/v) for a second extraction by using Ultraturax for 2 min at 9500 rpm. The mixture was centrifuged for 5 min at 9500 rpm (g= 4000) and the upper organic phase (containing the lipid) was transferred to the pear shaped flask by a Pasteur pipette. The organic phase in the pear shaped flask was evaporated using a rotary evaporator for 10 min at 40 °C and the flask containing the lipids was weighed to calculate the oil yield. The oil was transferred to a pre-weighed 4 ml glass vial and the flask was rinsed twice by a mixture of cyclohexane/ isopropanol (1ml, 87:13v/v) and the liquid was added to the glass vial. Each sample was filtered into a vial by adding sodium sulphate in a syringe that contained hexane pre-washed cotton and the oil sample was evaporated in water bath under nitrogen stream. All the samples were flushed with nitrogen and kept in the freezer (-30°C) until analysis.

3.2.5.3 Preparation of Methyl esters for fatty acids analysis

Methyl esters were prepared by transmethylation of the oil samples using 1% sulphuric acid in methanol and n-hexane according to the method described by Hughes et al., (1980) with some modifications. A 50-70 mg oil sample was transferred into the bottom of a 30 mL Kimble screw cap test tube and 1 mL of dichloromethane containing heptadesanoic acid (C:17) as internal standard was added to the oil sample. One mL methylating mixture (1% sulphuric acid in dry methanol) was added to each sample then the tube was flushed with nitrogen capped and placed in an oven over night at 50 °C. The tubes containing the
samples were allowed to cool to room temperature and then 5 ml of 5% NaCl was added to each tube and the tubes were inverted to mix the contents. 5 mL of hexane was added to each tube and the tubes were inverted several times to mix the contents and allow the aqueous and hexane layers to separate. The hexane layer that contains the methyl esters was transferred to another clean labelled tube. This step was repeated again using 3 mL of hexane and the hexane fractions were combined. A 5 mL of potassium bicarbonate (2%) was used to wash the hexane and the bottom layer was removed. Anhydrous sodium sulphate was added and mixed until the hexane was dried (snows up) which was indicated by the presence of none-crystallized sodium sulphate. The hexane layer was carefully transferred using Pasteur pipette into a fresh GLC vial, capped and stored at 4°C. When the sample was dry, 1.2 ml of hexane was added and the sample was ready for injection in GC.

3.2.5.4 Fatty acid separation and detection

Fatty acid methyl esters (FAME) were analysed by a GC-2010 (Shimadzu, Japan) equipped with an auto-sampler (AOC-20i, Shimadzu, Japan), a flame ionization detector (FID) and a column (JW-Innowax, 30 m * 0.25 mm ID * 0.25 μm Film Thickness), controlled by GCSolution (Version 2.300 SU4). The oven temperature was initially at 50 °C, then raised to 205 °C over 30 min and increased to 240 °C at a rate of 1°C/min and maintained at 240°C for 5 min. The injector and the detector temperatures were set at 230 and 250 °C, respectively. The sample size was 1 μl and the carrier gas (helium) was initially at 55.7 KPa, and then increased to 107 KPa and 200 KPa following the time for the change in the oven’s temperature. The split used was 30:1 with an initial column flow rate of 0.6 ml/min.
3.2.5.5 Standards and quantification

Heptadesanoic acid (C_{17}) (T0502-5G, Sigma Aldrich, Bellefonte, USA) at a concentration of 3 mg/ml was used as internal standard. Fatty acids were identified by comparing the retention times of FAME with standard of mixed known compositions (WE-411, GLA-411, GLA-411-EP, Nu-Check, Elysian, USA). Two replicate GC analyses were performed for each sample and the results were expressed in fatty acids % as mean values ± standard deviation.

3.2.6 Amino acid analysis

3.2.6.1 Preparation and extraction of samples

All the amino acids, except tryptophan, cysteine and methionine, were determined by HPLC analysis after acid hydrolysis. The hydrolysis method was modified from Fountoulakis & Lahm (1998). All the samples were freeze-dried and ground through a 0.5 mm sieve using an Ultra Centrifugal Mill ZM 100 (Retsch GmbH & Co., Germany). Samples were weighed (100 mg) into 20ml screw-cap culture tubes and then 5.0 mL of 6 M HCl and 10.0 µl of 0.50 M amino-butyric acid (internal standard) were added into each tube. The tubes were purged with nitrogen gas to remove the air and immediately capped. Tubes were heated on the Dry Block Heater (Ratek DBH10D, Ratek Instrument Pty. Ltd. Australia) for 20 hrs at 110°C. After being removed from the block heater, the tubes were cooled to room temperature, the hydrolysate was decanted into 250 ml round bottom flask, and the tube was rinsed twice with 5ml Milli-Q water.

The hydrolysate was evaporated to dryness using a rotary evaporator under vacuum at 40 °C. The dried material was re-dissolved in Milli-Q water and decanted into a 50 ml volumetric flask. The round bottom flask was then rinsed
twice with Milli-Q water and the volume was made up to 50 ml. The resulting 50ml hydrolysed sample was thoroughly mixed before filtering with a 0.45 µm syringe filter (cellulose acetate membrane, 13mm, Biolab, New Zealand) directly into a 2 ml HPLC vial and stored at –20°C until analysis.

3.2.6.2 HPLC analysis of amino acid

The HPLC analysis was performed on an Agilent 1100 series (Agilent Technologies, Walbronn Germany), including a degasser, a HPLC quaternary pump, an auto-sampler with a thermostat, heated column compartment and a fluorescence detector, controlled by ChemStation (version A10.02). The HPLC method was modified from Carducci et al., (1996). A Prodigy reverse phase column (250x4.6mm, 5µm) connected to a guard column (Phenomenex, New Zealand) was used for amino acids separation. The column temperature was kept at 40°C, and the auto-sampler tray was kept at 4 °C. Solvent A [disodium hydrogen phosphate 0.01M containing 0.8% of Tetrahydrofuran (THF), pH 7.5] and solvent B [20% solvent A, 40% methanol and 40% acetonitrile] were used. All the chemicals were analytical grade and solvents were HPLC grade (supplied by Biolab, New Zealand). The flow rate was 1.0ml/min, the pump gradient was 0% solvent B to 40% B from 0 to 14 min, 55%B at 22min, 100% B from 27min to 35min, decreased to 0% B at 36 min, and equilibrating at 0% B till 40min. The total run finished at 40min.

All the primary amino acids were derivatized by OPA (o-phthaldialdehyde 5mg dissolved by 125µl methanol, mixed with 5.0µl of 3-mercaptopropionic acid, then 1.12ml of 0.2M borate buffer pH=10 was added). The secondary amino acids were derivatized by FMOC (0.22mg of 9-fluorenylmethyl chlormate dissolved in 1.0ml acetonitrile). To increase the detection sensitivity, 3-MAP (10µl of 3-
mercaptopropionic acid and 990µl of 0.2M borate buffer pH=10) and IDA (26mg of iodoacetic acid dissolved with 500µl of 0.2M borate buffer pH=10) were used. Pre-column derivatization was carried out by programmed auto-sampler, which mixed 2.0µl of sample with 2.0µl of 3-MPA, 2.0µl IDA, 4.0µl of OPA and 1.0µl of FMOC, finally the mixture of 11.0µl was injected into the HPLC system (Heems and Luck, 1998). The detector was set at excitation 335nm and emission 440nm for detecting all the primary amino acids and switched to excitation 260 nm and emission 315nm at 26.0 min for detecting the secondary amino acids such as proline and hydroproline.

Cysteine and methionine were analysed as cysteic acid and methionine sulphone after oxidation of the sample with performic acid followed by hydrolysis with 6 M HCl. The method was modified from McDonald et al., (1985). Freeze-dried and grounded samples were weighed (100 mg) directly into 250 ml round bottom flasks. A 10.0 µl aliquot of 0.50 M amino-butyric acid was added to the flasks as an internal standard. Five millilitre performic acids (made by mixing 95 volumes of 99% formic acid with 5 volumes of 30% hydrogen peroxide on ice) were added to each sample. The flasks were then shaken to mix the sample with the acid and sealed with Parafilm™. The sealed flasks were placed on ice stored in a 4ºC fridge for 17 hours. After being removed from the fridge, 0.75 ml of HBr was added to each flask. When the reaction had ceased, the flasks were removed from the ice bath and 15 ml 6N HCl was added to each. The flask was then connected to a reflux condenser in an oil bath set at 110ºC and left to hydrolyse for 23 hours. Before removing the flasks from the oil bath, the condensing apparatus was carefully washed with Milli-Q water and the washings collected in the flask. The resulting liquid has evaporated in a rotary evaporator under vacuum in a water bath set at 40ºC. When the flask achieved dryness, the resulting residue was re-
dissolved in Milli-Q water and the residue was scraped off the inside of the flask into the water. The hydrolysate was transferred into a 50 ml volumetric flask and the round bottom flask was rinsed with Milli-Q water twice and the volume was made up to 50 ml. The sample was filtered with a 0.45 µm syringe filter into 2 ml HPLC vial, then stored in a freezer at –20 °C until analysis.

The HPLC system and the column used for cysteic acid and methionine sulphone analysis were the same as for amino acids analysis as described above. Solvent A was 0.02M sodium acetate buffer (pH=6.0), solvent B was methanol. The flow rate was 1.0ml/min, the pump gradient was 34% solvent B to 60% B from 0 to 5 min, 60%B at 6min, 100% B from 17min to 20 min, decreased to 34% B at 21 min, equilibrating at 34% B for 4min, and the whole run finished in 25 min. Both compounds were derivatized by OPA (o-phthaldialdehyde 5mg dissolved by 125µl methanol, mixed with 5.0µl of mercaptoethanol, then 1.12ml of 0.2M borate buffer pH=10 was added), injection volume was 6.0 µl which was mixed with 3.0 µl of sample and 3.0 µl of OPA by programmed auto-sampler. The detector was set at excitation 335nm and the emission 440nm.

**3.2.6.3 Standards and quantification**

A mixture standard of Asp, Glu, Ser, His, Gly, Thr, Agr, Ala, Tyr, Val, Phe, Ile, Lys, Leu, Pro, Tau, Cys, Met and Try (Sigma Aldrich, Aus.) at concentrations of 25, 50, 100, 250, 500 µM were used to construct standard curves. Sample contents were quantified by the comparison of the retention time and the peak areas of pure standards.
3.2.7 Cholesterol analysis

3.2.7.1 Method of cholesterol analysis

Cholesterol content of Mutton bird oil was analyzed using Liberman-Burchard method as described by Sabir et al. (2003).

Liberman-Burchard reagent: 0.5 ml of sulfuric acid dissolved in 10 ml of acetic anhydride. Covered and kept in ice bucket

3.2.7.2 Preparation of samples and standards

A 1 gram sample of oil was dissolved in 10 mL of chloroform. Then, 1 mL of the diluted sample was further diluted 10-folds to give a final dilution of 100 fold in chloroform. Standards were prepared as in Table 3.1.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard cholesterol solution</td>
<td>0.25</td>
<td>0.5</td>
<td>0.75</td>
<td>1.0</td>
<td>1.25</td>
<td>0</td>
</tr>
<tr>
<td>Chloroform</td>
<td>2.25</td>
<td>2</td>
<td>1.75</td>
<td>1.5</td>
<td>1.25</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*concentration= 1mg/ml

3.2.7.3 Cholesterol analysis

One ml of the Liberman-Burchard reagent 0.5 ml of sulfuric acid dissolved in 10 ml of acetic anhydride was added to 2.5 ml of sample /standard and the tubes were covered with black carbon paper and kept in dark for 15 minutes. The absorbance of the samples and the standards were read at 640 nm. The cholesterol concentrations were calculated from a constructed standard curve taking into account the dilution factor.
3.2.8 Statistical analysis

Data for proximate analysis, amino acids, fatty acids, cholesterol content, elements and DDTs analysis were analysed using one-way analysis of variance (ANOVA) using MINITAB (MINITAB®Release 14.1). Differences between means were determined using Fisher’s least significant difference.

3.3 Results and discussion

3.3.1 Proximate composition

Annual variation in the proximate composition of Mutton bird breast meat *Puffinus griseus* for years 2007 and 2008 is shown in Table 3.2. Higher ash and fat contents (P < 0.05) were found in 2008 samples compared with 2007 samples (Table 3.2). In contrast, protein content was lower (P < 0.05) in 2008 samples compared with 2007 samples. There were no differences in the moisture and carbohydrates contents over the two years.

Table 3.2: Mean ± standard deviation, deviation, n=20) of moisture, crude fat, ash, and protein and carbohydrate content of Mutton bird pectoral muscle obtained in 2007 and 2008

<table>
<thead>
<tr>
<th>Variable</th>
<th>Year</th>
<th>Δ</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>2007</td>
<td></td>
<td>54.52 ± 1.20</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td></td>
<td>53.97 ± 1.55</td>
</tr>
<tr>
<td>Fat % wet weight</td>
<td>2007</td>
<td></td>
<td>11.81 ± 1.59a</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td></td>
<td>13.01 ± 1.75b</td>
</tr>
<tr>
<td>Ash %</td>
<td>2007</td>
<td></td>
<td>10.26 ± 0.89a</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td></td>
<td>11.74 ± 0.64b</td>
</tr>
<tr>
<td>Protein%</td>
<td>2007</td>
<td></td>
<td>20.30 ± 0.83b</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td></td>
<td>18.49 ± 1.09a</td>
</tr>
<tr>
<td>Carbohydrates%</td>
<td>2007</td>
<td></td>
<td>2.97 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td></td>
<td>2.79 ± 0.93</td>
</tr>
</tbody>
</table>

A/b Means bearing a common superscripts letter are not different (p>0.05)
The comparison of proximate composition between New Zealand Mutton bird breast meat (MBBM) and other common meats (chicken, beef, lamb, goat, camel, fish and Australian Mutton bird) is shown in Table 3.3. Compared with other common meats, MBBM had lower moisture content (Barroeta, 2007; Almeida et al., 2006; Elgasim and Alkanhal, 1992 and Lee et al. (2008). The ash content of New Zealand MBBM was higher (Figure 3.2) than in other meats (Elgasim and Alkanhal, 1992; Lee et al., 2008 and Barroeta, 2007).

Table 3.3: Proximate composition of different animal meat

<table>
<thead>
<tr>
<th>Species</th>
<th>Moisture%</th>
<th>Protein%</th>
<th>Fat%</th>
<th>Ash%</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>NZ Mutton bird</td>
<td>53.97±1.55</td>
<td>18.49±1.09</td>
<td>13.01±1.75</td>
<td>11.74±0.64</td>
<td>present study</td>
</tr>
<tr>
<td>Beef (1)</td>
<td>74.48±1.08</td>
<td>21.17±0.16</td>
<td>3.08±0.07</td>
<td>ND</td>
<td>Almeida et al. (2006)</td>
</tr>
<tr>
<td>Beef (2)</td>
<td>73.4</td>
<td>20.4</td>
<td>4.7</td>
<td>1.5</td>
<td>Elgasim and Alkanhal (1992)</td>
</tr>
<tr>
<td>Lamb (1)</td>
<td>ND</td>
<td>19.28±0.51</td>
<td>6.85±0.77</td>
<td>0.91±0.04</td>
<td>Rowe et al. (1999)</td>
</tr>
<tr>
<td>Lamb (2)</td>
<td>68.96</td>
<td>23.39</td>
<td>4.56</td>
<td>1.17</td>
<td>Lee et al. (2008)</td>
</tr>
<tr>
<td>Lamb (3)</td>
<td>72.2</td>
<td>20.1</td>
<td>6.2</td>
<td>1.5</td>
<td>Elgasim and Alkanhal (1992)</td>
</tr>
<tr>
<td>Goat (1)</td>
<td>68.32</td>
<td>23.41</td>
<td>4.97</td>
<td>1.73</td>
<td>Lee et al. (2008)</td>
</tr>
<tr>
<td>Goat (2)</td>
<td>74.5</td>
<td>19.8</td>
<td>3.3</td>
<td>1.4</td>
<td>&quot;</td>
</tr>
<tr>
<td>Camel</td>
<td>77.2</td>
<td>19.3</td>
<td>2.6</td>
<td>0.9</td>
<td>&quot;</td>
</tr>
<tr>
<td>Fish</td>
<td>78.7</td>
<td>17.8</td>
<td>2.6</td>
<td>0.9</td>
<td>&quot;</td>
</tr>
<tr>
<td>Chicken (1)</td>
<td>77.49-1.04</td>
<td>18.83±0.09</td>
<td>4.08±0.60</td>
<td>ND</td>
<td>Almeida et al. (2006)</td>
</tr>
<tr>
<td>Chicken (2)</td>
<td>66.03±0.50</td>
<td>28.78±0.34</td>
<td>3.46±0.12</td>
<td>1.68±0.07</td>
<td>Barroeta (2007)</td>
</tr>
<tr>
<td>Chicken (3)</td>
<td>73.2</td>
<td>21.2</td>
<td>5.4</td>
<td>1.3</td>
<td>Elgasim and Alkanhal (1992)</td>
</tr>
<tr>
<td>Australian Mutton bird</td>
<td>ND</td>
<td>23.9</td>
<td>18.7</td>
<td>ND</td>
<td>Woodward et al. (1995)</td>
</tr>
</tbody>
</table>
The crude fat content was higher in MMBM (average 13.01%) compared with beef, lamb, goat, camel, fish and chicken (average 4.7, 6.85, 4.97, 2.6, 2.6 and 5.4%, respectively (Table 3.3) but lower compared with Australian Mutton bird (18.7%). The crude protein content of MBBM (18.49%) was lower than beef [(21.17%; Almeida et al., (2006)], lamb [(19.28%; Rowe et al. (1999)], goat (23.41%), camel (19.3%), chicken (28.78%, Barroeta 2007) and Australian Mutton bird [(23.9%, Woodward et al., (1995)]. But it was higher than in fish [(17.8%; Lee et al., (2008)] in comparison with the MBBM.

![Figure 3.2: Proximate analysis of Mutton bird and other common meats](image)

**3.3.2 Amino acid composition of Mutton bird breast meat**

**3.3.2.1 Amino Acid composition for samples collected in 2007 and 2008**

Mutton bird breast meat contains all eight essential amino acids and nine non-essential amino acids and therefore is an excellent choice for meeting daily protein needs of humans. The amino acid content in MBBM in the two years (2007-2008) is presented in Tables 3.4 and 3.5. The results show no remarkable differences in the individual amino acids over the two-year period. There were no significant differences (\( P > 0.05 \)) in the amino acid compositions of the protein in threonine,
arginine, leucine, lysine and alanine (P = 0.11, 0.08, 0.36, 0.21 and 0.19 respectively) as shown in Table 3.4. Montague et al. (1986) and Ogi et al. (1980) found that the diet from different seas could lead to differences in seabird’s proximate composition since it is well known that plankton species can vary from one ecosystem to another leading to differing dietary input which might explain the slight difference in the protein content found (Table 3.2). There were no differences in the contents of essential and non-essential amino acids over the two years (Table 3.4). However, the impact of the year of harvest was significant (P < 0.05) with higher levels of many of the amino acids in 2008 MBBM (93.6) compared the 2007 level (88.8) when expressed for 100 g protein (Table 3.5).

Table 3.4: A comparison of amino acid composition for Mutton bird breast between years (2007 and 2008) (g/100g fresh weight)

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential Amino Acids</td>
<td>2007</td>
<td>2008</td>
</tr>
<tr>
<td>Leu</td>
<td>1.52±0.36</td>
<td>1.49±1.50</td>
</tr>
<tr>
<td>Lys</td>
<td>1.81±0.21</td>
<td>1.90±1.86</td>
</tr>
<tr>
<td>Met</td>
<td>0.20±0.16</td>
<td>0.17±0.18</td>
</tr>
<tr>
<td>Phe</td>
<td>0.74±0.28</td>
<td>0.77±0.76</td>
</tr>
<tr>
<td>Thr</td>
<td>0.85±0.11</td>
<td>0.93±0.89</td>
</tr>
<tr>
<td>Val</td>
<td>0.84±0.38</td>
<td>0.83±0.84</td>
</tr>
<tr>
<td>Ile</td>
<td>0.82±0.44</td>
<td>0.83±0.83</td>
</tr>
<tr>
<td>His</td>
<td>0.55±0.15</td>
<td>0.59±0.57</td>
</tr>
<tr>
<td>Non Essential Amino Acids</td>
<td>2007</td>
<td>2008</td>
</tr>
<tr>
<td>Arg</td>
<td>1.37±0.08</td>
<td>1.24±1.31</td>
</tr>
<tr>
<td>Ala</td>
<td>1.07±0.19</td>
<td>1.00±1.03</td>
</tr>
<tr>
<td>Asp</td>
<td>1.60±0.40</td>
<td>1.63±1.62</td>
</tr>
<tr>
<td>Cys</td>
<td>0.06±0.30</td>
<td>0.06±0.06</td>
</tr>
<tr>
<td>Glu</td>
<td>2.56±0.43</td>
<td>2.53±2.55</td>
</tr>
<tr>
<td>Gly</td>
<td>0.83±0.47</td>
<td>0.83±0.83</td>
</tr>
<tr>
<td>Pro</td>
<td>0.78±0.48</td>
<td>0.78±0.78</td>
</tr>
<tr>
<td>Ser</td>
<td>0.74±0.38</td>
<td>0.73±0.73</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.56±0.32</td>
<td>0.54±0.55</td>
</tr>
</tbody>
</table>
The dominant essential amino acids in Mutton bird were lysine and leucine (10.06 and 8.15 g/100g protein, respectively) and among the non-essential amino acids relatively high amounts of glutamic and aspartic acids observed which accounted for 14.11 and 9.06% respectively, (Table 3.5).

Table 3.5: A comparison of amino acid composition for Mutton bird breast between years (2007 and 2008) (g/100 g protein).

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential Amino Acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>7.97±0.36</td>
<td>8.26±0.41</td>
</tr>
<tr>
<td>Lys</td>
<td>9.46±0.69a</td>
<td>10.56±0.56b</td>
</tr>
<tr>
<td>Met</td>
<td>0.94±0.43</td>
<td>0.91±0.29</td>
</tr>
<tr>
<td>Phe</td>
<td>3.89±0.35a</td>
<td>4.28±0.43b</td>
</tr>
<tr>
<td>Thr</td>
<td>4.46±0.18a</td>
<td>5.18±0.30b</td>
</tr>
<tr>
<td>Val</td>
<td>4.44±0.15</td>
<td>4.60±0.25</td>
</tr>
<tr>
<td>Ile</td>
<td>4.32±0.25a</td>
<td>4.60±0.35b</td>
</tr>
<tr>
<td>His</td>
<td>2.88±0.08a</td>
<td>3.26±0.14b</td>
</tr>
<tr>
<td>Non Essential Amino Acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>7.28±0.36</td>
<td>6.89±0.50</td>
</tr>
<tr>
<td>Ala</td>
<td>5.58±0.17</td>
<td>5.52±0.82</td>
</tr>
<tr>
<td>Asp</td>
<td>8.40±0.18a</td>
<td>9.06±0.49b</td>
</tr>
<tr>
<td>Cys</td>
<td>0.29±0.15</td>
<td>0.32±0.14</td>
</tr>
<tr>
<td>Glu</td>
<td>13.58±0.65</td>
<td>14.11±0.68</td>
</tr>
<tr>
<td>Gly</td>
<td>4.34±0.11a</td>
<td>4.64±0.32b</td>
</tr>
<tr>
<td>Pro</td>
<td>4.13±0.58</td>
<td>4.37±0.47</td>
</tr>
<tr>
<td>Ser</td>
<td>3.89±0.15</td>
<td>4.04±0.21</td>
</tr>
<tr>
<td>Tyr</td>
<td>2.96±0.17</td>
<td>3.03±0.20</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>88.82±3.06a</strong></td>
<td><strong>93.63±5.13b</strong></td>
</tr>
</tbody>
</table>

Amino acids are important components of meat. The amino acid composition of the MBBM in the present study was compared with that of other red meats (camel, lamb, goat, and beef) and white meats (chicken, Garganey, Pintail, Fish) (Table 3.6). Five essential amino acid contents (threonine, lysine, leucine, methionine and isoleucine) of the MBBM were at the high end of the scale compared to those found in other red and white meats. Similarly, seven non-essential amino acids (glutamine, asparagine, serine, glycine, alanine, proline and arginine) had the same or lower trend compared with other meat sources reported. The level of valine in
other sea birds (Garganey, Pintail) was higher than in Mutton bird. Mutton bird meat had higher tyrosine content than in chicken and fish, but lower tyrosine content than in Pintail and goat meats. The concentration of phenylalanine in pintail, chicken and goat was higher than in the Mutton bird (Table 3.6). The level of amino acids in Mutton bird and other meat sources can be visually compared in Figures 3.3 and 3.4.

Table 3.6: A comparison of the amino acid content of Mutton bird breast meat with other common meats (g/100g of protein)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Mutton bird</th>
<th>Other sea birds</th>
<th>Chicken</th>
<th>Fish</th>
<th>Camel</th>
<th>Beef</th>
<th>Lamb</th>
<th>Goat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Garganey¹</td>
<td>Pintail¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Essential Amino Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>8.16</td>
<td>5.44</td>
<td>5.43</td>
<td>6.14</td>
<td>6.66</td>
<td>7.92</td>
<td>9.34</td>
<td>9.10</td>
</tr>
<tr>
<td>Lys</td>
<td>10.07</td>
<td>6.23</td>
<td>6.98</td>
<td>6.23</td>
<td>9.91</td>
<td>8.39</td>
<td>7.96</td>
<td>8.00</td>
</tr>
<tr>
<td>Met</td>
<td>0.095</td>
<td>2.08</td>
<td>3.44</td>
<td>ND</td>
<td>4.03</td>
<td>3.24</td>
<td>2.30</td>
<td>3.09</td>
</tr>
<tr>
<td>Phe</td>
<td>4.10</td>
<td>5.62</td>
<td>6.31</td>
<td>5.96</td>
<td>3.77</td>
<td>4.43</td>
<td>4.92</td>
<td>4.59</td>
</tr>
<tr>
<td>Thr</td>
<td>4.85</td>
<td>4.97</td>
<td>4.93</td>
<td>3.29</td>
<td>5.15</td>
<td>4.53</td>
<td>4.78</td>
<td>4.00</td>
</tr>
<tr>
<td>Val</td>
<td>4.53</td>
<td>8.54</td>
<td>8.29</td>
<td>3.75</td>
<td>4.78</td>
<td>5.91</td>
<td>5.76</td>
<td>5.53</td>
</tr>
<tr>
<td>Ile</td>
<td>4.46</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>4.24</td>
<td>5.52</td>
<td>5.69</td>
<td>5.53</td>
</tr>
<tr>
<td>His</td>
<td>3.08</td>
<td>2.57</td>
<td>3.83</td>
<td>2.95</td>
<td>2.66</td>
<td>5.27</td>
<td>5.41</td>
<td>5.58</td>
</tr>
<tr>
<td>Non essential Amino Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>7.13</td>
<td>5.75</td>
<td>5.45</td>
<td>4.98</td>
<td>6.55</td>
<td>7.10</td>
<td>7.05</td>
<td>6.85</td>
</tr>
<tr>
<td>Ala</td>
<td>5.58</td>
<td>6.88</td>
<td>5.67</td>
<td>4.34</td>
<td>4.93</td>
<td>3.85</td>
<td>7.74</td>
<td>6.73</td>
</tr>
<tr>
<td>Asp</td>
<td>8.75</td>
<td>8.47</td>
<td>7.28</td>
<td>6.80</td>
<td>10.16</td>
<td>10.8</td>
<td>10.8</td>
<td>10.3</td>
</tr>
<tr>
<td>Cys</td>
<td>0.47</td>
<td>1.28</td>
<td>0.88</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Glu</td>
<td>13.95</td>
<td>14.86</td>
<td>15.81</td>
<td>11.25</td>
<td>15.04</td>
<td>18.6</td>
<td>16.5</td>
<td>17.9</td>
</tr>
<tr>
<td>Gly</td>
<td>4.50</td>
<td>4.92</td>
<td>5.93</td>
<td>3.13</td>
<td>4.78</td>
<td>6.11</td>
<td>6.23</td>
<td>5.49</td>
</tr>
<tr>
<td>Pro</td>
<td>4.27</td>
<td>3.25</td>
<td>2.23</td>
<td>3.38</td>
<td>1.91</td>
<td>3.87</td>
<td>4.54</td>
<td>3.81</td>
</tr>
<tr>
<td>Ser</td>
<td>3.96</td>
<td>4.78</td>
<td>3.58</td>
<td>2.92</td>
<td>3.58</td>
<td>3.18</td>
<td>4.22</td>
<td>2.98</td>
</tr>
<tr>
<td>Tyr</td>
<td>3.02</td>
<td>3.86</td>
<td>4.91</td>
<td>1.95</td>
<td>2.85</td>
<td>3.81</td>
<td>4.10</td>
<td>3.51</td>
</tr>
<tr>
<td>Total</td>
<td>ND</td>
<td>93.09</td>
<td>94.15</td>
<td>ND</td>
<td>ND</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Protein%</td>
<td>91.67</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>86.7</td>
<td>86.5</td>
<td>90.8</td>
</tr>
</tbody>
</table>

See text for description of the amino acids. ¹Khalifa and Nassar (2001); ²Elgasim and Alkanhal (1992); ³Mavromichalis et al. (2000); ⁴Rowe et al. (1999); ⁵González et al. (2007).

The main source for dietary protein of Mutton bird is krill which have a proximate composition of 60–80% protein, 7–26% lipid, and 12–17% ash on a dry weight basis (Grantham, 1977). The protein derived from krill is considered of high quality based on chemical analysis showing krill protein contains all the nine essential amino acids (Gigliotti et al., 2008). However, some authors suggested...
decreased digestibility of krill protein which may be related to the presence of the unavailable exoskeleton protein (Ikegamie et al., 1990).

![Figure 3.3: Amino acids for Mutton bird and other common white meats](image)

![Figure 3.4: Amino acids in Mutton bird breast meat and common red meats](image)

### 3.3.3 Fatty acid composition

The annual variations of fatty acid profile in Mutton bird breast meat for two years (2007 and 2008) are shown in Table 3.7. Generally, there were few significant variations in the content of saturated and monounsaturated fatty acids (MUFA) over the two years of sampling. Individually, no differences ($P > 0.05$) in the levels of the following fatty acids, C 12:0, C 16:0, C 17:0, C 18:0, C18:3(ω3), C18:3 (ω6) and C20:4(ω6) were found. The most relevant changes were detected in the concentration of MUFA. Significant differences were found in C14:1, C 18:1ω6, C
20:1 and C 24:1(ω9) \((P < 0.05)\). However, there was no significant variation \((P > 0.05)\) in the total monounsaturated fatty acid \((\text{MUFA})\) content over the two years sampling period. The percentages of PUFA in 2007 were found to be not different from those found in 2008 (Table 3.7). A higher \(\omega3/\omega6\) was found in 2007 NZMBBM samples compared to 2008.

### Table 3.7: Fatty acid compositions of Mutton birds’ breast for two years (2007 & 2008)

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>14:0</td>
<td>1.31 ± 0.18</td>
<td>1.32 ± 0.18</td>
</tr>
<tr>
<td>15:0</td>
<td>0.18 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.21 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>16:0</td>
<td>19.60 ± 0.78</td>
<td>19.13 ± 0.96</td>
</tr>
<tr>
<td>17:0</td>
<td>0.30 ± 0.05</td>
<td>0.44 ± 0.45</td>
</tr>
<tr>
<td>18:0</td>
<td>9.77 ± 1.30</td>
<td>9.99 ± 1.18</td>
</tr>
<tr>
<td>20:0</td>
<td>0.22 ± 0.03</td>
<td>0.26 ± 0.33</td>
</tr>
<tr>
<td>14:1</td>
<td>0.08 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.11 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>16:1</td>
<td>5.46 ± 1.35</td>
<td>5.28 ± 2.36</td>
</tr>
<tr>
<td>17:1</td>
<td>0.28 ± 0.08</td>
<td>0.30 ± 0.03</td>
</tr>
<tr>
<td>18:1(ω9)</td>
<td>34.03 ± 0.85</td>
<td>33.73 ± 0.82</td>
</tr>
<tr>
<td>18:1(ω6)</td>
<td>4.44 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.73 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>18:1(ω11)</td>
<td>0.53 ± 0.06</td>
<td>0.63 ± 0.28</td>
</tr>
<tr>
<td>20:1</td>
<td>7.01 ± 0.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.71 ± 1.54&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>22:1</td>
<td>1.07 ± 0.28</td>
<td>1.10 ± 0.16</td>
</tr>
<tr>
<td>24:1(ω9)</td>
<td>0.52 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.61 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>18:2</td>
<td>2.10 ± 0.15</td>
<td>1.80 ± 0.65</td>
</tr>
<tr>
<td>18:3(ω3)</td>
<td>0.18 ± 0.02</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>18:3(ω6)</td>
<td>0.08 ± 0.03</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>20:4(ω6)</td>
<td>0.79 ± 0.17</td>
<td>0.79 ± 0.17</td>
</tr>
<tr>
<td>20:5(ω3){EPA}</td>
<td>1.40 ± 0.29</td>
<td>1.46 ± 0.37</td>
</tr>
<tr>
<td>22:5(ω3){DHA}</td>
<td>0.26 ± 0.15</td>
<td>0.24 ± 0.14</td>
</tr>
<tr>
<td>22:6(ω3){DHA}</td>
<td>2.15 ± 0.51</td>
<td>2.03 ± 0.45</td>
</tr>
<tr>
<td>Others</td>
<td>8.28 ± 1.83</td>
<td>9.87 ± 2.83</td>
</tr>
<tr>
<td>SFA</td>
<td>31.39 ± 0.83</td>
<td>31.36 ± 1.04</td>
</tr>
<tr>
<td>MUFA</td>
<td>53.43 ± 1.94</td>
<td>52.20 ± 3.07</td>
</tr>
<tr>
<td>PUFA</td>
<td>6.91 ± 0.96</td>
<td>6.57 ± 1.37</td>
</tr>
<tr>
<td>SFA/PUFA</td>
<td>4.63 ± 0.69</td>
<td>5.02 ± 1.30</td>
</tr>
<tr>
<td>ω3</td>
<td>4.00 ± 0.88</td>
<td>3.90 ± 0.84</td>
</tr>
<tr>
<td>ω6</td>
<td>0.71 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.86 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ω3/ω6</td>
<td>5.68 ± 1.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.56 ± 0.61&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>A-b Mean values in each row bearing a common superscript letter are not significantly different \((p>0.05)\)</sup>
As stated previously, it was observed that the proportion of some fatty acids changed significantly over the two years (2007-2008) of sampling. Great variations were reported in the fat content and fatty acid composition of several marine organisms during a year (Gockse et al., 2004; Luzia et al., 2003; Shirai et al., 2002). It is important to investigate the influence of annual variation on the fat content and the fatty acid composition of the Mutton bird in order to estimate the impact of annual variation on n-3 fatty acids available from Mutton birds.

Mutton birds as marine animals are subjected to considerable environmental changes and fluctuations in the availability and compositions of their feed, which may affect the chemical composition of their muscles such as fatty acid profile. Furthermore, sea water temperature differences across the seasons which Mutton birds feed (e.g. during the migration season) may have an influence on the fatty acid composition of Mutton bird meat. The annual influence on the MBBM during (2007-2008) from the same geographic area is common and is probably affected by the different genetic cycle of the fish species. Diet, location and season are the major factors affecting the fatty acid composition (Gruger, 1967; chapter. 1) which may affect the nutritional and health status of chicks. Annual changes in water temperature and nutrients are the major factors affecting composition of fish muscle and the nutrient composition has a major being on feeding the chicks.

Seafood is generally the main source of n-3 PUFA in the human diet. For example, lipids from marine fish species are characterized by high levels of long-chain n-3 PUFA (Steffens, 1997). Mutton bird is a sea bird surviving on small marine organisms such as fish and krill. It is hypothesised that they would have high content of n-3 PUFA. However, this does not seem to be the case, the fatty acid profiles of Mutton bird pectoral muscle and other common white and red meats are
shown in Table 3.8. The fatty acid composition of Mutton bird were found to be 31.38\% saturated (SFA), 52.82\% MUFA and 6.74\% PUFA. The results of the fatty acid composition shows that Mutton bird breast meat is very rich in MUFA which was higher than in other common meats as shown in Figure 3.5 such as chick (34.31), lamb (31.37), beef (21.94) and fish (23.70) but it was lower than Australian Mutton bird (60.9) (Figure 3.5). Individually, the highest proportions of the fatty acids in Mutton bird muscle were C18:1ω9 (oleic acid) as a monounsaturated omega-9 fatty acid was 33.88\%. It is the highest amount in comparison with other common meats (lamb 30.3) followed by chicken 28.72, beef 16.52) and fish (9.83).

### Table 3.8: Comparison of Mutton bird fatty acid composition breast meat with other common meats

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>NZ Mutton bird</th>
<th>Chicken</th>
<th>Other seabirds</th>
<th>Lamb</th>
<th>Beef</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Arctic Fulmar</td>
<td>Australian Mutton bird</td>
<td>Previous Mutton bird</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:0</td>
<td>0.02±0.01</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.8</td>
</tr>
<tr>
<td>14:0</td>
<td>1.32±0.18</td>
<td>0.55</td>
<td>2.46</td>
<td>2.5</td>
<td>2.13</td>
<td>1.75</td>
</tr>
<tr>
<td>15:0</td>
<td>0.20±0.03</td>
<td>ND</td>
<td>ND</td>
<td>0.3</td>
<td>0.35</td>
<td>0.86-</td>
</tr>
<tr>
<td>16:0</td>
<td>19.37±0.8</td>
<td>23.02</td>
<td>9.55</td>
<td>18.1</td>
<td>23.8</td>
<td>19.23</td>
</tr>
<tr>
<td>17:0</td>
<td>0.37±0.25</td>
<td>17.21</td>
<td>0.28</td>
<td>0.2</td>
<td>0.03</td>
<td>2.12</td>
</tr>
<tr>
<td>18:0</td>
<td>9.88±1.24</td>
<td>10.06</td>
<td>3.00</td>
<td>5.1</td>
<td>7.02</td>
<td>30.11</td>
</tr>
<tr>
<td>20:0</td>
<td>0.24±0.18</td>
<td>ND</td>
<td>0.25</td>
<td>0.3</td>
<td>0.35</td>
<td>0.47</td>
</tr>
<tr>
<td>14:1</td>
<td>0.10±0.03</td>
<td>ND</td>
<td>ND</td>
<td>0.2</td>
<td>ND</td>
<td>2.2</td>
</tr>
<tr>
<td>16:1</td>
<td>5.37±1.86</td>
<td>3.61</td>
<td>ND</td>
<td>8.0</td>
<td>8.35</td>
<td>2.19</td>
</tr>
<tr>
<td>17:1</td>
<td>0.29±0.06</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.68</td>
</tr>
<tr>
<td>18:1ω9</td>
<td>33.88±0.8</td>
<td>28.72</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>30.3</td>
</tr>
<tr>
<td>18:1ω6</td>
<td>4.59±0.26</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>16.52</td>
</tr>
<tr>
<td>18:1ω11</td>
<td>0.58±0.17</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>9.83</td>
</tr>
<tr>
<td>20:1</td>
<td>6.36±1.18</td>
<td>0.79</td>
<td>ND</td>
<td>8.8</td>
<td>7.17</td>
<td>ND</td>
</tr>
<tr>
<td>22:1</td>
<td>1.09±0.22</td>
<td>ND</td>
<td>ND</td>
<td>6.6</td>
<td>1.03</td>
<td>ND</td>
</tr>
<tr>
<td>24:1ω9</td>
<td>0.57±0.12</td>
<td>ND</td>
<td>ND</td>
<td>0.6</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>18:2</td>
<td>1.95±0.4</td>
<td>17.02</td>
<td>ND</td>
<td>2.3</td>
<td>ND</td>
<td>20.0</td>
</tr>
<tr>
<td>Fatty Acid</td>
<td>18:3(ω3)</td>
<td>18:3(ω6)</td>
<td>20:4(ω6)</td>
<td>20:5(ω3){EPA}</td>
<td>22:5(ω3){DPA}</td>
<td>22:6(ω3){DHA}</td>
</tr>
<tr>
<td>------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td></td>
<td>0.18±0.02</td>
<td>0.04±0.03</td>
<td>0.79±0.17</td>
<td>1.43±0.33</td>
<td>0.25±0.15</td>
<td>2.09±0.48</td>
</tr>
<tr>
<td></td>
<td>2.03</td>
<td>0.21</td>
<td>2.15</td>
<td>0.47</td>
<td>0.47</td>
<td>2.14</td>
</tr>
<tr>
<td></td>
<td>0.53</td>
<td>ND</td>
<td>0.33</td>
<td>1.26</td>
<td>4.77</td>
<td>19.96</td>
</tr>
<tr>
<td></td>
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<td>ND</td>
<td>0.8</td>
<td>ND</td>
<td>ND</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>1.92</td>
<td>ND</td>
<td>0.7</td>
<td>ND</td>
<td>5.2</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>1.19</td>
<td>0.02</td>
<td>1.09</td>
<td>0.6</td>
<td>0.30</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>0.11</td>
<td>0.63</td>
<td>1.22</td>
<td>0.84</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>ND</td>
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<td></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

1= Woodward, et al. (1995); 2=Wang, et al. (2007); 3= Quigley et al. (1995); 4= Rowe et al. (1999); 5=González et al. (2007); 6= Costa et al. (2007); 7= Jahan et al. (2004).

Mutton bird pectoral muscle contained higher level of unsaturated fatty acids compared with lamb (5.36). However, Mutton bird pectoral muscle was low compared with fish (53.17) followed by beef (44.98%) and chicken (31.85%). It has been reported that n-3 PUFAs have various beneficial health effects that include reducing the risk of cardiovascular disease (Gigliotti et al., 2008). Among the PUFA, EPA (C20:5 n-3) and DHA (C22:6 n-3) are the dominant n-3 fatty acids in Mutton birds. These fatty acids are of great importance to humans because they have been shown to prevent the coronary heart disease (Conner, 2000; Mozaffarian et al., 2005). The fatty acid composition of marine organisms (fish) reflects the fatty acid composition of their natural foods (Grigorakis et al., 2002; Van et al., 1991). General recommendations for daily dietary intakes of DHA or EPA are 0.5 g for infants and 1 g/day for adults (Kris-Etherton et al. 2002).
Important nutritionally-essential \( n-3 \) fatty acids such as C18:2 (\( \alpha \)-linolenic acid (ALA)) was 2.10\% lower than chicken (17.02), beef (20.0) and Australian Mutton bird (2.3). EPA (C 20:5(n-3) was 1.43\%, higher than in other common meats (chicken (0.75), beef (1.22) and lamb (0.93). It is lower than in another sea bird (Arctic Fulmar) (1.95) and Australian Mutton bird (3.3). DHA C22:6(\( \omega \)3) is 2.09\% which was higher than lamb (0.30) and beef (0.07), but low compared to fish (30.84), Australian Mutton bird (5.2), Arctic Fulmar (4.77) and Chicken (2.14). DPA C22:5(\( \omega \)3) was 0.25\%, lower than in other common meats (chicken (0.47), Arctic Fulmar (1.26), and Australian Mutton bird (0.6), lamb (0.84), beef (1.81) and fish (1.29).

Saturated fatty acids in Mutton bird muscle were lower than in chicken (40.79\%), lamb (55.07\%) and beef (32.33\%), but it was higher compared with Australian Mutton bird (26.5\%) and fish (25.15\%). Among the saturated fatty acids, C 16:0 (palmitic acid) was the most abundant fatty acids in the lipid extracted from Mutton bird (19.37\%). Other meats had similar average values, e.g. Australian Mutton bird (18.1), beef (18.27\%), lamb (19.23\%) and fish (19.34\%), but lower than in chicken (23.03\%) and an earlier study on NZ Mutton bird (23.8). Mutton bird meat had higher C 16:1 (Palmitoleic Acid) at 5.37\% than in other meats (chicken (3.61), lamb (2.19) and beef (0.31). But, it was lower compared to a previous NZ MB (8.35) and Australian Mutton bird (8.0). SFA/PUFA in Mutton bird muscle was 4.83\%, a quite high compared with other common meats (chicken (0.92), lamb (0.10), beef (0.98) and fish (0.48).

Total \( n-3 \) fatty acids in Mutton bird meat were 3.95\%, lower than in chicken (4.94) and Australian Mutton bird (9.3). Also, the \( n-6 \) in Mutton bird muscle was 0.79, low compared with chicken (19.96) and Australian Mutton bird (3.3). The
interesting result of the comparison of Mutton bird meat with white meats (chicken and fish) is n-3/n-6 ratio in Mutton bird was (5.12), higher ten times than in chicken (0.50) but lower than in fish (17.96). The high n-3/n-6 ratio for MBBM in the present study was expected because of high seafood diet. Considering the Mutton bird muscle as a human food, the previous differences seen would be likely to provide an altered nutritional value. Since people nowadays have been paying particular attention to their diet and seek to choose healthy foods in their dieting regimes. Mutton bird meat which is rich in unsaturated fatty acids ranks first among these foods. MUFA of the long-chain unsaturated fatty acids contained in Mutton bird and n-3 fatty acids are the most important in terms of nutrition physiology. It has been found that n-3 fatty acids have a protective effect on heart and tissue related diseases in particular, with beneficial effects on hypertension, diabetes, brain growth and infant cancer (Simopoulos, 1991; Tapiero et al., 2002; Sidhu, 2003; Mahaffey, 2004). An increase in the human dietary n-3/n-3 fatty acid ratio is essential to help prevent coronary heart disease by reducing plasma lipids (Gökc et al., 2004). Therefore, Mutton bird beast meat rich in n-3 MUFA could be consumed as a source of n-3 fatty acids.

The beneficial effect of fish consumption on human health has been related, among other factors to the high content of n-3 fatty acids, particularly, EPA (C20:5n-3) and DHA (C22:6n-3). Results of clinical and epidemiological research suggest that EPA and DHA, found in fish and sea foods, possess extremely beneficial properties such as prevention of human coronary artery disease (Leaf and Weber, 1988). The n-3/n-6 ratio is a good index for comparing relative nutritional value. The present study indicates it is a good source in terms of n-3/n-6. It was observed that marine animals such as fish may need PUFA to provide adaptation to lower
water temperatures. This is the reason why cold sea fish are rich in n-3 fatty acids (Chanmugam et al., 1986).

![Figure 3.5: The fatty acid contents in different common red and white meats.](image)

### 3.3.4 Cholesterol content

The mean and standard deviation of cholesterol content in Mutton bird muscle is shown in Table 3.9. The average cholesterol level in Mutton bird was 184 mg/100g fresh meat in 2007 and 134 mg/100g fresh meat in 2008. The differences between the samples during two years are significant ($p=0.05$). Changes in the cholesterol composition of Mutton bird over the two years might have resulted from environmental factors. The magnitude of variation of the Mutton bird muscle cholesterol content between 2007 and 2008 appears to be high ($\approx 27\%$). Differences between their sexes and various feeding might be the most likely reason for some of the variations observed in the cholesterol contents. Cholesterol content in marine animal (e.g. fish) is influenced by several factors, among which the PUFA content plays an important role. According to Kinsella (1986), an increase in PUFA content is associated with a decrease of cholesterol content (Othman et al., 2000).
Table 3.9: Mean and standard deviation \((n=20)\) for cholesterol content of Mutton Bird’s breast meat mg/100 fresh weight for years 2007 and 2008.

<table>
<thead>
<tr>
<th>Year</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>184.4±37.37</td>
</tr>
<tr>
<td>2008</td>
<td>134.4±25.55</td>
</tr>
</tbody>
</table>

These differences of cholesterol concentrations in Mutton bird meat are probably due to the free-ranging animals living in different locations and eating different diets.

Table 3.10: Comparison of cholesterol content in Mutton bird meat with other common meats

<table>
<thead>
<tr>
<th>Species</th>
<th>Amounts (mg/100g)</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamb</td>
<td>62.03</td>
<td>Rowe et al (1999)</td>
</tr>
<tr>
<td>Beef</td>
<td>51.97</td>
<td>Rowe et al (1999)</td>
</tr>
<tr>
<td>Chicken</td>
<td>80.3</td>
<td>De Almeida et al (2006)</td>
</tr>
<tr>
<td>Chicken (2)</td>
<td>88.9</td>
<td>Barroeta (2007)</td>
</tr>
<tr>
<td>Fish</td>
<td>52.79</td>
<td>Moreira et al (2001)</td>
</tr>
<tr>
<td>NZ Mutton bird (2007)</td>
<td>184.4</td>
<td>the present study</td>
</tr>
<tr>
<td>NZ Mutton bird (2008)</td>
<td>134.4</td>
<td>the present study</td>
</tr>
</tbody>
</table>

Comparison of the cholesterol content in Mutton bird meat with other common meat is shown in Table 3.11. Meat and meat products are one of the main sources of cholesterol in the diet and they have been implicated in the development of coronary heart disease. Mutton bird was shown to contain significantly higher cholesterol contents (134.4-184.4) than other common meat such as chicken (80.3-88.9), lamb (62.3), fish (52.79) and beef (51.97). However, as shown in Figure 3.6, the cholesterol content of Mutton bird muscle in 2007 was similar to that reported for Australian Mutton Bird. Decreasing saturated fatty acid intake will reduce cholesterol intakes as it has been found in many studies (King and Gibney, 2003). In the USA, the recommendations for dietary cholesterol intake should be less than 300 mg/d (Piironen et al., 2002). As dietary cholesterol is known to affect serum
cholesterol (Mathew et al., 1999) information on the daily dietary intake of cholesterol can be quite important, especially to those with cardiovascular problems. The high values obtained for cholesterol content in Mutton bird meat (184 mg/100g) indicate that those with heart problems need to be aware of the level of cholesterol in this product. A large serve (200g of cholesterol) would present on the daily recommended limit for cholesterol.

![Figure 3.6: Comparison of cholesterol content of Mutton bird muscle with other common white and red meats.](image)

Overall, although, Mutton bird breast meat has a low crude protein compared with other meats, it contains all seventeen essential and non-essential amino acids that are required by humans particularly threonine, lysine, leucine, methionine and isoleucine which were very high as observed in the present study. In addition other non essential amino acids were found. It is considered that MBBM is a good source of n-3 PUFA for human diet. Moreover, it is rich in MUFA but poor in saturated fatty acids compared with other common meats. However, Mutton bird has a high cholesterol content that may affect some people especially those suffering from heart diseases.
Chapter 4
Toxicological studies on Mutton bird

4.1 Introduction

Human beings may be exposed to contaminants both of biological and chemical origin (Lievaart et al., 2005). In recent years, concerns have been raised about the bioaccumulation and magnification of persistent organic pollutants in fish and sea birds (Hites et al., 2004; Brambilla et al., 2008). Among the migratory seabirds are the genus *Puffinus* that includes Great Shearwater *Puffinus gravis*, Sooty Shearwater *Puffinus griseus* and Manx Shearwater *Puffinus puffinus* (Belton, 2000; Petry et al., 2007). The Sooty Shearwater (*Puffinus griseus*) is a medium-sized seabird that breeds in burrows on islands in South America, Australia and New Zealand. It is the most common Procellariiform seabird in south-eastern South American and New Zealand (Reyes-Arriagada et al., 2007). Sooty Shearwaters occur along the coast of New Zealand in a large number of colonies on surrounding islands (Hamilton et al., 1997). The species nests in New Zealand, Tasmania, the Falklands and islands surrounding Tierra del Fuego from November to April and carries out trans-equatorial migrations outside this period (Cooper et al., 1991; Hamilton et al., 1997) and migrates to the northern hemisphere during the southern winter (Cooper et al., 1991; Spear and Ainley, 1999).

The feeding habits and diet of Sooty Shearwater have been the focus of much research in New Zealand (Kitson et al., 2000; Cruz et al., 2001) during migration through the Northern Hemisphere (Marchant and Higgins, 1990, Shiomi and Ogi, 1992, Brown et al., 1981) with the aim of estimating food intake and its significance to reproduction. However, the role of their diet is of utmost importance since the accumulation of contaminants during their migration may
affect the health of the birds and their offsprings. Several substances obtained through diet, such as dioxins, mycotoxins, heavy metals, pesticides and polycyclic aromatic hydrocarbons, can impose negative biological effects on marine birds if they are in their diet. These compounds can be divided into three major classes: (1) compounds rapidly metabolized and excreted (e.g. chlorpyrifos); (2) compounds with detectable accumulation in the animal, (e.g. lindane); and (3) compounds with excessive accumulation due to minimal biotransformation in the animal, (e.g. DDT) (Kan and Meijer, 2007).

Heavy metals and pesticides such as DDT are the most common contaminants studied in marine birds. The use of persistent chlorinated pesticides such as DDT and dieldrin was common in the 1970s but is now prohibited or severely restricted in many countries (Barrie et al., 1992). Despite regulatory measures taken in the 1970s to reduce discharges of Hg and organochlorine compounds into the environment, organochlorine and metal contamination continue to be a problem in some areas due to their widespread distribution and extreme persistence (Jacknow et al., 1986; Braune et al., 1991). Contaminants, such as metals and metalloids, reach oceans through natural erosion, geochemical cycles and human activity. Once in the marine environment, they can enter the food web where bioaccumulation and/or biomagnification can occur. Top predators, such as marine birds, are particularly exposed to high levels of these trace elements through their food (Bearhop et al., 2000). Because of their trophic position and bioaccumulation capacities, seabirds are used as bioindicators for elemental exposure in the marine environment (Monteiro and Furness 1995; Gray 2002).

Pesticide residues in foods of animal origin which have been of greatest public and regulatory concern have been insecticides and fungicides (Waltner-Toews and
McEwen, 1994). These compounds can be classified on the basis of their chemical structure or the action they perform (Cantoni and Comi, 1997). Persistent organic chlorine compounds such as DDT have been implicated in chronic poisoning and shown to induce part in a number of pathological processes (Lembowicz et al., 1991; Sitarska et al., 1991). DDT and its metabolites are ubiquitous in the environment and are suspected to be contributing to adverse effects on the environment and human health. Exposure via inhalation or dermal contact has been considered to be negligible due to its low concentration in the atmosphere (U.S. Agency for Toxic Substance and Disease Registry, 2002) leaving diet as the major route for toxicity.

The widespread presence of organochlorine pesticide residues (OCPs) in food has arisen from their extensive agricultural application and industrial emission in the environment. One reason for the environmental persistence of these compounds is that microorganisms are either unable to degrade them or do so very slowly (Alexander, 1981). It has been estimated that over 90% of the DDT stored in the general population is derived from food, particularly fatty food of animal origin (meat, fish and dairy products) (WHO, 2004; WHO Regional Office for Europe, 2003). Fat solubility of those compounds is responsible for their varied concentrations in the tissues and their accumulation in the lipoproteins of the cell membranes thus changing their structure and permeability (Chowdhury et al., 1990; Antunes-Madeira et al., 1993). Many toxic organo pollutants such as lindane and heptachlor persist in the environment and tend to accumulate in the body fat of animals occupying a higher trophic level (Addison et al., 1984). Since trace elements and OCPs can potentially accumulate in Mutton bird as it is on the top of the marine food chain, the present study investigated the heavy metals and DDTs
concentrations in Mutton bird. Other minerals were also analysed as an overall part of the nutritional aspects of this meat.

4.2 Materials and methods

4.2.1 Determination of elements in Mutton bird pectoral muscle

The concentrations of 20 minerals (Cu, Zn, Fe, Mg, Mn, B, Co, Cr, Na, K, Li, S, P, Se, Pb, Cd, As, Ni, Al, Hg) in the pectoral muscles of Mutton bird were determined using AXIAL Varian 720 OES Series ICP (Inductively Coupled Plasma) Emission Spectrometer, after microwave digestion with nitric acid in a PTFE (Teflon) vessel (Okamoto, 1994). Mixed multi-element standard solution was prepared from 10 µg/g stock solutions.

Preparation and digestion of samples

Breast tissue samples were dried and then prepared using a microwave assisted acid digest method. A 0.5 g sample of dried Mutton bird breast was dissolved in 6 mL of HNO₃ (70%) and 2 mL of H₂O₂ (30%), and digested in a microwave digestion system (ETHOS SEL/ plus, Milestone S.r.l., Sorisole, Italy). Digestion conditions applied were 2 min at 250 W, 2 min at 0 W, 6 min at 250 W, 5 min at 400 W, 8 min at 550 W, then venting for 8 min. The digested solution was filtered with filter paper (Whatman No. 5) and made up to volume of 25 ml with deionised water. Blanks were carried out in the same way for background values to make sure the whole digestion process was correct. Fish protein certified reference material (DORM-3, National Research Council Canada) was digested under the same conditions and used as reference standard. The solutions were then analysed using a Varian ICP-OES 720.
Detection and quantification

AXIAL Varian 720 OES Series ICP (Inductively with a SP3 Auto Sampler (Varian, Inc. Palo Alto, USA) was used for element detection and quantification. Concentration of each element in the sample was detected by the absorption wavelengths (nm), the following absorption lines were used: sodium 589.592, potassium 766.491, calcium 317.933, magnesium 279.553, phosphorus 213.618, sulphur 181.972, iron 238.204, copper 327.395, zinc 213.857, manganese 257.610, aluminium 167.019, arsenic 188.980, cadmium 214.439, boron 249.772, cobalt 238.892, chromium 267.716, nickel 231.604, and lead 220.353.

Metal concentrations were calculated by comparing with a standard calibration curve which was generated by plotting the intensity of peak against known concentrations of standard. The limits of detection for Na, K, Ca, Mg, P, S, Fe, Cu, Zn, Mn, Al, As, B, Cd, Co, Cr, Ni and Pb were 1.547, 12.239, 0.305, 0.374, 0.108, 0.085, 0.413, 0.960, 213.857, 0.117, 0.77, 1.504, 1.224, 0.258, 0.678, 0.672, 0.810, 4.109 ppb (μg/kg), respectively. The results were expressed as mg/g and/or mg/kg Mutton bird muscle weight and as mean values ± standard deviation.

4.2.2 Determination of Hg in Mutton bird muscle

The Hg determination was carried out according to the method described by Cominos et al (2001).

Materials

Hydrochloric acid (HCL) 30%, nitric acid (HNO₃) 65%, sodium hydroxide (Na OH) pellets, amidosulfonic acid (WGKI), stock mercury standard solution (1000
µg/ml) in 0.5 M HNO₃ and sodium borohydride (Na BH₄) 98%. Then HCL acid solution, 5 M, Na BH₄ solution (2.0 g Na BH₄ and 2.5 g Na OH were dissolved in 500 ml deionized water) freshly was prepared. The amidosulfonic acid solution 10% w/v in deionized water and mercury standard working solution, 10 µg/µl in 0.5 M HNO₃ were also prepared.

**Preparation of standards**

Aqueous standards for analysis were prepared by dispensing 0, 50, 100, 150, 200 and 250 µg/µl standard working solution into six quartz decomposition vessels individually. A 2 ml concentrated HNO₃ and 0.5 ml concentrated HCL were added to each vessel. The standards were digested in the microwave and diluted with deionised water to 50 ml volume to give 0, 10, 20, 30, 40 and 50 µg/ml final calibration solutions.

**Preparation of samples**

A 500 mg of dried Mutton bird breast muscle was placed into a quartz decomposition vessel and 2ml concentrated HNO₃ and 0.5 ml concentrated HCL were added. The sample was digested in the HTS microwave devices and diluted with deionised water to 50 ml volume to give 0, 10, 20, 30, 40 and 50 µg/ml final calibration solutions.

**Protocol of microwave digestion:**

The decomposition vessels were tightly plugged with a special seal. These were placed in bomb jackets which in turn were hand tightly sealed by a screw cap. The bomb jackets with screw cap were placed in protected jackets and into the microwave oven. The door of the microwave oven was closed and the power selector switch set to 8 units and the decomposition time to 10 min. The “low
intensity“ time of the exhaust device was set to 10 min equal to the “decomposition” time of the microwave oven for the escaping fumes to be sucked away. The “high Intensity” time of the exhaust device was set also to 10 min to cool the bomb jacket. The digests (standards and samples) were diluted with deionized water to 10 ml volume and then 2 ml of 10% w/v amidosulfonic acid solution was added dropwise to deactivate NO\(_2\) fumes. The aqueous standards and tissue digests were diluted to the respective final volumes (50 ml) with deionized water.

**Determination of Hg by HVG/AAS**

The Hg was analyzed using the Hydride Vapour Generator (HVG) attached with Atomic Absorption Spectrophotometer (AAS). The sample solution, HCl and NaBH\(_4\) were fed with a peristaltic pump into a manifold. The Hg was vaporized as free Hg into a reaction coil. The Hg vapour was swept by the inert carrier gas to a gas/liquid separator. The liquid phase was drained and the gas phase containing the mercury vapour was swept into an absorption cell which afforded the measurement of Hg without heating.

**4.3 DDTs analysis**

These studies were carried out by me in the laboratory of Dr Khalid Othman, Al-Qassim University, Al-Qassim, Saudi Arabia during my holidays in December 2007 and in February 2009.

**Chemicals and materials**

Analytical grade standards of organochlorine compounds, p,p'-DDT (100%), p,p'-DDD (99%), p,p'-DDE (99.47%), p,p'-DDA (98%), lindane (95.10%),
methoxychlor (99.10%), aldrin (99.40%), chlordane (99.90%) and dicofol (88%) were obtained from Environmental Protection Agency (EPA, NC, USA). Water SPE-20G Column Processor designed vacuum manifold capable of processing up to 20 solid phase extraction columns was purchased from Waters, USA, while solid phase extraction column (SPE), aminopropyl (NH$_2$) cartridge (Waters SPE™, C18, 500 mg per column) was purchased from Supleco, USA. Acetonitrile, toluene, acetone and hexane of HPLC grades were purchased from BDH Company. Sodium chloride and anhydrous sodium sulfate were of analytical grade. Ultra-pure deionized water of 15 MΩ cm resistivity was obtained from a water purification system (PURELAB Option-R, ELGA, UK) and used throughout this study. All other chemicals used in this study were of the highest grade available.

**Calibration curves**

Individual stock solutions of standards were prepared by dissolving 10 mg of each compound in 10 ml hexane. DDA was dissolved first in 1 ml acetone and then completed with 9 ml hexane. A mixed standard solution was prepared from the individual stock solutions with concentrations of 100 mg/L. Working solutions were prepared by adding proper amount of 100 mg/L of the mixed standard solution to give a final concentration of 0.1, 0.2, 0.5, 1.0 and 2.0 mg/L.

**Sample preparation**

Samples were extracted according to the method of Chen *et al.* (2009) with some modifications. Three grams of dried Mutton bird breast muscle sample was placed in 75 ml amber bottle and mixed vigorously for 1 min with 3 ml of ultra pure water. A 20 ml portion of acetonitrile was added and shaken vigorously for 15 min. A 5 g of NaCl was added and shaken for 2 min, and then the mixture was shaken
for 24 hrs at room temperature. The mixture was filtrated under vacuum through porcelain funnel and the filtrate was chilled at -20°C for 20 min to freeze lipid. The cold extract was immediately filtered through filter paper to remove frozen lipids and the precipitated lipids were re-dissolved in 10 ml acetonitrile and filtered again. The filtrate was evaporated to dryness by gentle stream of nitrogen and then re-dissolved in 1 ml of acetonitrile-toluene (3:1) prior to solid phase extraction.

**Clean-up by NH$_2$ SPE**

Anhydrous sodium sulphate (ca 1cm) was placed on the top of NH$_2$ SPE cartridge. The cartridge was conditioned with 10 ml acetonitrile-toluene (3:1) and then the concentrated extract was added to the cartridge. The remaining extract in the flask was quantitatively transferred with 2x2 ml acetonitrile-toluene (3:1). A reservoir (100 ml) was attached to the cartridge and then extracts were eluted with 25 ml of acetonitrile-toluene (3:1) under vacuum using manifold at a rate of 5 ml/ min into glass vials (30 ml). After elution, solvent that had been passed through the extraction column was forcibly removed from the column by vacuum aspiration under increased vacuum. The eluted sample was evaporated to dryness under gentle stream of nitrogen and then re-dissolved in 1 ml of hexane, agitated by vortex and subjected for GC-MS analysis.

**Gas chromatography-mass spectrometry (GC-MS)**

Chromatographic analysis was performed using a Shimadzu GCMS-QP5050 capillary gas chromatograph, coupled to mass spectrometer equipped with split/splitless injector at split ratio of 1: 49 with. The chromatographic separation of the organochlorines was accomplished with DB-5 M5 fused silica capillary column (30 m, 0.25mm i.d., 0.25 mm) coated with a 5% biphenyl- 95%
dimethylsiloxane stationary phase in combination with the following oven temperature programme: initial temperature 59 °C, held for 0.30 min, 15 °C/min ramp to 150 °C, followed by 4 °C/min to 275 °C. The carrier gas (helium, 99.999%) flow rate was set to 1 ml/min with column inlet pressure of 54.8 kPa and linear velocity of 36.10 cm/sec. The mass spectrometer was operated in electron ionization mode with a transfer line temperature of 275 °C, manifold temperature of 40 °C, trap temperature of 200 °C, ion source of 230 °C, solvent cut time of 2 min., detector gain of 1 kV, sampling rate of 1 sec. and selected ion monitoring (SIM) mode. The ion energy for electron impact (EI) was always 70 eV. Mass detection was performed in the single ion monitoring (SIM) mode. The selected ion groups in SIM mode are listed in Table 4.1. All organochlorines were identified by retention time and specific ions, and identified by the external standard method.

Table 4.1: Retention times, molecular weight and fragment ions selected for confirmation compounds using GC-MS.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Retention time (Rt) min.</th>
<th>MW</th>
<th>M/Z1</th>
<th>M/Z2</th>
<th>M/Z3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lindane</td>
<td>19.167</td>
<td>288</td>
<td>109</td>
<td>181</td>
<td>219</td>
</tr>
<tr>
<td>Methoxychlor</td>
<td>34.053</td>
<td>344</td>
<td>152</td>
<td>212</td>
<td>227</td>
</tr>
<tr>
<td>Aldrin</td>
<td>24.819</td>
<td>378</td>
<td>73</td>
<td>81</td>
<td>117</td>
</tr>
<tr>
<td>Chlordane</td>
<td>20.680</td>
<td>406</td>
<td>237</td>
<td>272</td>
<td>375</td>
</tr>
<tr>
<td>DDT</td>
<td>21.100</td>
<td>352</td>
<td>165</td>
<td>235</td>
<td>237</td>
</tr>
<tr>
<td>DDE</td>
<td>29.333</td>
<td>316</td>
<td>176</td>
<td>246</td>
<td>318</td>
</tr>
<tr>
<td>DDD</td>
<td>20.800</td>
<td>318</td>
<td>165</td>
<td>199</td>
<td>235</td>
</tr>
<tr>
<td>DDA</td>
<td>21.350</td>
<td>280</td>
<td>165</td>
<td>235</td>
<td>280</td>
</tr>
<tr>
<td>Dicofol</td>
<td>24.700</td>
<td>368</td>
<td>111</td>
<td>139</td>
<td>251</td>
</tr>
</tbody>
</table>
4.3.1 Results and discussion

4.3.2. Trace elements in Mutton bird breast meat

Annual variations of heavy metals in Mutton bird muscle

Essential, beneficial and toxic metal concentrations (wet weight basis and dry weight basis mg/kg for the sake of comparison with literature values) of New Zealand Mutton bird breast muscle for 2007 and 2008 are presented in Table 4.2. No significant annual variation (P > 0.05) was seen in the following seven trace elements: As, Cd, Cr, Cu, Ni, Pb and Se, whereas significant annual differences were found in the remaining metals (P < 0.05). MBBM had significantly lower Ag, B, Fe, Mn, Zn, Ca, K, Mg, P and S concentration in 2008 compared with 2007 values. The concentrations of Al, Hg, Li and Na were higher (P < 0.05) in 2008 Mutton bird samples compared with 2007. Because Mutton birds prey on fish, krill, molluscs and crustaceans, they could have higher levels of trace elements in their bodies than their prey. Mutton birds, like fish and shellfish are particularly susceptible to trace element accumulation due to their feeding regime and behaviour. The notable variations in trace elements of MBBM between two years may be explained by several factors including the feeding behaviour, location, Mutton bird age/sex/size, the ecosystem and season that might affect the accumulation pattern (Farkas et al., 2003; Durrieu et al., 2005; Bekhit et al., 2009).
Table 4.2: Heavy metal concentrations in New Zealand Mutton bird breast meat for 2007 and 2008

<table>
<thead>
<tr>
<th>Element</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh weight basis</td>
<td>Dry weight basis</td>
</tr>
<tr>
<td>Ag</td>
<td>0.40±0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.90±0.82&lt;sup&gt;X&lt;/sup&gt;</td>
</tr>
<tr>
<td>Al</td>
<td>0.72±0.59</td>
<td>1.35±1.10&lt;sup&gt;Y&lt;/sup&gt;</td>
</tr>
<tr>
<td>As</td>
<td>0.23±0.07</td>
<td>0.50±0.14</td>
</tr>
<tr>
<td>B</td>
<td>0.73±0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.60±1.96&lt;sup&gt;X&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cd</td>
<td>0.03±0.02</td>
<td>0.07±0.06</td>
</tr>
<tr>
<td>Co</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cr</td>
<td>0.06±0.04</td>
<td>0.13±0.08</td>
</tr>
<tr>
<td>Cu</td>
<td>4.37±0.35</td>
<td>9.59±0.84</td>
</tr>
<tr>
<td>Fe</td>
<td>45.07±4.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.69±12.04&lt;sup&gt;X&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hg</td>
<td>0.02±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05±0.11&lt;sup&gt;X&lt;/sup&gt;</td>
</tr>
<tr>
<td>Li</td>
<td>12.48±1.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.59±3.03&lt;sup&gt;Y&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mn</td>
<td>0.40±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.85±0.13&lt;sup&gt;X&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ni</td>
<td>0.17±0.22</td>
<td>0.36±0.46</td>
</tr>
<tr>
<td>Pb</td>
<td>0.02±0.03</td>
<td>0.03±0.05</td>
</tr>
<tr>
<td>Se</td>
<td>0.91±0.12</td>
<td>2.02±0.29</td>
</tr>
<tr>
<td>Zn</td>
<td>21.83±1.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.67±3.42&lt;sup&gt;X&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Macro Elements mg/g

<table>
<thead>
<tr>
<th>Element</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh weight basis</td>
<td>Dry weight basis</td>
</tr>
<tr>
<td>Ca</td>
<td>0.35±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78±0.18&lt;sup&gt;X&lt;/sup&gt;</td>
</tr>
<tr>
<td>K</td>
<td>2.42±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.27±0.26&lt;sup&gt;X&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mg</td>
<td>0.24±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.52±0.03&lt;sup&gt;X&lt;/sup&gt;</td>
</tr>
<tr>
<td>Na</td>
<td>30.77±4.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.28±8.36&lt;sup&gt;X&lt;/sup&gt;</td>
</tr>
<tr>
<td>P</td>
<td>1.83±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.96±0.27&lt;sup&gt;X&lt;/sup&gt;</td>
</tr>
<tr>
<td>S</td>
<td>2.26±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.90±0.24&lt;sup&gt;X&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

ND = below detection limit

Superscripts a and b: means of the elemental concentration in 2007 and 2008 on a wet weight basis are shown in columns 1 and 3. For wet weight values, in a given row, values in column 1 and 3 with different superscript letters (a,b) are significantly different (P < 0.05). Superscripts X and Y: means of the elemental concentration in 2007 and 2008 on a dry weight basis are shown in columns 2 and 4. For these values, in a given row, values in column 2 and 4 with different superscript letters (X, Y) are significantly different (P < 0.05).

The concentrations of As, Cd, Pb and Hg in MBBM (Table 4.2) were much lower than the maximum allowable level for metal contaminants (mg/kg material) in fish and shellfish of As (2), Cd (2), Pb (0.5 and Hg (0.5) mg/kg (NZFSA, 2004). The Cu levels were lower than the generally accepted levels for abalone and lobster (5
and 10 mg/kg) but higher than the acceptable value for fish (0.5 mg/kg). Lithium content was (27.59 - 33.01 mg/kg dry weight) higher than the proposed provisional recommended maximum daily intake (1.0 mg Li/day for 70 kg adult), but a daily intake of 10 mg/day have been reported without evidence of adverse effects (2). Zn was similar to the range acceptable to seafood (5-25 mg/kg fresh weight). Trace elements are classified as either essential elements (e.g., Zn, Cu, and Fe) or toxic elements (e.g., Hg, Cd, and Pb). Both deficiency and excess intake of essential elements have important consequences on human health. As top predators, Mutton birds may be the end reservoir of the bioaccumulation of trace elements in a food chain causing them to be accumulated hazardous levels. The essential and beneficial mineral elements are K, Ca, Mg, B, Fe, Mn, Zn, Cu, Ni, S, Na, Co, P, Cr and Se. The toxic metals are Pb, Al, Cd, Hg, Li and Ag. Essentially, minerals are classified further into two types. Some minerals that are needed in relatively large amounts (in mg/kg body weight quantities) are referred to as macro-minerals such as (Ca, P, K, Na and S). Others needed in smaller amounts (in mg/kg body weight) are denoted as micro-minerals or trace elements (Zn, Cu, Fe, Mn and Se) (McDowell, 2003).

In the following section, the importance and the availability of different elements will be discussed in relation to their availability in Mutton bird compared with other common meat sources.
Comparison of heavy metal concentration in Mutton bird breast meat with other common meats

Table 4.3: Trace element concentrations of Mutton bird breast meat and other common meats (mg/kg)

<table>
<thead>
<tr>
<th>Element</th>
<th>Mutton Bird</th>
<th>Fish (1,2,8)</th>
<th>Australian Mutton Bird (10)</th>
<th>Beef (5,6,9)</th>
<th>Lamb (5,7)</th>
<th>Chicken (3)</th>
<th>Sea Bird (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2007</td>
<td>2008</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ag</td>
<td>0.90</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Al</td>
<td>1.35</td>
<td>3.30</td>
<td>10.9</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>As</td>
<td>0.50</td>
<td>0.55</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>B</td>
<td>1.60</td>
<td>0.07</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ca</td>
<td>779.18</td>
<td>475.14</td>
<td>6</td>
<td>46</td>
<td>2600</td>
<td>12.5</td>
<td>3.3</td>
</tr>
<tr>
<td>Cd</td>
<td>0.07</td>
<td>0.08</td>
<td>0.3</td>
<td>ND</td>
<td>0.0019</td>
<td>0.23</td>
<td>0.12</td>
</tr>
<tr>
<td>Co</td>
<td>ND</td>
<td>ND</td>
<td>5.4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cr</td>
<td>0.13</td>
<td>0.14</td>
<td>34.7 (Ww)</td>
<td>ND</td>
<td>ND</td>
<td>0.33</td>
<td>ND</td>
</tr>
<tr>
<td>Cu</td>
<td>9.59</td>
<td>8.93</td>
<td>2.34 (Ww)</td>
<td>ND</td>
<td>70</td>
<td>0.10</td>
<td>0.33</td>
</tr>
<tr>
<td>Fe</td>
<td>96.69</td>
<td>75.32</td>
<td>3.6</td>
<td>4.5</td>
<td>1.93</td>
<td>1.98</td>
<td>92.72</td>
</tr>
<tr>
<td>K</td>
<td>5273.51</td>
<td>4778.60</td>
<td>282</td>
<td>190</td>
<td>710</td>
<td>324</td>
<td>1.3</td>
</tr>
<tr>
<td>Li</td>
<td>27.59</td>
<td>33.01</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Mg</td>
<td>519.95</td>
<td>401.80</td>
<td>27</td>
<td>33</td>
<td>931</td>
<td>20.8</td>
<td>ND</td>
</tr>
<tr>
<td>Mn</td>
<td>0.85</td>
<td>0.63</td>
<td>3.66</td>
<td>ND</td>
<td>0.8</td>
<td>0.01</td>
<td>1.38</td>
</tr>
<tr>
<td>Ni</td>
<td>0.36</td>
<td>0.65</td>
<td>19.4 (Ww)</td>
<td>ND</td>
<td>2</td>
<td>7.30</td>
<td>ND</td>
</tr>
<tr>
<td>P</td>
<td>3962.84</td>
<td>2800.03</td>
<td>ND</td>
<td>ND</td>
<td>1.1</td>
<td>3.6</td>
<td>ND</td>
</tr>
<tr>
<td>Pb</td>
<td>0.03</td>
<td>0.12</td>
<td>0.065</td>
<td>ND</td>
<td>ND</td>
<td>0.002</td>
<td>0.98</td>
</tr>
<tr>
<td>S</td>
<td>4902.45</td>
<td>4155.41</td>
<td>174</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Se</td>
<td>2.02</td>
<td>2.04</td>
<td>1.4</td>
<td>0.078</td>
<td>ND</td>
<td>ND</td>
<td>36.1</td>
</tr>
<tr>
<td>Zn</td>
<td>47.67</td>
<td>31.59</td>
<td>25.6 (Ww)</td>
<td>2.5</td>
<td>4.09</td>
<td>0.243</td>
<td>6.12</td>
</tr>
<tr>
<td>Na</td>
<td>68276.0</td>
<td>77318.71</td>
<td>ND</td>
<td>150</td>
<td>0.85</td>
<td>2.1</td>
<td>ND</td>
</tr>
<tr>
<td>Hg</td>
<td>0.188</td>
<td>0.188</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.13</td>
</tr>
</tbody>
</table>


Silver is a non-essential element and normally occurs in animal and human tissues only in trace amounts. It is observed in nature (Earth’s crust) in elemental form and is found in soils as a result of geochemical processes at concentrations ranging from less than 0.01 to 5 mg/kg, with an average concentration of 0.1 mg/kg (Boyle, 1968; Smith and Carson, 1977). The WHO estimated that the level of Ag in urban
air does not exceed 0.00005 mg/m (WHO, 1984). Therefore, food is considered to be the main source of Ag for persons not occupationally exposed to this element. However, little information is available on the levels of Ag in food. Ag has been evaluated in mushrooms at levels of hundred milligrams per kilogram. In wheat flour and bran Ag was found at 0.4 and 1.0 mg/kg dry weight respectively. In the present study, Ag in MBBM in 2007 was 0.90 mg/kg wet weight which is higher than those reported for any of the other meats. However, in 2008, Ag levels were below detection, indicating that Ag accumulation in Mutton birds are probably influenced by the environmental factors rather than natural accumulation/deposition in the muscles.

Calcium is an essential mineral which gives strength to bones and teeth and it plays a key role as an intracellular second messenger (Granner, 2000). Adequate Ca intake has been associated with reduced risk of osteoporosis, hypertension, colon cancer, and lead absorption (Weaver, 1998). The MBBM had significantly higher concentrations of Ca (779.18-475.14 mg/kg dry weight) than other meats such as fish (6 mg/kg dry weight), lamb (12.5 mg/kg), chicken (3.3 mg/kg dry weight) and Australian Mutton birds (46 mg/kg dry weight). But it was lower than in beef (2600 mg/kg dry weight). Phosphorus, the second most abundant element (after Ca) present in our bodies with around 85% found in the bones and teeth (Newton, Haas, 1984; McDowell, 2003). Phosphorus is required in large amounts for skeletal integrity, and is absorbed more efficiently than Ca. Phosphorus is an important to the utilization of carbohydrates and fats for energy production and also in protein synthesis for the growth, maintenance, and repair of all tissues and cells (Newton and Haas, 1984). Most muscle protein foods such as meats, fish, and chicken are high in P. According to Institute of Medicine in Washington, (1997), the recommended dietary intakes of phosphorus are as follows: from 4 to 8 years it
is 500 mg/day and for adults: 700 mg/day. In the present study, P was very high (3962.84-2800.03 mg/kg dry wet weight) compared to other meats particularly beef and chicken that have 1.1 and 3.6 mg/kg respectively. This suggest lower serving requirement to achieve the recommended daily intake.

Potassium is an essential mineral for good nutrition and health. The recommended daily K intake is 4.7 grams/ day. It has many functions such as the transmission of nerve impulses and carbohydrate and protein metabolism. Dietary K may play a role in decreasing blood pressure (Anderson et al., 2008). Ascherio et al., (2009) stated that high K intake reduces the risk of stroke in a large cohort of men followed up for 8 years. The MBBM contains large amounts of K (5273.51-4778.60 mg/kg dry wet weight) compared to red meats (beef (710mg/kg dry weight) and lamb (324mg/kg dry weight) and white meat (fish (282), Australian Mutton birds (190 mg/kg dry weight) and chicken (1.3 mg/kg dry weight).

The highest amount of a mineral in the present study was Na which was 68227.60-77318.71 mg/kg dry weight. This level is extremely large compared to other meat (Australian Mutton birds (150 mg/kg), beef (0.85 mg/kg) and chicken (2.1 mg/kg dry weight)). The noticeable level in sodium is due to the use of sodium chloride which is added during processing to preserve Mutton birds. Sodium chloride has some benefits (e.g. improves flavour, texture and shelf life of meat products). Meat itself contains sodium but the amount is less than 100 mg Na per 100 g (Gillette, 1985). The recommended daily dietary salt intake is about 6 g NaCl/day/person [(Aho et al., (1980); WHO, (1990)]. This recommendation was given because a higher intake of Na can lead to Ca excretion and also has been associated with hypertension. Hypertension is a major risk factor in the development of cardiovascular disease (Appel et al., 1997). Also, the correlation between high Na
intake and the development of hypertension was reported (MacGregor and de Wardener, 2002).

Furthermore, Tuomilehto et al. (2001) found that high sodium intake correlated with mortality and risk of coronary heart disease, independent of other cardiovascular risk factors, including blood pressure. It should be noted that while the Na concentrations are very high in Mutton birds, the level will reduce substantially during the preparation of the meals (a hot water boiling step to de-salt the bird’s carcasses are traditionally carried out before cooking). Therefore, the availability of Na in the Mutton bird at the level found in Table 4.3 may not be of great consequence nutritionally but it is an assurance of the preservative action taken to stabilize the product during storage. Sulphur concentration in MBBM is very high (4902.45 - 4155.41 mg/kg dry weight) compared with other meats (Table 4.3). Sulphur is a major component that commonly found in seawater and an essential macro-element present in the body of animal tissues as a constituent of amino acids (methionine, cysteine and taurine), enzymes, vitamins and other biomolecules (Komarnisky et al., 2003; Hannelore et al., 2008). This element is ubiquitous in crustacean tissues (Barrento et al., 2009) which normally constitute a large part of Mutton birds’ diet.

Trace elements that are found in human and animals tissues and required in small amounts in human and animals nutrition are Zn, Cu, Fe, Mn and Se (McDowell, 2003). Although, these trace elements have a variety of biochemical functions in all living organisms, they can be toxic when taken in excess. They are available in high concentrations in MBBM as shown in Table 1 and 2. Zinc is widespread in living organisms due to its biological significance. Thus, its deficiency can lead to loss of appetite, growth retardation, skin changes and immunological
abnormalities. In addition, Zn has a major role in immune function (Keen and Gershwin, 1990), and has been associated with increased morbidity and mortality (Kincaid et al., 1997).

Zinc concentration in the Mutton bird breast meat as evaluated in the current study is 47.67 - 31.59 mg/kg dry weight which is higher than other common meats such as beef (4.09 mg/kg dry weight), lamb (0.243 mg/kg dry weight), fish (25.6 mg/kg dry weight), Australian Mutton birds (2.5 mg/kg dry weight) and chicken (6.12 mg/kg dry weight), while it was lower than in other sea birds [( Barau’s Petrel (119 mg/kg dry weight), Audubon’s shearwater (209 mg/kg dry weight) and White-tailed tropicbird (528 mg/kg)]. The recommended daily intake is 15mg Zn for adult males and 12mg Zn for adult females (Dogan Uluozlu et al., 2009). Regarding the toxic level of Zn, a 45 mg/day limit as determined by the WHO in 1996 as the maximum tolerable limit for the adult population [(WHO, (1999); Bekhit et al., (2009)]. The permissible limit of 150 mg /kg is set by NZFSA for chicken meat) (Dogan Uluozlu et al., 2009). There is no maximum level specified for Zn in the Australia New Zealand Food Standards Code for fish and seafood. A generally accepted level is 5 mg/kg fresh weight of fish or 25 mg/kg fresh weight of certain seafood (NZFSA. Food Standards Code, Food Safety Standard 1.4.1; Food Standards Australia: New Zealand, 2004). The recommended daily allowance (RDI) of Zn is 10 and 15 mg/day in children and adults, respectively (NAS-NRC, 1974).

Although Cu is an essential element, it can be toxic at high concentration and the maximum limit intake for humans was set from 1 to 10 mg/day (WHO, 1996). It is known to be both vital and toxic for many biological systems and may enter the food from soil through mineralization (Koc et al., (2008); Dogan Uluozlu et al.,
Copper deficiency in cows increased the risk of health disorders and growth retardation and in calves’ diarrhoea (Enjalbert et al., 2006). The copper content of MBBM as observed in the present research was high (8.93 - 9.59 mg/kg dry weight) compared to other white and red meats (fish (2.34 mg/kg dry weight), beef (0.07 mg/kg dry weight), lamb (0.10 mg/kg dry weight) and chicken (0.33 mg/kg dry weight). However, it was lower than in other seabirds Barau’s Petrel (143mg/kg dry weight), Audubon’s shearwater (11.1 mg/kg dry weight) and White-tailed tropicbird 31.5 mg/kg dry weight). The toxic limit of Cu to humans is 30 mg/kg (FAO, 1983). The acceptable range of oral intake is believed to be in the range of 2-3 mg/day for adults (WHO, 1996). The generally accepted levels for Cu in sea foods are 0.5, 5, and 10 mg/kg for fish, abalone, and lobster, respectively (Fabris et al., 2006; NZFSA, 2004). Cu levels in MBBM were about 30-50% of the toxic limit of 30 mg/kg for Cu set by the FAO (FAO, 1983).

Iron is a mineral essential for life and for our diets. It is known that adequate Fe in a diet is very important for decreasing the incidence of anaemia. Iron deficiency affects two billion people (Viteri 1998) and occurs when its demand is high such as in growth, high menstrual loss, and pregnancy, and the intake is quantitatively inadequate or contains elements that render the Fe unavailable to be absorbed (Lynch et al., 1996). There is no information about maximum Fe levels in chicken samples in Turkish standards (Dogan Uluzulu et al., 2009). Demirezen and Uruc (2006) have indicated that chicken meat (22.07–97.72 mg.kg) that is close to the Fe content in the current study which was 75.32 - 96.69-mg/kg dry weight. It is higher than in fish (3.6 mg/kg dry weight), Australian Mutton birds (4.5 mg/kg), beef (1.93 mg/kg) and lamb (1.98 mg/kg). However, it is lower than in other seabirds, Barau’s Petrel, Audubon’s shearwater and White-tailed tropicbird that have 201, 206, 337 mg/kg respectively. Beef is generally regarded as the richest
common dietary source of both Fe and Zn (Sandstead, 2000). However, MBBM as shown in this study is richer in Fe and Zn than in beef.

Selenium is recognized as an essential micronutrient in humans and animals and it is playing important biological roles as an antioxidant, as a regulator of thyroid hormone metabolism or as anti-carcinogenic agent (Pappa et al., 2006). It has been observed that high protein food represents a rich source of Se (Klapec et al., 1998). Low concentrations of Se can cause anomalies in organisms and high concentrations are toxic (Pappa et al., 2006). The adequate daily dietary Se intake ranges from 50 to 200µg, with an average value of 55 µg for adult humans (Dogan Uluozlu et al., 2009). MBBM had a high concentration of Se (2.02-204 mg/kg dry weight) compared with other white meats such as fish (1.4 mg/kg) and Australian Mutton birds (0.08 mg/kg). But it is lower than in other meats such as Barau’s Petrel, Audubon’s shearwater and White-tailed tropicbird which have 36.1, 48.5, and 43.7 mg/kg dry weight respectively.

Manganese (Mn) is one of the most abundant metals in the Earth’s crust. It participates in the regulation of different ways of control on glucose homeostasis (Korc, 1988). The proportion of Mn absorbed from food is around 3-4% (Keen et al., 1984). The US National Academy of Sciences (1980) recommends 2.5-5 mg/day and, the WHO, 1994) recommends 2-9 mg/day for an adult. Biego et al., (1998) estimated the quantity of Mn supplied by foods at 2.5 mg/day. This value is comparable to the one estimated by Buchet et al., (1983) 2.6 mg/day. The Institute of Medicine, Gaithersburg, Maryland, USA recommends that intake of Mn from food and dietary supplements should not exceed the tolerable daily upper limit of 11mg per day. The intake of Mn or the tolerable daily upper limit is 2-11mg per day (Anonymous, 2003; Dogan Uluozlu et al., 2009). In the present study, Mn
content in Mutton bird muscle is 0.85-0.63 mg/kg dry weight, similar to beef (0.8 mg/kg) however, it is lower than in fish (3.66 mg/kg) and in three sea birds, Barau’s Petrel, Audubon’s shearwater and White-tailed tropicbird with values of 1.38, 1.46 and 1.60 mg/kg respectively, whereas, it is high in comparison with chicken meat which is 0.01 mg/kg).

In general all types of foods are good source of Mn (Rumawas et al., 2006). It is an essential cofactor for multiple enzymes involved in glucose metabolism and is hypothesized to play a role in glucose homeostasis, insulin action and in the development of type 2 diabetes [Saris et al., (2000; Larsson and Wolk, (2007)]. Magnesium may reduce tissue glucose uptake by interfering with insulin signalling pathways and by promoting peripheral insulin resistance by decreasing cellular glucose utilization (Takaya et al., 2004). Its deficiency may lead to prolonged vomiting, diarrhoea and other gastrointestinal disorders. Fung et al., (2003) found that higher Mg intake was associated with lower fasting insulin concentrations among women without diabetes. The median intake of Mg from both foods and supplements was 298 mg/d for men and 281 mg/d for women (Rumawas et al., 2006). MBBM has very high amounts of Mg (401.80 - 519.95-mg/kg dry weight) compared with other meats such as beef (930 mg/kg), fish (27 mg/kg dry weight), Australian Mutton bird (33 mg/kg dry weight) and chicken (20.8 mg/kg dry weight).

The current study analysed some beneficial elements such as B which is widely distributed in nature and is an essential element. Boron is completely absorbed as boric acid (Biego et al., 1998). Moore, (1997) estimated the dietary exposure to B for an adult typically ranges from 0.25 to 3.1 mg/day with an average of 1.5 mg/day. Biego et al (1998) estimated a daily average B intake of 1.6 mg, which is
comparable to the amount determined by (Varo and Koivistoinen, 1980) (1.7 mg/day) that was lower than that reported by Zook and Lehmann, (1965) of (2.1-4.3 mg/day). In MBBM, there is a small amount of B about 1.60 mg/kg dry weight. Chromium is a essential mineral, widely distributed in the Earth’s crust, required by the body in trace amounts, distributed widely in the food supply so, most foods provide only small amounts (less than 2 micrograms), it is necessary for insulin activity (Anderson, 1988; Jeejeebhoy et al., 1997). Cr concentrations are close to 1 mg/kg of food. It is usually found in its trivalent form in biological and food samples (Ducros, 1992) while the hexavalent form is the most toxic. Ducros (1992) considers that 0.5-2% of Cr from food is absorbed. Cr concentration in MBBM (0.06-0.07 mg/kg dry weight) was more than the detection limit of <0.05 mg/kg and was well below the level of concern (13 mg/kg) reported by the U.S. for chronic seafood consumers (Bekhit et al., 2009). But in fish meat, Cr was higher; about 34.7 mg/kg wet weight.

Toxic heavy metal concentrations were also investigated in the present study such as Hg, Pb, Li, Cd and Al in MBBM and the values are shown in Table 4.2. Mercury is a heavy metal that is naturally present in the environment. It is an environmental pollutant that exists in three forms: elemental or metallic Hg, inorganic Hg compounds and organic Hg. Mercury finds its way into water sources, where it is converted by microorganisms to the highly toxic form, organic methyl Hg (Virtane et al., 2007). This form bio-accumulates in the aquatic food chain and is found in all species of fish and fish-eating animals (such as MBBM). It was observed that fish consumption is the main source of dietary Hg intake (Guven et al., 1999). Thus, frequent consumption of seafood can increase the amount acquired through diet (Yamashita et al., 2005). In humans, major exposure to methyl Hg occurs via food, with the major sources being fish and fish products.
Exposure to a high level of Hg is known to accumulate not only in the internal organs of small marine organisms, but also in the muscle tissue (Endo et al., 2007). Mercury content in MBBM was 0.188 mg/kg dry weight, higher than in other seabirds, Barau’s Petrel, Audubon’s shearwater with values of 0.13, 0.06 mg/kg respectively. However, it is lower than in White-tailed tropicbird (0.30 mg/kg dry weight). In 1997, the US EPA reduced the recommended safe daily intakes of methyl Hg from 0.5 to 0.1 mg/kg body weight (Virtane et al., 2007).

Lead is known as a poison of the new millennia and has recently been the focus of public health regulations in most of the developed world (Bjerregaard et al., 2004). It is present in the environment because of air, soil and water pollution (Alberti-Fidanz et al., 2001). It has been suggested that there are toxicological effects even in children at low levels of Pb exposure (Winneke et al., 1996). The daily intake of Pb was 0.25 mg/day-1) for a 70-kg adult (WHO, 1993; Santos et al., 2004). The Pb content found in the Chilean meats is (0.112 mg/kg, wet weight). The maximum limits permitted in the EU for meat is 0.02 mg/kg wet weight. FAO/WHO (1993) has established the Provisional Tolerable Weekly Intake (PTWI) for Pb as 25 mg per kg body weight for both adults and children, which approximate to 1700 mg/day for a 68 kg person (Munoz et al., 2005). The uptake of Pb depends on a number of factors such as concurrent ingestion of food, interaction with other substances and the chemical form.

Lead concentrations in MBBM are (0.03-0.12 mg/kg dry weight) higher than in fish (0.07 mg/kg dry weight) and in lamb (0.002 mg/kg DW) but it was lower than in chicken meat (0.98 mg/kg DW). This level is clearly higher compared to pb levels in seabird meat from Greenland (Dietz et al. 1996) that reported Pb concentrations below the detection limit, 0.02 mg/g wet wt. Lead concentration in
waterfowl breast muscle was reported to be 11% of the measurements above 0.5 mg/g wet wt. (Scheuhammer et al., 1998). The maximum Pb level recommended by FSAN is 0.5 mg/ kg (Bekhit et al., 2009); 0.1 mg/kg according to Turkish Food Codex (Romeo, 1987) and the provisional tolerable daily intake for a 60 kg adult is 0.215 mg Pb/day (WHO, 1999; Bekhit et al., 2009).

Lithium is an ultramicroelement, in soil and is probably bound as organo-mineral compounds (Pais, 1980; Vetter, 2005). It is known to cause nephrogenic diabetes insipidus (NDI) in some people (Angrist et al. 1970; Phatak et al., 2006). Li-deficiency in certain insulin-sensitive tissues may be associated with blood glucose imbalance in Chinese hamsters (Hu et al., 1997). Li supplements have a positive effect on productivity of pigs and may stimulate the production of new brain cells and therefore have been used to treat strokes and Alzheimer’s disease (Kokorev, et al, 1996). Low dosages of Li generally have a beneficial effect on human behaviour (Vetter, 2005). Increasing human Li intake by supplementation (lithiation of drinking water) was suggested as a possible means of reduction of crime, suicide and drug-dependency (Schrauzer and Shrestha, 1990). Lithium appears to play an especially important role during the early fetal development as evidenced by the high Li content of the embryo during the early gestational period. Lithium appears to be minimally present in common foods (Donahoo et al., 2009) and but found in variable amounts in foods such as grains and vegetables. Human dietary Li intake depends on location and the type of foods consumed and varies over a wide range. It has been suggested that the accepted limit (provisional RDA) of Li as essential for a 70 kg adult is 1000 µg/day (Schrauzer, 2002). In the current study, Li concentrations were observed in high amounts (27.59 - 33.01 mg/kg DW) in MBBM.
Nickel is considered an essential element for the catalytic activity of some enzymes in vegetables or bacteria (Nielsen, 1984). It is possibly not essential for humans and absorption from the gastrointestinal tract is about 3% (Elinder et al., 1994). Nickel can cause respiratory problems and is a carcinogen (Anonymous, 2003). It is reported that the maximum Ni levels in foods to be 0.2 mg/kg (Dogan Uluozlu et al., 2009). The highest Ni content in meat and chicken are 2.08 µg/g and 1.67ug/g (Onianwa, et al., 2000). From our results, Ni contents in MBBM range from 0.17 to 0.30 mg/kg wet weight which on the average is above the maximum recorded Ni level. But, it was below Ni concentration in fish (19.4 mg/kg dry weight), beef (2 mg/kg dry weight) and chicken meat (7.30 mg/kg dry weight).

Cadmium is toxic to many organisms. It has been considered that food is one of the principal environmental sources of Cd (Baykov et al., 1996). Many toxicological effects of Cd are attenuated in the presence of Se (Barrento et al., 2009). Cadmium may accumulate in the human body and may cause kidney dysfunction, skeletal damage and reproductive deficiencies (Dogan Uluozlu et al., 2009). The minimum permissible limit of Ca in meat is 0.5 mg kg-1 (Anonymous, 2001). The provisional tolerable weekly intake of 0.007 mg Cd /kg (body wt) has been recommended by the FAO/WHO (WHO, 2005). In the current study, MBBM had 0.07-0.08 mg/kg DW which is similar to that observed in Audubon’s shearwater but higher than in the lamb (0.0019 mg/kg). But, it was low compared to other common meats such as fish (0.3 mg/kg), chicken meat (0.23 mg/kg) and in meats of two other sea birds [(Barau’s Petrel (0.12 mg/kg) and White-tailed tropicbird (0.37 mg/kg)]. For chicken meat, the maximum Cd content was shown to be 6.09 mg/kg in meat. It has been reported in Nigeria, that the range of 0.05–0.09 mg/kg (Onianwa et al., 2000) and 1–2mg/kg in Canada (Dabeka et al., 1995). Cadmium
levels in chicken products from Turkey were lower than the maximum permitted levels (Dogan Uluozlu et al., 2009).

Aluminium is not considered to be an essential element in humans. It is the most widespread metal on the Earth’s surface (8% of Earth’s crust) (Joshi et al., 2003). Exposure to Al has been implicated in a number of human pathologies including encephalopathy/dialysis dementia, Parkinson disease and Alzheimer’s disease (Narin et al., 2004; Shokrollahi et al., 2008). Al toxicity is well known in patients with longstanding chronic renal failure (Meiri et al., 1993). Recently, Al has been associated with various bone (osteomalacia) and neurological failures (Alzheimer’s disease) (Grant et al., 2002). Al is distributed in the whole food chain as result of its high presence in nature (Gerhardsson et al., 1994). Most foods contain small amounts of Al (Biego et al., 1998). The permissible Al dose for an adult is quite high (60mg per day) (Dogan Uluozlu et al., 2009). Al absorption is difficult to estimate. The degree of absorption has been estimated to be at least 0.2% (Elinder et al., 1994). The possible correlation between increased tissue Al content due to Al intake via the diet and human pathologies such as osteomalacia and neurodegenerative disorders (encephalopathy, dementia, Alzheimer’s disease, and Parkinson disease) has been (Narin et al., 2004; Shokrollahi et al., 2008). Although there are several potential routes of exposure to this element, the diet is the main source of Al in the healthy population. Normal intake ranges from 6 to 14 mg/day (Gramiccioni et al., 1996). The Al analysis in foods is difficult because of their low concentrations and problems in their collection, storage, processing, and final determination without outside contamination (López et al., 2002). In this study, it was found that Al concentrations in MBBM were 1.35 - 3.30 mg/kg DW, which is lower than in the fish (10.9 mg/kg).
4.3.2.1 Organochlorines in Mutton bird breast meat

The present study is considered the second paper which has reported about occurrence of these compounds in the Mutton bird meat in New Zealand. The measured residuals of DDTs and their metabolisms in breast tissues of MBBM samples for two years (2007-2008) in New Zealand are shown in Table 4.4. As shown in the results obtained in this study, DDT could not be detected in any of the samples. However, ultimate DDT metabolite DDA (2,2-bis(4-chlorophenyl)-acetic acid) was found in nine samples 1,3,4,10, 11, 14 and 15 with values of 2.19±0.56, 3.16±.34, 1.30±0.10, 0.77±0.09, 25.58±1.85, 5.56±.35, 22.27±0.88, 17.02±1.21 and 1.28±0.09 ng/g) respectively. In addition, three other types of organochlorins (Aldrin, Lindane and Methoxychlor) are also found in some samples. Aldrin was found in samples 1, 3,4,5,6,13,14,15 and 17 with values of 37.78±2.44, 7.32±1.10, 3.01±0.20, 10.50±0.37, 6.71±0.44, 38.03±1.20, 17.28±0.85, and 17.28±0.85 and 66.45±1.56 ng/g respectively). Lindane was observed in samples 10, 11 and 19 with values of 93.02±2.56, 11.67±0.73 and 0.163±0.01 ng/g respectively). Methoxychlor was detected only in one sample, sample 19 with the value of 0.694±0.05).
Table 4.4: Levels (ng/g = ppb) of organochlorines in the Mutton bird breast meat collected over two years (2007-2008).

<table>
<thead>
<tr>
<th>Sample#</th>
<th>Lindane</th>
<th>Methoxychlor</th>
<th>Aldrin</th>
<th>Chlordane</th>
<th>DDT</th>
<th>DDE</th>
<th>DDD</th>
<th>DDA</th>
<th>Dicofol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>37.78±2.44</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2.19±0.56</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>3</td>
<td>ND</td>
<td>ND</td>
<td>7.32±1.10</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>3.16±0.34</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>ND</td>
<td>ND</td>
<td>3.01±0.20</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.30±0.10</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>10.50±0.37</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.77±0.09</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>ND</td>
<td>ND</td>
<td>6.71±0.44</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
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<td>8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
<td>ND</td>
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<tr>
<td>9</td>
<td>0.86</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>93.02±2.56</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>25.58±1.85</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>11.67±0.73</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>5.56±3.5</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>13</td>
<td>ND</td>
<td>ND</td>
<td>38.03±1.20</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>22.27±0.88</td>
<td>ND</td>
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<tr>
<td>14</td>
<td>ND</td>
<td>ND</td>
<td>17.28±0.85</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>17.02±1.21</td>
<td>ND</td>
</tr>
<tr>
<td>15</td>
<td>ND</td>
<td>ND</td>
<td>45.17±0.99</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.28±0.09</td>
<td>ND</td>
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<td>ND</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>17</td>
<td>ND</td>
<td>ND</td>
<td>66.45±1.56</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
</tr>
<tr>
<td>18</td>
<td>ND</td>
<td>ND</td>
<td>0.163±0.01</td>
<td>0.694±0.05</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>19</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>20</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

1 Each value represents the mean for three replicates ± SD              ND means not detected.

Kunisue et al. (2003) reported that in all the migratory birds from the Philippines and Vietnam, DDT was the most dominant organochlorines. Historically, Bogan and Bourne (1972) examined the great shearwater (*P. gravis*) from Trinidad and the Manx shearwater (*P. puffinus*) from Bhum in Inverness and Scotland. They were found to have small amounts of DDE in their muscle tissues. A chick bird, probably about 4 weeks of age, was observed to contain the same amounts of organochlorine compounds in the fat as in the adult birds but in much smaller amounts in chicks. Due to this, they had almost certainly never left its burrow and were being fed by the parent birds; it would seem that these compounds are probably derived from the regurgitated food of the parent birds (Dacre, 1974). Dacre, (1974) found that the mean level of total DDT compounds was 17.3 ppm, and this consisted about 78% pp'-DDE which is more than twice that reported by Risebrough et al. (1967). These latter workers found a very high (32 ppm of
total DDT of which 92% was pp'-DDE) in the fat of a slender-billed shearwater of Australia (Risebrough et al., 1968) indicating different accumulation pattern can be expected in different sea bird species.

In comparing Mutton bird meat with other meats in relation to DDT type compounds, Table 4.5 shows the different variations between them. The concentration of DDT metabolites in MBBM were 17.56 ng/g and this is similar to a previous study of Mutton bird NZ which was conducted by Dacre, (1974) and in cattle meat (17.90 ng/g; Sallam and Morshedy, 2008). But, it is very low compared to that observed in Slender-billed shearwater (32 ng/g) (Risebrough et al., 1968) and sheep meat (20.30 ng/g); Sallam and Morshedy, 2008). However, the values in chicken, fish and camel meats were 0.123, 0.93 and 13.90 ng/g respectively which was lower than in MBBM observed in the current study.

Table 4.5: A comparison of DDT type compounds (DDT+ DDE+ DDA) between Mutton breast bird muscle and other common meats:

<table>
<thead>
<tr>
<th>Species</th>
<th>Amounts(ng/g)</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>0.123</td>
<td>Tao et al., 2009.</td>
</tr>
<tr>
<td>Blue shark</td>
<td>0.93</td>
<td>Silva et al., 2007.</td>
</tr>
<tr>
<td>Camel</td>
<td>13.9</td>
<td>Sallam and Morshedy, 2008.</td>
</tr>
<tr>
<td>Cattle</td>
<td>17.9</td>
<td>Sallam and Morshedy, 2008</td>
</tr>
<tr>
<td>Sheep</td>
<td>20.3</td>
<td>Sallam and Morshedy, 2008</td>
</tr>
<tr>
<td>Previous Mutton bird</td>
<td>17.3</td>
<td>Dacre, 1974.</td>
</tr>
<tr>
<td>Slender-billed shearwater</td>
<td>32</td>
<td>Risebrough et al., 1968.</td>
</tr>
<tr>
<td>NZ Mutton bird</td>
<td>17.5607</td>
<td>Present study</td>
</tr>
</tbody>
</table>

It has been stated that fish consumption is the main source of human exposure to different environmental contaminants like DDT (Smith and Gangoli, 2002; Sidhu, 2003). For example, the average concentration of DDT in blue shark was 0.93 ng/g wet weight. (0.4–2.1 ng/g) and in the swordfishes was 2.47 ng/g wet weight. (0.15 – 10.53 ng/g). The amounts of DDT in different meat types with MBBM were
below the toxic limit compared with the maximum limit which is 75 ng g$^{-1}$ wet mass established in Belgium (Maes et al., 2008). The reference value of Acceptable Daily Intake (ADI) was proposed by the WHO is 20 µg/kg (FAO/WHO, 2001; ASTDR, 2002). Figures 4.1 and 4.2 demonstrated that MBBM has high concentrations (25.81 ng/g of Aldrin in comparison to sheep meat (0.198 ng/g wet weight). Lindane also was found very high (34.95 ng/g) in MBBM as it is comparative with other meats such as camel (0.33 ng/g), cattle (0.72 ng/g) and sheep (0.45 ng/g) but they are still in the safe limit.

Figure 4.1: Aldrin concentrations in mutton bird meat and sheep meat
Figures 4.2: Lindane concentration in Mutton bird muscle and other common meats

In summary, MBBM is considered a good source of many minerals such as Na, Cu, Zn and Fe and would be demandable for the human nutrition. However, some toxicological metals have been observed such as Li in high amount and Hg and Pb in tissue amounts. In addition the ultimate metabolite of DDT which is DDA was observed in second sample also. Aldrin and Lindane were found in small amounts. These findings are significant because of such concentration of toxic heavy metals and organochlorins could accumulate in MBBM which has a relatively low fat content, it raises concern for the concentration of these chemicals in tissues with higher fat concentration because these metals and organochlorins are lipophilic.
Chapter 5
General Discussion

5.1 Introduction

The increasing nutritional and health awareness of consumers and their demands for wholesome and nutritious food free from pollutants and other health compromising compounds exerts more pressure on the food industry to identify and control potential contaminants in their products. Consumers purchase fresh meats such as beef, lamb, chicken and fish based on their own perception of the quality of the product and the dietary information available on the product. Although a great deal of information is available on the nutritional value of common meat sources such as beef, pork, poultry and fish, little is known about other traditional meats; such as Mutton birds in New Zealand. Better understanding of the nutritional impact due to the consumption of such food and its contribution to diet can be elicited by the investigation of the basic nutritional composition (Null, 1978). With this in mind, the first part of the present work was carried out to investigate the nutritional value of breast meat of NZ Mutton bird. The information generated from this section was helpful to evaluate its nutritional value compared to other common meats and to estimate its nutritional contribution to the diet of its consumers. Furthermore, a toxicological evaluation for the breast meat of NZ Mutton bird was carried out to complement the nutritional part of the study. This section was important since the Mutton birds survive on marine organisms which can be a potential source of contaminants.

Mutton birds are wild seabirds annually harvested by Maori people according to the customary rights agreement set by Treaty of Waitangi. As mentioned earlier, the adult birds’ sole diet is based on krill and other seafood (small fish) that are
potentially rich in n-3 fatty acids and other marine bioactive compounds. The samples used in the present study were chicks that depend on their parents for their food. The fatty acid content of the diet has an influence on the body fat of the consumer. Therefore, the nutritional value (fatty and amino acids and cholesterol content) of Mutton bird (breast only) was determined and compared with common meat to explore the nutrition value of this New Zealand delicacy. The concentrations of Ag, Al, As, Cd, Cr, Cu, Hg, Mn, Pb, Se, Zn, K, Ca, Mg, B, Fe, Ni, S, Na and Co in Mutton birds were determined for two years (2007 and 2008) to evaluate their contribution to nutrition and its safety for human consumption as well as the annual variation in these elements. Furthermore, DDT, its metabolites and aldin and lindane concentration were also investigated in Mutton bird breast in the same samples.

5.2 Nutritional values in MBBM

The nutritional value (proximate composition, amino and fatty acids, cholesterol content and beneficial minerals) of MBBM was studied for two harvesting seasons (2007 and 2008) to investigate the impact of annual variation. The moisture and carbohydrates contents ranged between 54.0 to 55.0 % and 2.8 to 3.0 %, respectively, and no annual effects were found in these components. The values for fat and ash contents were higher and protein content was lower for birds harvested in 2007 compared with those from 2008. A range of 11.8 to 13.0 for fat, 10.3 to 11.7 for ash and 18.5 to 20.3 % for protein was found in MBBM. The proximate composition of the NZ Mutton Bird muscle was compared to other white (chicken, fish and Australian Mutton bird) and red meats (lamb, beef, goat and camel). It was found that MBBM is similar to Australian Mutton bird with a higher fat
concentration 13.0 and 18.7%, respectively than in other common meats, with evidence high of monounsaturated fatty acids.

The ash content of NZ Mutton Bird Muscle is very high (11.7%) compared with other meat sources due to the salting step required for preservation. The higher ash concentration observed in this study is due to the sodium (in salt) and hydration of meat. This potentially affected the concentrations of elements in Mutton birds and increased their concentrations compared with other meats. Generally speaking, consumers prefer fresh meats that are juicy upon cooking and with high moisture content. The water holding capacity of meat plays a major role in dictating several eating (e.g. tenderness and juiciness) and keeping (e.g. shelf life and microbial growth) qualities. Mutton bird meat has a low moisture content compared to other meats due to a salting preservation process being used at harvest to extend the shelf life. This may be an important step for the product stability during storage, but it will have very little consequence on the quality of the meat as it is normally prepared for cooking in such a way to bring the salt and moisture content to a level that is acceptable to consumers.

Eighteen essential and nonessential amino acids were determined in NZ Mutton bird muscle. Although there were differences in the protein content of MBBM between the two years (2007-2008), no significant annual variation was observed in the amino acids concentrations. Despite the protein content in NZ Mutton bird muscle (18.5%) being similar or slightly lower than other common meats, NZ mutton bird muscle protein had higher concentrations of five essential amino acids (Thr, Lys, Leu, Met and Ile) compared to those found in other common sources of meats. Tyrosine (a non essential amino acid) content in Mutton bird meat was higher than in common meats (chicken, garganey, camel, fish, lamb and beef), but
less than that found in Pintail and goat meats. Similar trends were found in another seven non-essential amino acids (Glu, Asp, Ser, Gly, Ala, Pro and Arg). Amino acids play an important role in human and animal nutrition with functional roles in reproduction and disease prevention. Leucine, Pro, Arg and Glu are necessary for maintenance, growth, reproduction, and immunity in organisms, through maximizing the efficiency of food utilization, enhancing protein Accrual, reducing adiposity, and improving health (Suenaga et al. 2008). The present study support that Mutton bird meat has a high protein quality that could in fact be better than some of the more common non-animal sources.

Mutton birds like other sea birds survive on small marine organisms such as fish, plankton and krill (Kerry et al., 1983) that are known to contain high amounts of unsaturated fatty acids. The fat content of NZ Mutton bird breast muscle was higher (13.0%) than in other common meats. The hypothesis of the present study was that Mutton bird may have an interesting fatty acid profile that contributes to human nutrition in favourable way. Over 50% of the fatty acids were MUFA (52.8%) which is higher than the reported values for all the other meats except Australian Mutton bird meat. The SFA concentration (31.4%) was less than those found in poultry, pork, lamb and beef. These results are considered as a positive outcome, because the unsaturated fatty acids reduce the amount of saturated fatty acids. Also dietary MUFA may lead to decrease in blood cholesterol concentration, and this is beneficial to human health.

The ratios of PUFA/SFA and n-6/n-3 are widely used to evaluate the nutritional value of fat for human consumption. According to current nutritional recommendations, the PUFA/SFA ratio in human diets should be above 0.45 and the n-6/n-3 ratio should not exceed 4.0 (British Department of Health, 1994). The
ratio of n-6/n-3 in NZ mutton bird muscle was 5.12 which exceed the recommended level, however, the ratio of PUFA/SFA was 4.83, which is high compared to other common meats and is regarded as a positive aspect of mutton bird meat. Nutrition advisers recommend a higher intake of PUFA, especially n-3 PUFA at the expense of n-6 PUFA (British Department of Health, 1994). Among the polyunsaturated fatty acids, EPA (eicosapentaenoic acid, C20:5 n-3) and DHA (docosahexaenoic acid, C22:6 n-3) were the dominant n-3 fatty acids in Mutton birds (the EPA is 1.43%, higher than other common meats and DHA is 2.09%). These two fatty acids are of great importance to human Nutrition because they have shown to prevent coronary heart disease (Mozaffarian et al., 2005).

The general recommendations for daily dietary intakes of DHA and EPA are 0.5 g/day for infants and 1 g/day for adults (Kris-Etherton et al., 2002). The amount of EPA and DHA in Mutton bird muscle for 2007 is 0.37/100 g and for 2008 0.32/100 g which indicates that about 150 g of MBBM will fulfil the recommended daily dietary intake for infants and twice this amount will be sufficient to satisfy adult recommended level. Scientific evidence and nutritional guidelines recommend a reduction in total fat intake, particularly of SFA (World Health Organization, 2003) that are associated with an increased risk of obesity, hypercholesterolemia and some cancers (Wood et al., 2004). The total SFA in Mutton bird muscle was low compared with chicken, lamb and beef but higher than the values detected in Australian Mutton bird and in fish. The most abundant SFA in Mutton bird was palmitic acid (C 16:0) at 19.4%.

Overall, the increased MUFA and lower SFA suggest that Mutton bird meat is potentially better than other red meats in terms of nutritional and keeping quality. Higher PUFA content in meat may adversely affect its sensory qualities during
storage due to the susceptibility of PUFA to oxidation. MBBM had a high concentration of cholesterol (184 mg/100g) in 2007, which is similar to what was reported in the Australian Mutton bird (185 mg/100g) but a lower cholesterol content in 2008 (135mg/100g). This cholesterol content is very high compared with other common meats and suggests that those with heart problems should avoid eating Mutton bird meat frequently because one serve of 200 g of MBBM will give the daily recommended amount of cholesterol. However, the presence of high amounts of UFA particularly MUFA may reduce this problem as UFA can contribute positively to the metabolism of cholesterol ((Mozaffarian et al., 2005).

The ash content of New Zealand MBBM was higher than in other common meats and this reflects the increase in metals as expected. The concentrations of twenty two metals in the breast muscle of the Mutton bird were investigated in order to examine safety and the annual variation in these elements in samples collected in 2007 and 2008. Annual variation over the two years was observed in the studied elements. Several factors can cause these differences such as feeding behaviour, location, age, sex and size of birds, their foods (krill, molluscs and crustaceans) and also the amount and type of salt used in the salting step. MBBM had a very high content of some essential elements (Ca, Fe, K, Mg, P, S and Na) compared with other common meats. The Cu levels were lower than the generally accepted levels for abalone and lobster (5 and 10 mg/kg) but higher than the acceptable value for fish (0.5 mg/kg). Lithium content in a 100 g MBBM was higher than the proposed provisional recommended maximum daily intake (1.0 mg Li/day for 70kg adult), but this may not be of significant importance since a daily intake of 10 mg/day have been reported without evidence of adverse effects (Schrauzer, 2002). The Zn was similar to the range acceptable in seafood (5-25 mg/kg). The concentrations of Zn and Fe in MBBM were higher than those found in red meat.
Overall, the macro- and micro-elemental profile of the Mutton bird breast suggests a good nutritional minerals profile without any hazardous reservations.

5.3 Toxicological studies in MBBM

This study evaluated chemical contaminants such as toxic heavy metals and pesticides particularly DDT and its metabolites. The concentrations of As, Cd, Pb and Hg in MBBM were much lower than the maximum allowable level of metal contaminants (mg/kg material) in fish and shellfish of As (2), Cd (2), Pb (0.5 and 2,) and Hg (0.5). The preliminary data (not reported in the present study) indicated that higher levels of Cd and other metals in the Mutton bird skin may compromise the overall safety of consuming the Mutton birds and these need to be investigated in future work. However, the mineral profile of MBBM suggests a good nutritional mineral profile without any hazardous reservations.

With regard to DDT, there were very small amounts of DDT metabolites in Mutton bird breast meat particularly aldrin, lindane and methoxychlor as shown in chapter 4. The average contents of DDT metabolites in the current study (17.56 ng/g) was similar to that reported earlier for NZ Mutton bird (Dacre, 1974). This suggesting the persistence of DDTs and its metabolites in the MBBM at a level that was similar to those found in other red meats but at a much higher level than those found in several fish species. However, these results were found in the lean breast meat which may not reflect the overall level of DDT and their metabolites because these organochlorins are lipophilic compounds. Therefore, other organs such as skin would be suspected to have a high concentration. Thus, a more comprehensive study for the distribution of DDTs and their metabolites are needed to assure the safety of this unique meat source.
5.4 Conclusion

The current study investigated both the nutritional value and toxicological status of Mutton bird muscle over two years (2007 and 2008) to study the annual variation of these parameters. The nutritional value and toxicological status of NZ Mutton bird muscle were compared to other common white and red meats. The nutritional value of Mutton bird muscle was similar to or superior to the traditionally protein sources such as seafood and red meat. It may even have an advantage over the other common meats (beef, lamb, fish and chicken) due to its high intramuscular protein and MUFA (omega n-3 and n-6). However, its high cholesterol contents may represent a risk factor for some people. Annual variations existed in Mutton bird which may not be of nutritional consequence but might be a useful indicator for ecological events such as feed availability and other environmental factors. Mutton bird is a good source of minerals such as Zn and Fe compared with other meat sources. Apart from one sample in 2007, all the samples were below the recommended maximum limit or level of concern of toxic minerals. The concentrations of DDT and its metabolites were very low and their levels in NZ Mutton bird muscle are lower than that of several common meats. However, for future work, it is recommended that the evaluation of the pollutants be evaluated in different parts of the bird and/or the whole bird to assure the safety of those annual delicacies.
Chapter 6

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