EVALUATION OF PROTEIN UTILISATION OF VACUUM MICROWAVE, AIR, AND FREEZE-DRIED *EUPHAUSIA PACIFICA* IN A RAT MODEL

by

CORRIE LORRAINE LANDYMORE

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Department of Food Science

The University of British Columbia
Vancouver, Canada

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ABSTRACT

Euphausia pacifica was dried using vacuum microwave blanching at a high temperature followed by air-drying (VH), vacuum microwave dehydrating (VMD) at a low temperature (VL), freeze-drying (FD), and hydrolysis plus freeze-drying (HZ). The effect of the drying method on the protein utilisation by young rats was evaluated. Five groups of rats were each fed an isocaloric diet containing 25.9 ± 0.2 % protein from one of five sources. The protein sources were casein (CA) as the control protein, and the four dehydrated krill samples (VH, VL, FD, and HZ). The results showed that the inclusion of the dehydrated *Euphausia pacifica* as the only source of protein in the feeds resulted in significantly reduced ($p \leq 0.05$) body weight gains, feed intakes, protein efficiency ratios (P.E.R.), and nitrogen balances as compared to the CA control group. The nitrogen balance of the HZ group was significantly higher ($p \leq 0.05$) than those of the VL and FD groups. There were no significant differences ($p \leq 0.05$) in the apparent digestibilities of the CA, VL, FD, and HZ feeds. The VH feed had an apparent digestibility that was significantly lower ($p \leq 0.05$) than the other experimental feeds. No significant differences ($p \geq 0.05$) amongst the experimental groups were noted for apparent biological values (B.V.), apparent net protein utilisations (N.P.U.), or apparent urinary nitrogen losses. The available lysine content of the VH feed was significantly lower ($p \leq 0.05$) than the other feeds. Only the FD group had a plasma α-amino nitrogen level significantly lower ($p \leq 0.05$) than the CA control group; however, it was not statistically different ($p \geq 0.05$) from the other krill meal fed groups. The rates of proteolysis of the VL, FD, and HZ feeds with added chitinase were all significantly faster ($p \leq 0.05$), and the VH feed showed a trend towards being faster, than the rates of proteolysis of these feeds without the added chitinase. Fluoride levels in the VH, VL, FD, and HZ feeds were correlated with a reduction ($p \leq 0.05$) the rats' feed intakes and weight gains.

Drying *Euphausia pacifica* significantly reduced ($p \leq 0.05$) or destroyed protease activity. Overall, fluoride present in the krill meals was correlated to a decrease ($p \leq 0.05$) in the rats' feed intake, which affected the values of the biological methods used for assessing protein quality and utilisation. The method used to dehydrate *Euphausia pacifica* did not statistically ($p \geq 0.05$) affect protein utilisation in the rat model.

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LIST OF ABBREVIATIONS

Abbreviation	Word
α	alpha
a_g	acceleration due to gravity
a_w	water activity
ANOVA	analysis of variance
A.O.A.C.	Association of Official Analytical Chemists
β	beta
B.V.	biological value(s)
^oC	degree(s) centigrade
^{14}C	radioactive carbon
C_i	molar concentration
©	copyright
CA	casein
CH	rat chow
cm	centimetre(s)
db	dry basis
ddH_2O	distilled deionised water
ε	epsilon
EC	Enzyme Commission
EDTA	ethylenediaminetetraacetic acid
E. crystallorophias	*Euphausia crystallorophias*

E. pacifica	*Euphausia pacifica*
E. superba	*Euphausia superba*
F^-	fluoride
°F	degree(s) Fahrenheit
F.A.O.	Food and Agriculture Organisation of the United Nations
FD	freeze-dried
FDNB	fluorodinitrobenzene
g	gram(s)
g %	gram(s) percent (1 g/100 mg)
GHz	gigahertz
HF	hydrofluoric acid
HZ	hydrolysed, freeze-dried
J	joule(s)
J/g	joule(s) per gram (gross energy)
kg	kilogram(s)
kg/cm^2	kilogram(s) per square centimetre
kJ	kilojoule(s)
km	kilometre(s)
kW	kilowatt(s)
L	litre(s)
LD_{50}	dose required to kill 50 % of the population
M	molar
m^3	cubic metre(s)

mg	milligram(s)
mg %	milligram(s) percent (1mg/100 mg)
MHz	megahertz
mil	$1/1000^{th}$ of an inch
mL	millilitre(s)
mM	millimolar
mm	millimetre(s)
mm Hg	millimetres mercury
mol	mole(s)
mV	millivolt(s)
M.W.	molecular weight
n	number of samples
NAG	*N*-acetyl-β-D-glucosamine
nm	nanometre(s)
NPN	non-protein nitrogen
N.P.U.	net protein utilisation(s)
P.E.R.	protein efficiency ratio(s)
pH	$-\log_{10}$concentration of hydrogen ions
pI	isoelectric point
pk_a	$-\log_{10}$ concentration of dissociation constant
psi	pounds per square inch
R- / R′-	side group; denotes organic compounds
®	trademark name - registered

r	correlation coefficient
r^2	coefficient of determination
rpm	revolutions per minute
RNA	ribonucleic acid
RW	raw
s	second(s)
SEM	standard error of the mean
TCA	trichloroacetic acid
TISAB	total ionic strength adjustment buffer
TNBS	trinitrobenzenesulfonic acid
μequivalent	microequivalent(s)
μg	microgram(s)
μL	microlitre(s)
μmol(e)	micromole(s)
U.B.C.	The University of British Columbia
U.N.U.	United Nations Union
U.S.D.A.	United States Department of Agriculture
UV	ultraviolet
VH	vacuum microwave blanched at a high temperature and air-dried
VL	vacuum microwave dehydrated at a low temperature
VMD	vacuum microwave dehydration
W	watt(s)
W.H.O.	World Health Organisation

ω	omega
wb	wet basis
w/v	weight in volume
$	American dollar
/	per
%	percent; percentage
±	plus or minus
=	equals
≤	less than or equal to
≥	greater than or equal to

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DEDICATION

I dedicate this achievement to my parents, Art and Marlene Landymore, without whom I would have never accomplished the feats I once perceived as insurmountable. Throughout the years they have patiently and gently guided, inspired, and enlightened, and thus, provided me with the framework necessary to produce this thesis. They have taught me the value of an education; that it is a privilege, not a right, and that with it we are obliged to apply our knowledge to the betterment of society. By example, they have shown me the importance of always completing your commitments and endeavours to the best of your ability, and to be proud of your accomplishments without being arrogant. Most importantly, my parents have been unceasing in their love, and support.

Thank you Mum and Dad.

All my love.

I. INTRODUCTION

For centuries, the successful commercial harvesting of marine fish and shellfish, have made these common food items. Zooplankton on the other hand, have not been considered a major food or protein resource and have been harvested only to a very limited extent. However, interest in an euphausiid market is now evident as a growing number of nations engage in commercial fishing of euphausiids (McWhinnie and Denys, 1978; Nicol, 1997).

Euphausiids, or krill, have been viewed as possibly the world's largest single untapped source of harvestable marine protein (Nicol, 1997). Due to the abundance of krill, its possible exploitation for food and feed purposes has intrigued food and fishery scientists for many years. With the development of industrial methods for rearing salmonids, researchers are faced with the task of manufacturing high quality, balanced feeds (Kotik *et al.*, 1979).

The fishery for North Pacific krill (*Euphausia pacifica*) reached levels of over 100 000 tonnes a year in the waters off Japan in the early 1990's. As of late, the harvest for this species is somewhat smaller at approximately 60 000 tonnes per year. The majority of the krill catch is used in aquaculture; however, krill is also used for domestic animal feed (cattle, poultry, pigs, and mink), for aquarium fish feed, for sport fishing bait (Nicol, 1997), and for human consumption. The flavour of some krill products is said to resemble the flavour of crab (Burukovskiy, 1967; Pierce *et al.*, 1969).

The chemical compositions and nutrient values of krill products and fish-protein-concentrate have been reported to be equivalent (McWhinnie and Denys, 1978). *Euphausia pacifica* contains more than 15 % protein by wet weight. This, coupled with the full array of amino acids, gives krill an excellent cost to protein ratio. Due to the perishability of raw krill, dry meals are preferred products for inclusion in formulated feeds. Durance and Liu (1996a)

showed that dried krill prepared by vacuum microwave dehydration had excellent colour, odour, and rehydration potential.

This research was undertaken to investigate the protein utilisation of dehydrated *Euphausia pacifica* by young rats and to compare the effects of various drying methods on the protein quality of this crustacean.

The objectives of this study were to evaluate the effects of vacuum microwave blanching at a high temperature followed by air-drying, vacuum microwave dehydration at a low temperature, freeze-drying, and hydrolysing plus freeze-drying on the physical properties, nutritive values, and enzyme activities of *Euphausia pacifica*, as well as to assess the potential toxicity of fluoride in dehydrated *Euphausia pacifica* and to determine if the different dehydration methods influenced the overall toxicity of fluoride in *Euphausia pacifica*.

The major hypothesis of this research was that the drying methods affected the nutritional quality of the *Euphausia pacifica*.

Testing was conducted to determine if there was a difference between the protein efficiency ratios (P.E.R.s) of the dehydrated *Euphausia pacifica* meals fed to young rats and the P.E.R. of the control meal fed to young rats; and to determine if there was a difference between the P.E.R.s of the dehydrated *Euphausia pacifica* meals fed to young rats. Testing was conducted to determine if there was a difference between the nitrogen balances of the young rats fed the dehydrated *Euphausia pacifica* meals and the nitrogen balance of the young rats fed the control meal; and to determine if there was a difference between the nitrogen balances of the young rats fed the dehydrated *Euphausia pacifica* meals.

Since krill fishing season in British Columbia's waters only runs from October to January, this research will determine if krill can be dried in a manner that preserves its nutritional value and its enzyme activity and thus, enhances protein utilisation by young animals.

LITERATURE REVIEW

II. LITERATURE REVIEW

A. *Euphausia pacifica*

1. Introduction

Euphausia pacifica (*E. pacifica*) or krill (from the Norwegian word "krill", meaning young fry) (Figure 2.1) are classified as belonging to the phylum Arthropoda, subphylum Crustacea, class Malacostraca, subclass Eumalacostraca, superorder Eucarida, order Euphausiacea, and family Euphausiidae (Barnes, 1980).

The family Euphausiidae contains ten genera, seven of which are found in the Northeast Pacific. Currently, eighty-six species of euphausiids are found world-wide in the marine environment. Twenty species of euphausiids inhabit the waters of British Columbia, with the biomass being dominated by five. These five are *Euphausia pacifica*, *Thysanoessa spinifera*, *Thysanoessa inspinata*, *Thysanoessa longipes*, and *Thysanoessa rashii*. *Euphausia pacifica* accounts for 70 % to 100 % of the euphausiid biomass, an estimated 4 550 tonnes, in the Strait of Georgia (Jervis Inlet) where the commercial fishery, licensed in the "Z-F" category since 1983, occurs (Fisheries and Oceans, 2001). British Columbia's annual coast-wide catch quota is 500 tonnes (Fisheries and Oceans, 2001).

Euphausia pacifica is restricted to the North Pacific from latitude 30°N to latitude 50°N (Pierce *et al.*, 1969). Populations are densest in the North Pacific Drift, the Aleutian Current, and off the southeast coast of California in the California Current. It is the dominant euphausiid within 480 km to 640 km off the coast of Point Conception, California and is very abundant northward up to the Bering Sea (Kathman *et al.*, 1986). A swarm may horizontally cover the equivalent of several city blocks, and vertically may form a layer five or more metres thick. The surface waters contain the greatest concentration of krill and may reach densities of

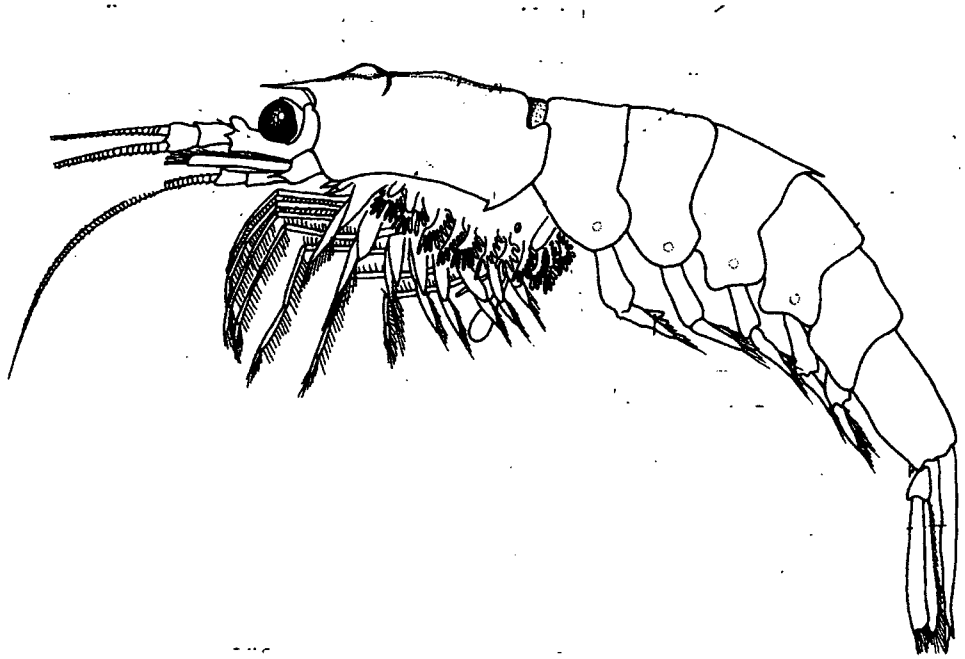


Figure 2.1. A euphausiid.
Scale: 1 cm represents 1.9 mm.

63 000 individuals/m^3 (Barnes, 1980). This species is most common in the upper 300 metres of ocean waters during the spring in British Columbia (Kathman *et al.*, 1986).

Euphausia pacifica lives about five to ten years (Nicol, 1997), and reaches a total length of approximately 24 mm. Feeding mostly at night, krill are filter feeders, although *E. pacifica* can be actively predaceous. They consume detritus, algae, chaetognaths, echinoderms, and crustaceans. In turn, they are important food items for whales (blue, fin, humpback, baleen, and right), seals, birds (penguin), fish (hake, catfish, halibut, herring, trout, and salmon), squid, eels, and decapods (lobsters and crayfish) (Fisheries and Oceans Canada, 1997).

Krill, like all crustaceans, grow by moulting. Sub-adult Antarctic krill (*Euphausia superba*) moult regularly every fourteen to seventeen days (Buchholz, 1989). Growth rates of the adults vary in accordance with food and temperature, but generally, body volume and weight are directly related to body length. Differences in weight and length have been correlated with different seasons (Kathman *et al.*, 1986).

2. World Markets

a. Uses

Euphausiids have long been recognised as an important food item for whales, fish, and birds wherever they represent a major portion of the plankton biomass. This is especially well documented in the North Atlantic and in the Antarctic (Sahrhage, 1988). The total estimated global biomass of krill has been reported to be between 62 million tonnes and 137 million tonnes (Environment News Science, 1999). Euphausiids have now become economically important as a protein supplement for both human and animal (especially fish) consumption, and are sold fresh, frozen, freeze-dried, air-dried, spray-dried, and pickled throughout the world. In British Columbia, euphausiids have been harvested in the Strait of Georgia and in adjacent inlets since

1970 (Kathman *et al.*, 1986). Currently, the majority of the world's krill catch is used in aquaculture as fish feed - fresh, frozen, or processed. The krill is generally extruded into pellet form and added to the feed as a nutritional supplement; most producers add between 1 % and 20 % of krill (wet weight) to their prepared feeds (Argent Chemical Laboratories, 2001). Krill is also used for aquarium fish feed - ground and frozen or freeze-dried, for cat treats, for domestic animal feed (cattle, poultry, swine, and mink) (Nicol, 1997), for sport fishing bait, and even for human consumption - fresh boiled, or peeled and frozen, frozen protein concentrates (FPC), and pastes (Fisheries and Oceans Canada, 1997). As human food however, krill is most commonly used as a flavourant.

b. Harvesting

Krill have been regarded as possibly the largest single untapped source of harvestable marine protein, yet they have only been commercially harvested in a small number of locations. The best-known krill fishery is for Antarctic krill (*Euphausia superba*). The catch of Antarctic krill reached over half a million tonnes a year in the 1980's, when the Soviet Union was the major fishing nation, but it has since undergone a series of fluctuations, and in 1995/96 only 95 000 tonnes were harvested. As of late, Japanese vessels (64 % of the total krill fishery fleet), Polish vessels (21 %), and Ukrainian vessels (14 %) catch the majority of the krill (Nicol, 1997). The fishery for North Pacific krill (*Euphausia pacifica*) is less well known, but in the early 1990's, in the waters off Japan, it had reached levels of over 100 000 tonnes a year. In recent times, the harvest has been somewhat smaller at approximately 60 000 tonnes per annum (Nicol, 1997).

Krill as a product has a number of problems, which have restricted the growth of the industry. These include rapid spoiling, a small size, and high fluoride levels. As well, our lack

of knowledge concerning krill abundance, biology (longevity), sustainability, and interactions with other species in the ecosystem, poses a problem for fishery management.

There are however, a number of valuable components found in krill, such as protein, chitin, proteolytic enzymes, pigmenting carotenoids, and fatty acids. The development of the use of these is a pointer to future expansion of krill fisheries (Nicol, 1997).

Krill fisheries require particularly careful management because of the central ecological role of these species in most of the world's marine ecosystems, but krill remain one of the world's few marine stocks that could sustain far higher levels of exploitation (Nicol, 1995).

c. Processing

At present, only three methods, namely, freeze-drying, air-drying, and hydrolysis with enzymes followed by spray drying are consistently used for the dehydration of krill for the market. These processes yield a product with poor colouring and a small particle size, as the krill is often broken or powdered. With freeze-drying, the krill are frozen shortly after they are caught and then dried at a convenient time. The final commodity, usually in block form, is brittle and easily broken, and in many instances, the krill are crushed into a powder. Freeze-dried krill have a very low moisture content (2 % to 10 %), high protein retention, a pale red colour, and initially, a mild aroma, though they oxidize quickly to take on a fishy odour and a flat or oxidized flavour. Air-drying requires fresh krill to be immediately blanched and then either dried in trays or ground and spray dried. The resultant product, which may be in whole or broken form if tray dried, or in powder form if spray dried, has a high moisture content (greater than 12 %), a yellow to pale red colour, a very weak aroma, and a bland flavour. Blanching and air-drying both significantly reduce protein quality. The texture, colour, flavour, and aroma are important characteristics of dried krill and generally reflect the quality of the product (Lin *et al.*,

1998a). Prices for the final product vary from $60.00 U.S. per kg to $75.00 U.S. per kg depending on the quality, the quantity, and the type of drying method used (Argent Chemical Laboratories, 2001).

3. Chemical Composition

Yanase (1974) reported the proximate constituents of raw, whole krill to be as follows: water 80 %, crude protein 12 %, crude lipid 3 %, chitin and carbohydrate 2 %, and ash 3 %.

a. Moisture Content

Weight loss caused by heating is equal to the weight of water in animal tissue or foods, but in the case of krill, the weight loss caused by heating indicates a 1 % to 2 % higher value than the actual weight of water in krill tissue. The reason for this is that there are low amounts of volatile components formed when krill is heated (Suzuki, 1981). The average moisture content for raw krill is 80 %. The average moisture contents for processed krill are as follows: cooked 78 %, paste 71 %, meal 10 %, and powder 5 %. These values do vary slightly with time due to effects from collection, handling throughout, mode of preparation, storage, and packaging (McWhinnie and Denys, 1978).

b. Protein Content

Fresh, whole krill contains a high amount of water-soluble protein (50 % to 60 % on a dry matter basis). Of the total protein in krill, 58 % is sarcoplasmic protein (water-soluble) (6.1 % wet weight of krill) and 17 % is myofibrillar protein (salt-soluble) (3.7 % wet weight of krill) (Budzinski and Dutkiewicz, 1985). Non-protein nitrogen accounts for 21 % of the nitrogen in krill (Budzinski and Dutkiewicz, 1985).

(i). Amino Acid Content

The nutritional value of krill protein rests in its amino acid content (Table 2.1). The potential of krill as a food source is enhanced by its high concentration of essential amino acids. The essential amino acids constitute 45 % of the total amino acids of krill (Suzuki, 1981). Krill, as compared to many other foods, is considered high in tryptophan, threonine, lysine, methionine, cysteine, phenylalanine, alanine, and glycine (Suzuki, 1981).

(ii). Nutritive Value

Judging from the amount of protein and the amino acid content, the nutritive value of krill as a food or feed product is considered high. Feeding tests have been conducted with experimental animals. Arai *et al.* (1976) fed dehydrated krill powder to rats for fifty-one days and compared the weight gains, the digestibility, and the protein efficiency ratios (P.E.R.) to a casein-fed group. No significant differences ($p \geq 0.05$) involving weight gains or P.E.R.s were found. The digestibility of the crude protein from krill was deemed better than the digestibility of casein (Arai *et al.*, 1976).

Obatake (1980) fed boiled krill to rats for five months. The animals' body weights, internal organ weights, serum cholesterol, and serum iron were measured. It was reported that at a level of 10 % protein in the feed, no differences between the krill and casein-fed groups were found. The nutritive values of the whole, boiled krill were as follows: P.E.R., 3.16; biological value (B.V.), 75.0 %; and net protein utilisation (N.P.U.), 69.8 % (Obatake, 1980).

Iwatani *et al.* (1977) also examined the nutritive values of boiled krill, boiled krill without viscera, and defatted, freeze-dried, raw krill. The results found krill protein to have a nutritional value equal to casein. Raw krill was found to have a lower nutritional value than

Table 2.1. Amino acid content of *Euphausia superba* (Horbowska *et al.*, 1979).

	Euphausia superba **(mg amino acid/100 mg protein)**
Amino Acids	
Alanine	4.68
Arginine	6.32
Aspartic Acid	9.55
Cysteine	1.03
Glutamic Acid	12.52
Glycine	4.00
Histidine	2.51
Isoleucine	6.97
Leucine	4.34
Lysine	7.62
Methionine	2.55
Phenylalanine	4.36
Proline	4.12
Serine	3.79
Threonine	3.84
Tryptophan	1.02
Tyrosine	3.73
Valine	3.95

boiled krill, however, the value improved when the viscera were removed or when the protein was isolated (Iwatani *et al.*, 1977).

c. Lipid Content

From various studies, it has been observed that the lipid content of krill varies with the age of the animal and the season of its harvest. The lipid concentrations increase with the krill's age but after spawning the levels rapidly decrease. Almost 70 % of the lipids in krill are located in membranes found under the shell (Budzinski and Dutkiewicz, 1985). In one study, it was found that in *E. superba* captured in December the lipid content was at a level of 13.3 % dry weight basis, while in January it was at 27.1 % dry weight basis (McWhinnie and Denys, 1978). In a second study, it was found that in *E. superba* captured in December the lipid content was at a level of 4.2 % dry weight basis, while in March it was at 30.5 % dry weight basis (Burukovskiy, 1967). These differences may be related to phytoplankton availability (McWhinnie and Denys, 1978).

The lipid classes include phospholipids, free fatty acids, monoglycerides, diglycerides, and triglycerides, as well as sterols and their esters (Budzinski and Dutkiewicz, 1985). The natural level of free fatty acids in krill is about 4 %, however, contents as high as 31 % of the total lipids have been observed. These higher levels are believed to be a result of lipase and phospholipase activity (Ellingsen and Mohr, 1981). Approximately 43 % of krill lipid is saturated fatty acids and 56 % is unsaturated fatty acids (with an iodine value of 110 to 190) (Grantham, 1977). The relatively high level of unsaturated fatty acids in krill will undergo rapid oxidation and lead to rancidity (McWhinnie and Denys, 1978). The unsaturated long-chain fatty acids, linoleic acid, linolenic acid, and arachidonic acid are present in high quantities in the lipid fraction. Bottino (1974) reported that the fatty acid composition of krill was similar to that of

phytoplankton collected at the same site, suggesting that krill incorporate the lipid from their food with little metabolic conversion.

The fatty acids present in *E. pacifica* as compared to canola oil are shown in Table 2.2. The content of phospholipids in krill lipids have been reported to range from 30 % (Arai *et al.*, 1976) to 58 % (Bottino, 1974). Unsaponifiable matter in the krill oil has been determined to be between 4.7 % and 13.1 %, about half of which is cholesterol. The total cholesterol content was found to be 1.7 % to 7.6 % of krill lipid and 0.06 % to 0.07 % of krill tissue (Suzuki, 1981).

d. Carbohydrate Content

Carbohydrates account for approximately 2 % of the wet weight of krill (McWhinnie and Denys, 1978). Chitin represents approximately 1.5 % of the wet weight of krill (Suzuki, 1981).

e. Ash and Mineral Contents

Mineral levels in krill are comparable to those of most crustaceans and other animals. Ash derived from krill is high in sodium, magnesium, phosphorus, and calcium. Over thirty elements can be detected in krill. According to Kinumaki (1980), 54.5 mg % of calcium was found in the shell, and 3.7 mg % to 7.3 mg % in the remainder of the body. Copper was detected at a level of 3.8 mg % in the meat and at 8.7 mg % in the shell. Zinc was not detected in either portion (Kinumaki, 1980). Soevik and Braekkan (1979), by analysing the whole body of Antarctic krill (*Euphausia superba*) and Atlantic krill (*Meganyctiphanes norvegica*) detected very high values of fluoride, 1 330 μg/g to 2 400 μg/g (lipid free, dry matter) in the samples. These values are twelve to twenty-two times the permissible limit of 110 μg/g whole body (db) of fluoride in shellfish (Soevik and Braekkan, 1979). According to the report, the value was

Table 2.2. Percent fatty acids in *Euphausia pacifica* and Canola Oil.

	Products (%)	
	E. pacifica[1]	Canola Oil[2]
Fatty Acids		
4:0	-	7.1
12:0	-	-
14:0	3.8 - 4.7	-
16:0	22.8 - 28.5	4.0
16:1	6.4 - 8.1	0.2
18:0	1.4 - 2.5	1.8
18:1	13.9 - 15.1	56.1
18:2	1.9 - 2.9	20.3
18:3	0.6 - 1.2	9.3
20:0	0.5 - 1.0	0.7
20:5	27.9 - 30.9	-
20:1	1.0 - 2.5	1.7
22:0	0.0 - 0.8	0.4
22:1	1.2 - 1.3	0.6
22:6	6.4 - 16.1	-
24:0	-	0.2

[1] Data are expressed as a range of percentages (Pierce *et al.*, 1969).
[2] Data are expressed as percentages (U.S.D.A., 2001).

relatively low in the muscle at 570 μg F/g (db), but high in the exoskeleton, carapace, and the cephalothorax (Soevik and Braekkan, 1979). These authors stated that: "this would make krill in any form, even peeled, fail to comply with requirements for human consumption."

Christians *et al.* (1981) proved that fluoride migrates from the shell to the muscle in frozen krill. The lowering of the temperature to -40°C stops this process. Similarly, boiling of the raw material arrests the migration (Christians *et al.*, 1981). During the Fifth Polish Antarctic expedition in 1981, the muscle of freshly caught krill was shown to contain about 40 μg/g fluoride (db). The presence of fluoride in the krill shell was also established, however, the mechanism of fluoride binding in the shell has not been fully explained. There are no papers on the remineralisation of fluoride from the exuviae and dead animals or on the pathway of fluoride in the Antarctic food chain (Budzinski and Dutkiewicz, 1985).

f. Vitamin and Carotenoid Contents

Every euphausiid synthesises (in the liver) vitamin A. This vitamin is made from pro-vitamin A, which is obtained from the diet. The vitamin A is stored by krill in moderately high concentrations (Kathman *et al.*, 1986). The majority of the pro-vitamin A found in krill is in the form of astaxanthin (a carotenoid), which is highly concentrated in the eyes. *Euphausia pacifica* contains on average, 80 μg/g (db) to 120 μg/g (db) of astaxanthin in its tissues (Suzuki, 1981; Argent Chemical Laboratories, 1999; and Mori *et al.*, 1976). This is a highly marketable and profitable quality.

Watanabe *et al.* (1976) found that krill contains (in wet tissue) between 164 μg vitamin E/100 g body weight and 781 μg vitamin E/100 g body weight. Yanase (1971) reported that krill, on a wet weight basis, contains 1.6 μg riboflavin/g body weight, 1.1 μg vitamin B_6/g body weight, 0.16 μg vitamin B_{12}/g body weight, 15 μg panthothenate/g body weight,

70 μg niacin/g body weight, and 0.10 μg biotin/g body weight.

B. Chitin

1. Structure

Chitin (poly-*N*-acetyl-β-D-glucosamine) (Figure 2.2, (Windholz, 1976)), a polymer, is the second most abundant organic material in nature. It is second only to cellulose, to which it is structurally related. Chitin is a polysaccharide consisting predominantly of unbranched chains of β-(1-4)-2-acetamido-2-deoxy-D-glucose residues (also named *N*-acetyl-β-D-glucosamine (NAG)). In chitin, as compared to cellulose, the acetamido residues have replaced the C-2 hydroxyl groups (Saborowski *et al.*, 1993).

2. Chitin and Krill

Chitin, which contains 6.89 % nitrogen (Pierce *et al.*, 1969), is a principal component of exoskeletons of krill, shellfish, and insects and it can be degraded by bacteria, fungi, and many invertebrates (Jeuniaux, 1966). Chitin represents approximately 5 % to 10 % of the dry weight of krill (McWhinnie and Denys, 1978; Suzuki, 1981; Pastuszewska *et al.*, 1983). In crustaceans, protein is often associated with the chitin and calcium carbonate in the shell matrix. The protein can amount to 30 % to 40 % of the organic matter in the shell (Richards, 1951; Tofon, 1948).

The results of previous experiments indicate that some of the negative effects of krill meal on animal performance may be related to the presence of the shell fraction and are probably caused by a high fluoride content in the shells (Pastuszewska *et al.*, 1983).

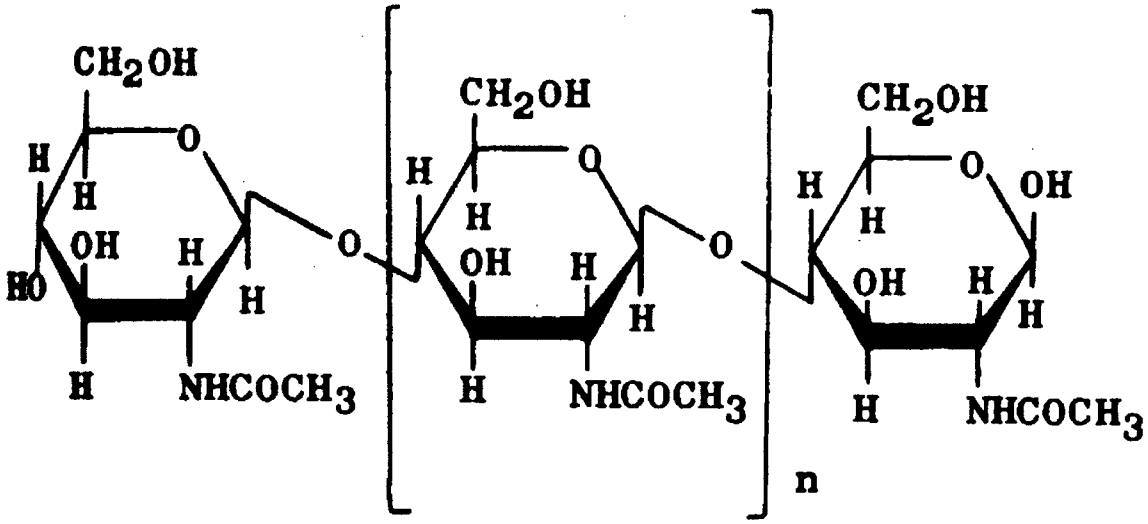


Figure 2.2. Chemical structure of chitin.

High activities of chitinolytic enzymes are present in the stomach and integument of *Euphausia superba* and *Meganyctiphanes norvegica*, where they act as digestive and moulting enzymes, respectively (Saborowski and Buchholz, 1991). The natural degradation of chitin to *N*-acetyl-β-D-glucosamine (NAG) is performed by a two-enzyme system consisting of an endochitinase, namely, poly-β-(1,4)-2-acetamido-2-deoxy-D-glucoside gluconohydrolase - otherwise known as chitinase, (EC 3.2.1.12) and an exochitinase, namely, *N*-acetyl-β-D-glucosaminidase or NAGase, (EC 3.2.1.29). The endochitinase hydrolyses the chitin to oligomers (Powning and Irzykiewicz, 1965), which are subsequently degraded to aminosugars by NAGase (Saborowski *et al.*, 1993).

Chitin is a waste product of the seafood processing industry with an estimated 1.2×10^5 tonnes annually accessible on a world-wide basis, krill chitin accounts for 5.6×10^4 tonnes (Knorr, 1991). Present production of chitin is concentrated mainly in Japan and in the United States. Prices for the final product vary depending on the quality, the quantity, and the type of derivatives ordered.

3. Laboratory Studies

The effects of dietary chitin supplements on growth and function of gerbils (Watkins and Knorr, 1983) and rats (Sugano *et al.*, 1980) have been studied. Up to 8.5 % chitin addition showed no growth inhibition.

The chitin content in meals produced from different catches of whole krill have been measured and found to range from 2.5 % dry matter to 7.3 % dry matter (Pastuszewska *et al.*, 1983). Considerable efficiency of digestion of fungal chitin was found in pigs and rats (Smith *et al.*, 1975) and according to Kühl *et al.* (1978), krill chitin was partially digested by rats and its nitrogen absorbed. These authors suggested that the decomposition of chitin takes place mainly

in the caecum due to bacterial activity, as the weight of the caecum in animals given feeds containing krill shells was greatly increased (Pastuszewska *et al.*, 1983).

C. Fluoride

1. Introduction

Fluorine (relative atomic mass of 19), the most electronegative of all elements, is ubiquitous. It is estimated as being the seventeenth most abundant element of the earth, the thirteenth of the crust, and the twelfth of the ocean (Eagers, 1969). It rarely occurs in its elemental state, but combines chemically with all elements except oxygen and nitrogen, and therefore, reacts vigorously with most organic compounds. Fluorine reacts with non-metallic elements to form covalent compounds, as well as metallic elements to form readily soluble compounds that are usually ionic, both in the crystalline state and in solution. Fluoride ions have a strong tendency to form complexes with heavy metal ions in aqueous solutions. Fluorine, found in many compounds, is widely distributed in nature and occurs in varying amounts in the atmosphere, soil, water, vegetation, and in body tissues (National Academy of Sciences, 1974). Fluorine constitutes approximately 0.06 % to 0.09 % by weight of the upper layers of the lithosphere (Koritnig, 1951).

Animals normally ingest some fluoride (2 mg F^-/kg body weight to 20 mg F^-/kg body weight) without adverse effects (Suttie, 1969). Indeed, fluoride in small amounts may be beneficial, but it is harmful when ingested in excess (National Academy of Sciences, 1974). The most common sources from which animals consume excessive fluoride are feed supplements and mineral mixtures, or water and forages/soils subjected to air-borne contamination from nearby industrial operations such as aluminium and steel mills, and enamel factories (National Academy of Sciences, 1974).

Most waters naturally contain some fluoride. In river and irrigation waters, the amounts vary widely from 0.1 mg F^-/L to 100 mg F^-/L (World Health Organisation, 1970), being the lowest in calcareous regions. Certain spring waters, especially hot springs, contain relatively high amounts of fluoride, between 25 mg F^-/L and 50 mg F^-/L, which have been derived from local mineral deposits (Neuhold and Sigler, 1960). The fluoride concentration in seawater averages 1.3 mg F^-/L (Mason, 1974), which continuously exposes marine plants and animals to fluoride. Most of the fluoride in seawater has come from rivers. The seas lose fluoride in the form of aerosols to the atmosphere, by precipitation as insoluble fluorides, and by incorporation in the carbonate- or phosphate-containing tissues of living organisms (Carpenter, 1969).

Fresh mackerel, salmon, sardines, and codfish have fluoride concentrations that range approximately from 6 μg F^-/g whole body (wb) to 27 μg F^-/g whole body (wb) (Muhler, 1970). Sands *et al.* (1998) examined a range of Antarctic marine crustaceans and found that euphausiids had the highest overall fluoride concentrations; levels up to 5 477 μg F^-/g dry weight were reported found in the exoskeleton of *E. crystallorophias*, while copepods had the lowest fluoride levels at 11 μg F^-/g whole body (wb). Sands *et al.* (1998) also reported that the fluoride in *E. superba* was concentrated in the exoskeleton, but that it was not evenly distributed. The exoskeleton of the head, carapace, and abdomen contained the highest concentrations of fluoride - the mouthparts were reported to have almost 13 000 μg F^-/g dry weight.

2. Fluoride Toxicosis

a. Definitions and Symptoms

The term fluoride toxicosis is used to describe a condition that results from an excessive ingestion of fluoride. Consumption can induce either an acute toxicosis or a debilitating chronic condition that is referred to as chronic fluoride toxicity, or fluorosis.

Acute fluoride toxicosis is relatively rare and has most frequently resulted from accidental ingestion of high levels of fluoride compounds, such as sodium fluorosilicate and sodium fluoride. The term acute is used to mean 'of short duration', as applied to materials that are inhaled, absorbed, or ingested in a single dose. It refers to a single exposure measured in seconds, minutes, or hours. The rapidity with which the symptoms appear depends upon the amount of fluoride encountered. In rats, the twenty-four hour LD_{50} values of sodium fluoride or sodium monofluorophosphate are reported as being 98 mg F^-/kg body weight and 102 mg F^-/kg body weight respectively (Grunninger *et al.*, 1988).

The onset of chronic fluoride toxicosis is usually insidious. The term chronic means, 'of long duration', as applied to materials which are inhaled, absorbed, or ingested over prolonged or repeated exposures. It is measured in days, months, or years, is usually progressive, and subject to varied manifestations. Symptoms in livestock develop progressively at total dietary fluoride concentrations above 20 mg F^-/kg body weight to 30 mg F^-/kg body weight (World Health Organisation, 1984). Some of the symptoms of the disease may be confused with those of other trace element toxicoses or deficiencies.

High fluoride contents in the blood and the urine from a daily dietary intake of 3.0 mg F^-/kg body weight may result in various symptoms. These include: dental defects, the teeth are modified in shape, size, colour, orientation, and structure; osseous lesions, bones become chalky, rough, and porous; motor restlessness/spasms; stiffness and lameness from inflamed joints; gastroenteritis due to the necrosis of mucosa of the digestive tract; nausea and vomiting; anorexia and weight loss; weakness and a general unthriftiness; drowsiness or a profound desire to sleep; dry hair; alopecia; thick, dry, non-pliable skin (Shupe *et al.*, 1963b); excessive salivation and thirst; reduced milk production (in females); incontinence of urine and faeces; degeneration of the kidneys and liver, changes in the size of endocrine glands such as the

thyroid, parathyroid and suprarenal (Eagers, 1969); and cardiac failure. It is difficult to define a precise point at which fluoride ingestion becomes harmful as it can vary from case to case and may be influenced by several factors. These factors include the amount of fluoride ingested, the duration of ingestion, the solubility of the fluoride ingested, the presence of dietary nutrients or additives that may alleviate the toxicity, and the animal's species, age, general level of nutrition, stress factors, and individual biological responses (National Academy of Sciences, 1974).

The impairment of feed intake is not in itself a good diagnostic aid, as many toxicities and deficiencies such as phosphorus and vitamin D (Eagers, 1969), are now known to bring about the same result. Analytical determinations of the components of the diet, the degree of dental fluorosis, the amount of fluoride in the bone and in the urine, and the presence of intermittent lameness and lesions (National Academy of Sciences, 1974) are all of particular importance in the diagnosis of fluoride toxicosis.

b. Influence of Dietary Components

Several cations are known to have an influence on the toxicity of fluoride. Aluminium salts exert a protective effect against fluoride in rats (Becker *et al.*, 1950), sheep (Becker *et al.*, 1950), and cattle (Majumdar and Ray, 1946) by reducing the absorption of fluoride from the intestinal tract (Becker *et al.*, 1950). Calcium salts function similarly, converting soluble fluoride ions to calcium fluoride (CaF_2). Sodium chloride added to the feeds of rats depresses the skeletal uptake of radioactive fluoride (Ericsson, 1968). Ericsson (1968) suggests that competition between chloride and fluoride ions for transport across the gastric or intestinal wall may account for the observed reduction.

The lipid level in the diet is another factor that influences fluoride toxicity (Underwood, 1971) and has been attributed, in part, to increased retention of fluoride in the heart, kidneys, and

skeletal tissues in chicks (Bixler and Muhler, 1960). Increasing the lipid content using corn oil, cottonseed oil, or lard from 5 % to 20 % in high fluoride rations has been reported to aggravate the growth-retarding effects of fluoride in the rat (Büttner and Muhler, 1958) and in the chick (Bixler and Muhler, 1960). It also resulted in a higher fluoride retention in the whole carcasses, specifically in the femurs and the soft tissues (Bixler and Muhler, 1960). These effects are unrelated to the chain length of the fatty acid (Miller and Phillips, 1956). The drastic increase in faecal lipase activity in the intestine (Suttie and Phillips, 1960) is probably of additional significance. It seems logical to assume that this phenomenon becomes increasingly deleterious to the fluorotic animal as its dependence upon dietary fat as a source of energy is raised (Underwood, 1971).

3. Relative Toxicity of Various Forms of Fluoride

Under most conditions, the more soluble forms of fluoride, such as sodium fluoride, are more toxic per unit of fluoride than are the insoluble compounds, such as calcium fluoride. Mitchell and Edman (1952) and Hobbs *et al.* (1954) have ranked the relative toxicity, as determined in rats, of various substances per unit fluorine as follows: sodium fluoride, 110 mg/kg to 190 mg/kg; cryolite (Na_3AlF_6), 150 mg/kg to 230 mg/kg; rock phosphate (for example, fluorapatite [$CaF_2{\cdot}3Ca_3(PO_4)_3$]), 120 mg/kg to 160 mg/kg; and calcium fluoride 230 mg/kg to 2300 mg/kg of the dry diet. Particle size may also have a bearing on toxicity. It has been found that the finer the particle size of cryolite, the more its toxicity approaches that of sodium fluoride (Lawrenz and Mitchell, 1941).

3. Metabolism of Fluoride

The concentration of free fluoride is generally considered more significant than total

fluoride in assessing the effects of excessive fluoride intake on normal metabolism; however, if fluoride binds to an enzyme, to a substrate, or to the co-factor of given enzymatic processes, the bound fluoride will affect the overall process (National Academy of Sciences, 1974).

Most commonly, fluoride is absorbed and enters the body fluids by way of the lungs or the gastrointestinal tract. The half-time for gastric absorption is thirty minutes (National Research Council, 1993). Fluoride may also be released within the body because of the biotransformation of fluorine-containing organic molecules such as the volatile anaesthetics (halothane and methoxyflurane). Plasma is regarded as the 'central compartment' because it is this fluid from which, and into which, the ion must pass for its subsequent distribution and elimination. The quantitatively important fates of absorbed fluoride are uptake by calcified tissues and excretion in the urine (Whitford, 1989).

Several dietary, environmental, physiologic, and pathologic variables can quantitatively affect the metabolic pattern of fluoride. Observations of humans (Carlson *et al.*, 1960a) and rats (Stookey *et al.*, 1962) have suggested that 75 % to 90 % of fluoride is readily absorbed in the stomach by simple diffusion and that the absorption is inversely related to the pH of the gastric contents (hydrofluoric acid (HF), pK_a= 3.4, is the permeating moiety) (Whitford and Pashley, 1984). Hydrofluoric acid has been shown to induce adverse structural and functional effects in the gastric mucosa of rats and dogs at concentrations of 190 μg F^-/mL. These effects range from the loss of the mucous layer and scattered desquamation of mucous cells, to widespread erosion of the gastric mucosa (Whitford, 1990). Absorption across the oral and gastric mucosae is strongly pH-dependent. Studies with rats indicate that fluoride absorption across the intestinal mucosa is not pH-dependent (Nopakun and Messer, 1989). Absorbed fluoride is distributed rapidly throughout the body as the fluoride ion, in a pattern similar to that of chloride (in the chloride space). It readily crosses cell membranes (Underwood, 1971). Approximately half the

fluoride that is absorbed is excreted in the urine. The urinary excretion of fluoride promptly increases with an increase in dietary intake (Whitford, 1989). It is generally believed that the bulk of the faecal fluoride, which approximates 10 % to 25 % of the daily intake of fluoride (National Research Council, 1993), was never absorbed from the gastrointestinal tract. Under most conditions, fluoride elimination in sweat (approximately 0.02 μg F^-/mL to 0.06 μg F^-/mL), saliva, nails, and hair are very small fractions of all that is excreted (National Research Council, 1993). The remainder of the fluoride is incorporated primarily in the calcified tissues. These are associated with 99 % of the body's fluoride (Whitford, 1989).

5. Fluoride and Enzymes

Little is known about the *in vivo* effects the low levels of fluoride, which occur naturally in body fluids and soft tissues, have on enzymes and the various facets of general metabolism of the living organism (World Health Organisation, 1970). What is known, is that fluoride ions have both activating and inhibiting effects on enzymes (World Health Organisation, 1984). It has been reported that at certain 'optimal' concentrations (U.S. Environmental Protection Agency, 1980; Wiseman, 1970), fluoride activates a variety of physiological processes.

Fluoride at a concentration of 0.18 μg F^-/mL in serum will stabilise and activate several isolated, as well as membrane-bound enzyme systems, while at a concentration of 0.3 μg F^-/mL, fluoride in serum will inhibit many enzymes (World Health Organisation, 1984). The ions may exert a direct action on the enzymes, but more frequently, the effect is indirect by their complexing with the metals of enzymes. Not much is known in regards to the mechanisms of activation of these processes by fluoride, but it is likely that fluoride, through its ability to complex certain metals, unmasks active surfaces of the enzyme that were previously covered by calcium or other metals (World Health Organisation, 1984). When a particular metabolic

pathway is blocked by fluoride, the cellular organisation can stimulate and exploit an alternative pathway for cellular function that may appear as activation by fluoride of the enzyme concerned (World Health Organisation, 1984).

6. Fluoride Homeostasis

Fluoride is concentrated in skeletal and dental tissues as the inorganic mineral fluorapatite. Only minor concentrations of fluoride occur in body fluids and soft tissues. An individual on a long-term, relatively constant fluoride intake reaches equilibrium between intake and retention. At this point, uptake by the skeletal tissue is reduced and the concentration of fluoride in the urine approximates that of the fluoride in the diet (Zipkin *et al.*, 1956).

7. Fluoride Retention

The levels of fluoride in the bones and teeth increase in proportion to the amount, form, duration, and continuity of the fluoride intake, and with the age of the animal. The relationship is logarithmic rather than linear. The rate of deposition is dependent on the fluoride content of the bone. The rate is also dependent on the age of the animal, as the rate of deposition of fluoride in the bones of older animals is less than in younger animals (Miller and Phillips, 1956). Balance studies in a number of subjects over several weeks of observation suggest that fluoride retention may range from 35 % to 50 % (World Health Organisation, 1984).

The quantities of fluoride retained in the body tend to be larger with increases in the dietary level of fluoride. The retention may range from about 12 % between 30 mg F^-/kg diet and 40 mg F^-/kg diet, to 57 % at about 100 mg F^-/kg diet (Cass, 1961).

8. Plasma Fluoride

Approximately 75 % of the total fluoride content found in blood is in the plasma, 5 % of which is bound to protein (Carlson *et al.*, 1960b). The ionic concentration of fluoride in rat serum has been reported to be 0.01 μg F^-/mL to 0.04 μg F^-/mL, representing 15 % to 70 % of the total plasma fluoride (Singer and Armstrong, 1964). Peak plasma concentrations usually occur within thirty minutes to sixty minutes of absorption. The elimination rate of fluoride from plasma exceeds the absorption rate after the bulk of the fluoride has been absorbed (Whitford, 1989). Plasma fluoride content is maintained within narrow limits, even when there is considerable variation of dietary intake. This is done by regulatory mechanisms involving principally the skeletal and renal tissues (Singer and Armstrong, 1964). The ability of body-regulating mechanisms to maintain a constant internal environment is influenced by such factors as the skeletal fluoride load, the physiologic statuses of the skeleton and kidneys, previous fluoride exposure, and the rate and amount of fluoride consumption. The regulatory mechanisms can be overridden by sustained, increased intakes, but a new equilibrium between bone, plasma, and body fluids quickly re-establishes. This has been observed with rats during periods of high fluoride intake, or during long periods of food deprivation (Singer and Armstrong, 1964). In rats, significant increases in plasma fluoride concentrations following large, incremental dietary fluoride intakes have been reported. Simon and Suttie (1968) found concentrations of up to 3.3 μg F^-/mL plasma of rats receiving 600 mg F^-/kg diet as NaF.

9. Soft Tissue Fluoride

The fluoride level in the plasma tends to be reflected within the various soft tissues of the body (Carlson *et al.*, 1960). A steady-state distribution between extracellular fluids or plasma and intracellular fluids is rapidly established (Whitford, 1989). The fluoride concentrations of

several of the body fluids, including saliva, bile, and urine, are also related to those of plasma in a steady-state manner. The mechanism underlying the transmembrane migration of fluoride appears to be the diffusion equilibrium of hydrofluoric acid (Whitford, 1989).

It has been demonstrated that the adjustment of the fluoride content of muscle tissue does not vary directly with plasma fluoride levels (Armstrong *et al.*, 1966) and may be more efficiently regulated than that of plasma. In these studies, rats fed a diet containing 0.5 mg F^-/kg had a mean total plasma fluoride content of 0.17 μg F^-/mL, whereas the plasma fluoride of animals receiving food containing 100 mg F^-/kg was only 0.47 μg F^-/mL. The fluoride content of fresh muscle in the two groups of animals was however, very similar, 0.20 μg F^-/g and 0.21 μg F^-/g respectively (National Academy of Sciences, 1974).

10. Role of the Kidney in Fluoride Regulation

The kidney is important in regulating the fluoride content of body fluids, although it is apparently secondary to the skeletal tissues in this respect. The clearance of fluoride by the kidney is directly related to urinary pH (Whitford *et al.*, 1976) and increases with urine volume. Urinary fluoride excretion as F^- and HF occurs by glomerular filtration (Whitford and Pashley, 1991).

It has been shown that the dog kidney, in producing urine, can concentrate fluoride from the plasma by a factor of ten to twenty, and that it functions effectively even when the plasma fluoride is elevated to a level more than ten times that of normal (Carlson *et al.*, 1960c). Studies on the physiologic responses of the rat to a large fluoride challenge provide evidence that rats can control plasma fluoride and dispose of fluoride by urinary excretion. The kidney appears to function normally under considerable stress and variation in fluoride intake (Yeh *et al.*, 1970).

The consumption of water by sheep and swine tends to increase with an increase in the concentration of fluoride in the diet (Eagers, 1969).

A correlation can be demonstrated among the concentration of fluoride in the urine, the amount of fluoride in the dry matter consumed, and the duration of fluoride ingestion, suggesting that during periods of relatively constant fluoride supply, there exists an almost steady-state relationship between absorbed fluoride and excreted fluoride (Shupe *et al.*, 1963a).

The degree of saturation of the skeletal tissue of animals maintained on a constant level of fluoride is a factor that affects the amount of fluoride excreted in the urine (National Academy of Sciences, 1974), as urinary fluoride also arises from the mobilisation and the excretion of a portion of the fluoride present in the skeleton.

D. Dehydration Techniques

1. Introduction

Dehydration is one of the oldest methods of food preservation and it represents a very important aspect of food processing. Although 'dry' and 'dehydrated' are normally considered synonyms, in the field of food process science they are accorded different meanings. Drying is the more general term and may be applied equally to passive drying of plants in the field, to solar drying, to wind drying, as well as to the more technologically advanced drying technologies such as baking and frying. Dehydration on the other hand is generally reserved specifically for the more advanced drying processes that are aided by mechanical or electromagnetic devices (Durance, 2000).

Dehydration is defined as the application of heat under controlled conditions to remove the majority of the water normally present in a food by vapourisation, or in the case of freeze-drying, by sublimation. This definition excludes other unit operations that remove water from

food, for example, mechanical separations. Dehydration is a combined heat- and mass-transfer operation and involves the transport of heat to and within the food, and the transport of water in the food and then away from it. Water may be transported in the food either as bulk liquid or as vapour. The main purpose of dehydration is to extend the shelf-life of food by a reduction in water activity. This inhibits microbial growth and enzyme activity, but the product temperature is usually insufficient to cause enzyme inactivation.

Consumer demands for processed products that keep their original characteristics are growing. In industrial terms, this means the development of operations that lead to the minimisation of the adverse effects of processing. In the particular case of food drying, this means loss of volatiles and flavours, changes in colour and texture, and possible decreases in nutritional value. Thermal damage incurred by a product during drying is directly proportional to the temperature and time involved. Microbial activity and residual enzyme activity are also important factors to consider in dehydration. Rehydration properties of dehydrated foodstuffs are often of crucial importance (Niijhuis *et al.*, 1997).

The quality of dehydrated foods is dependent, in part, on changes occurring during processing and storage. Some of these changes involve modification of the physical structure. These modifications affect texture, rehydratability, and appearance. Other changes are due to chemical reactions, but these are also affected by physical structure, primarily due to effects on diffusivities of reactants and of reaction products (Niijhuis *et al.*, 1997).

One study of energy, labour, and capital costs conducted by Durance and Liu (1996a) on a commercial pilot-scale drying operation indicated that costs of vacuum microwave dehydration are about 15 % greater than convection drying. By comparison, freeze-drying costs have been reported to be up to 400 % that of convection drying (Fellows, 1988). These figures do vary widely however, depending upon local energy, labour, and fixed costs (Lin *et al.*, 1998a).

2. Air-Drying

Foodstuffs may be dried in air, in superheated steam, in vacuum, in inert gas, and by the direct application of heat. Air is generally used as the drying medium because it is plentiful, convenient, and the overheating of the food can be controlled. As well, no elaborate moisture recovery system is required with air, as is needed with other gases (Desrosier, 1959). Air is used to conduct heat to the food being dried and to carry liberated moisture vapour from the food. As the surface dries, more liquid diffuses to the surface from the interior of the food. The evaporating water leaves behind a residue of precipitated solutes on the food surface - a phenomenon known as case hardening (Durance, 2000). In hot-air convection dryers, water evaporates from the surface of the food, and energy transfer to the surface is usually efficient. However, mass transfer of liquid water from the centre of the food piece to its surface is slow and rate limiting (Durance, 2000).

In regards to the volume of air required in air-drying, more air is required to conduct heat to the food to evaporate the water present than is needed to transport the vapour from the chamber. If the air entering is not dry, or if air leaving the dehydration chamber is not saturated with moisture vapour, the volume of air required is altered. As a rule, five to seven times as much air is required to heat food as is needed to carry the moisture vapour from the food. The moisture capacity of air is dependent upon the temperature (Desrosier, 1959). The heat required to evaporate 453.6 grams of water from food at common dehydration temperatures is 1 161 kJ. The heat of vapourisation is temperature dependent. Air velocities ranging from 91 metres per minute to 304 metres per minute are commonly employed in air-drying. The drying rate increases as the velocity of air flowing over the food increases. The rate also increases the greater the surface area, the more porous the surface, the higher the temperature of the air, and the greater the temperature drop, provided that case hardening does not develop. Almost as

much time may be consumed in reducing the final 6 % moisture as is required to bring the moisture content down from 80 % to 6 %. The drying time increases rapidly as the final moisture content approaches its equilibrium value (Desrosier, 1959).

Hot-air drying is generally carried out at temperatures of 60°C to 90°C in order to achieve efficient drying. Drying times vary from a few hours to fifteen hours or more, depending upon the temperature, piece size, and product. Several hours at these temperatures brings about substantial flavour volatile losses. As water vapour is removed from foods, volatile components involved in flavour and aroma are also depleted. Although dry food may retain intense flavour due to high relative concentration in the dry product, the absolute mass of original flavour compounds is reduced. Enzyme activity may also lead to colour and flavour changes. Oxidation reactions between unsaturated food components and atmospheric oxygen during the drying process can reduce the concentrations of some nutrients such as vitamin A precursors, vitamin C, and certain amino acids. Finally, hot-air drying causes structural changes in the food tissue that impact on texture and rehydration behaviour. Case hardening is characteristic of air-drying and is largely unavoidable with this technology (Durance, 2000).

Durance and Liu (1996a) blanched and air-dried krill. The resultant product had a high moisture content (greater than 12 %), was in both whole and broken form, had a yellow to pale red colour, a very mild, weak aroma, and very little flavour. The blanching and air-drying of the krill significantly reduced ($p \leq 0.05$) its protein content.

Air-drying, because of the low cost, is the most popular commercial drying technique used to preserve perishable products. However, it yields a product with low nutritional values, and poor sensory and rehydration properties (Loch-Bonazzi, 1992). The quality may be improved by the optimisation of the processing conditions (Niijhuis *et al.*, 1997).

3. Freeze-Drying

Freeze-drying is the most prominent example of separation by sublimation. In freeze-drying, water is removed, under vacuum, as a vapour from a frozen substance. The water passes from the solid phase directly into the vapour phase without becoming a liquid *en route*; consequently, it is necessary that the temperature of the sublimation zone in a material being freeze-dried be held below the triple point temperature of the water (0°C, 4.58 mm Hg) or aqueous solution in the material being dried (King, 1971). Water vapour is continuously removed from food by keeping the pressure in the freeze-dryer cabinet below the vapour pressure at the surface of the ice, removing vapour with a vacuum pump, and condensing it on refrigeration coils. As drying proceeds, a sublimation front moves into the food. The latent heat of sublimation (approximately 2.8 kJ/g of water) (King, 1971) is conducted through the food to the sublimation front. Water vapour travels out of the food through channels formed by the sublimed ice and is removed. The rate of drying depends mostly on the resistance of the food to heat transfer and, to a lesser extent, on the resistance to vapour flow (mass transfer) from the sublimation front. Energy transfer is rate limiting for freeze-drying. Commercial freeze-drying times are typically eight to twenty-four hours (Durance, 2000).

Freeze-drying yields a high quality product. The structural rigidity afforded by the frozen material at the surface where sublimation occurs is one reason for the high quality (Durance, 2000). This rigidity largely prevents the collapse of the solid matrix remaining after drying. The result is a dried product with a porous, non-shrunken structure, which facilitates rapid and nearly complete rehydration when water is added to the substance at a later time. As well, the food does not suffer from oxidation during the drying process (Durance, 2000). Other benefits from freeze-drying lie in the low processing temperatures, the relative absence of liquid water, and the rapid transition of any local region of the material being dried from a fully hydrated to a nearly

completely dehydrated state. This rapid transition minimises the extent of various degradative reactions, which often occur during drying, such as non-enzymatic browning (Maillard reactions), protein denaturation, and enzymatic reactions. The low temperatures involved also help to minimise these reactions and reduce those transport rates that control the loss of volatile flavour and aroma species. The lack of the water phase helps to minimise degradative reactions and discourages the transport of soluble species from one region to another within the substance being dried. Case hardening is avoided because water passing through the food as a vapour does not carry entrained solutes (Durance, 2000). Freeze-drying is generally acknowledged to allow the greatest rehydration potential of any food drying technique, both in terms of the rate and of the amount of water uptake (Barbosa-Canovas and Vega-Mercado, 1996).

Durance and Liu (1996a) freeze-dried krill. They found that the freeze-dried krill had a very low moisture content, due to the nature of the drying process, exhibited a pale red colour, initially had a mild aroma, but oxidized quickly to take on a fishy odour, and had a flat or oxidized flavour. The protein retention of the freeze-dried krill was excellent.

As the equipment is large and expensive, capital costs of industrial scale installations are very high. Energy costs are also higher than other drying methods because both freezing and evaporation of water from the frozen phase are energy intensive. In addition, freeze-drying is usually a batch system. Air and vacuum microwave systems can be either batch or continuous, a distinction that substantially reduces labour costs (Durance, 2000).

4. Vacuum Microwave Dehydration

The combination of vacuum and microwave technology can overcome many of the limitations of conventional dehydration technologies. Vacuum microwave dehydration is not a new concept, but until recently, it had not been successfully applied to commercial food

dehydration operations. The combination of modern computer control systems, more economical microwave equipment, and advances in vacuum microwave process science have now made commercial success possible (Durance, 2000), (Figure 2.3).

Vacuum microwave dehydration offers an alternative way to obtain high quality dehydrated products. The low temperature and fast mass transfer conferred by the vacuum (pressure below atmospheric pressure but above the triple point of water) (Yongsawatdiguul and Gunasekaran, 1996a), combined with rapid energy transfer and high internal vapour pressure generated by microwave heating, result in a very rapid, low temperature drying process. This may allow for increased retention of flavour volatiles and nutrients (Yousif *et al.*, 1999; 2000; and Lin *et al.*, 1998a). Moreover, the low levels of oxygen during drying may inhibit oxidation, and therefore, the colour and the nutrient content of products can be largely preserved (Lin *et al.*, 1998a). The texture of a food material may also be modified by vacuum microwave dehydration. The microwave power and pressure can be manipulated to expand the structure of some products, yielding a structure and a texture that are unobtainable by other techniques. In some cases, it may be possible to create a food texture that is similar to that produced by frying (Durance and Liu, 1996b).

Microwaves provide the fastest means available of transferring energy into the interior of biological solids (Durance, 2000). Microwaves are defined as electromagnetic waves in the frequency range of 300 MHz to 300 GHz. There are four narrow bands allocated for use in the microwave range. The two bands most commonly used are 915 MHz and 2 450 MHz.

Microwave energy is not a form of heat. Heat is a secondary effect of an electromagnetic field interacting with matter, such as food. The microwave field changes direction 2 450 million times per second in 2 450 MHz microwave ovens. The conversion of microwave energy into

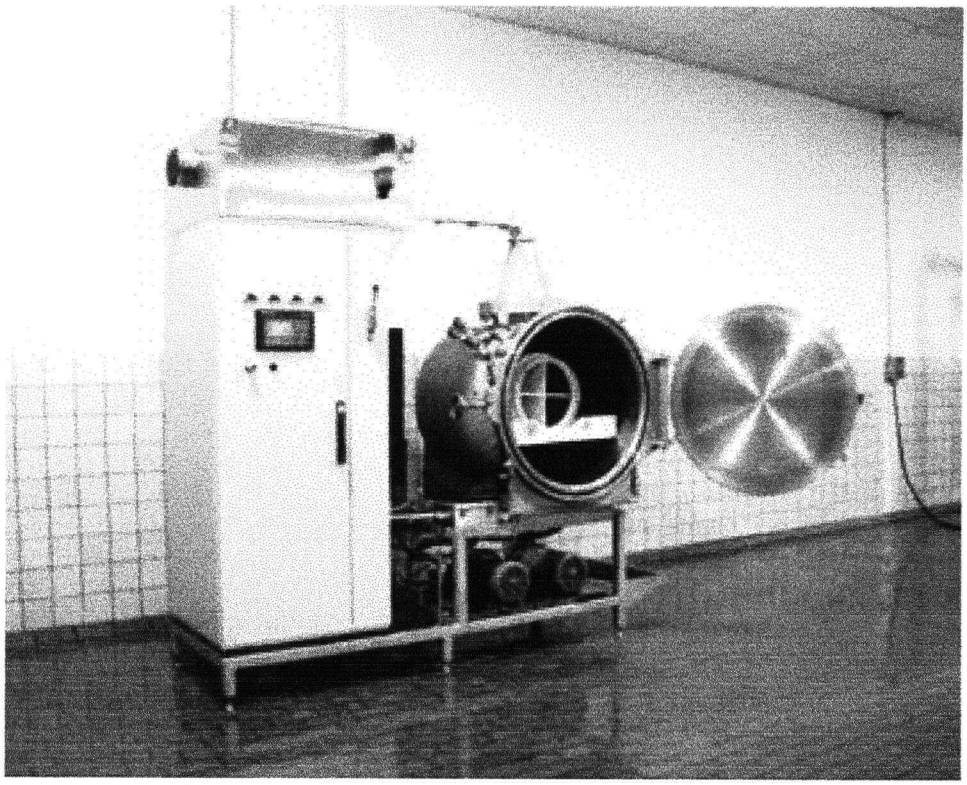


Figure 2.3. Commercial vacuum microwave dehydrator. Photograph courtesy of EnWave Corporation, (Port Coquitlam, B.C.).

heat is explained by two phenomena. First, molecules with a permanent dipolar moment rotate or vibrate in the rapidly changing electric field. When molecules rotate in a field that changes polarity at a frequency of millions of times per second, heat is evolved because of friction forces between the molecules. Second, there is charge drift under the action of the field (ionic conduction). When the ions drift, due to the electric field, they collide with each other and heat is evolved due to friction (Niijhuis *et al.*, 1997).

Water molecules are polar. This means that they can rotate under the influence of an alternating electrical field. Foodstuffs usually contain anywhere between 10 % and 97 % water. Thus, many foods are very well suited for drying with microwave energy. Microwave drying is favourable compared to hot air-drying. This is because in hot air-drying the product surface becomes dry and the dried food layer is a poor conductor of heat in the dehydration process. Microwaves are able to penetrate a dry surface layer and heat the food in high moisture regions. This promotes mass transport and increases the rate of drying. Drawbacks of microwave heating include the following: uneven heating (overheating of the edges of the foodstuff), the difficulty in controlling mass transport by controlling power input, as too rapid a mass transport may cause damage to the food's texture by "puffing" (Niijhuis *et al.*, 1997), and the high cost of building the microwave generators.

In microwave dehydration, most water is evaporated *in situ* within the food and diffuses to the surface as a vapour. Once the boiling point of the solution in the food is reached, positive steam pressure quickly develops within the food, which forces vapour to the surface (Durance, 2000). The available microwave power and the dielectric properties of the food, which define the efficiency of conversion of microwaves to heat, primarily determine the drying rates of microwave processes. Drying time is determined by the ratio of microwave power to the amount of water to be evaporated. If a drying time of five minutes is desired, the operator may simply

reduce the load size. Thus, microwave dehydration can proceed very rapidly. The only limitations are imposed by the microwave power density (W/m^3) within the chamber and the limits of microwave penetration into the load of wet food material. Excessively high power density may cause microwave arcing in the vacuum chamber, particularly if the load of microwave absorbing material in the chamber is small (Durance, 2000).

Unfortunately, drying by microwaves at ambient pressure occurs at too high a temperature for most food products as the food rapidly heats to the boiling point. By combining microwave technology with vacuum technology, one can retain the speed of the microwave but avoid the high temperatures (Durance, 2000). Durance (2000) reported that in a microwave dryer at 760 mm Hg, the boiling point of pure water was 100°C, and at 200 mm Hg, the boiling point of pure water was 68°C.

Evaporation of moisture within the food is rapid during microwave drying, as water boiling within the food results in a large vapour pressure differential between the centre and the surface of the product. Moderate levels of vacuum can reduce the boiling point within the drying chamber. When a vacuum and microwave heating are combined, food can be dried very quickly without exposure to high temperatures, thus, the high and intermediate moisture periods, when enzyme activity is a problem, are traversed before significant quality degradation occurs. During drying in a vacuum, the evaporation of moisture is so rapid that the product is kept cool; this slows bacterial and enzymatic deterioration (Burgess *et al.*, 1967). The drying process using a vacuum in combination with microwaves usually requires minutes, rather than hours as required for air-drying, or days as needed for freeze-drying (Lin *et al.*, 1998a). Oxidative deterioration may also be reduced due to the low partial pressure of oxygen within the drying chamber; therefore, texture, form, colour, flavour, and nutrient properties of products can be largely preserved. To date, vacuum microwave dehydration has been successfully used in the

dehydration of animal materials such as shrimp (Lin *et al.*, 1998b), and krill (Durance, 1997; and Durance and Liu, 1996a), as well as plant materials such as potato chips (Durance and Liu, 1996b), carrots (Lin *et al.*, 1998a), cranberries (Yongsawatdiguul and Gunasekaran, 1996a; 1996b), sweet basil (Yousif *et al.*, 1999), and *Echinacea purpurea* (Kim *et al.*, 2000).

Capital costs of vacuum microwave dryers are not well established, as only a few commercial systems are in operation. However, as a rough estimate, capital costs can be expected to be between $6.00 U.S. and $12.00 U.S. per watt of microwave power.

E. Proteins

1. Introduction

The name protein is derived from the Greek word *proteos*, meaning first or primary, which aptly describes its importance. Next to water, proteins are the main building blocks of animal tissues. Every living cell contains proteins, which appear in many different forms and amounts. These molecules, which account for about 50 % of the organic material in the body, play critical roles in almost every physiological process. Proteins are large complex molecules composed of amino acid subunits linked together by peptide bonds. Eight to twelve of the amino acids, depending on the species and physiological state of the animal, cannot be synthesised in adequate amounts by the body tissues and must be supplied in the diet. These are called "essential" (or indispensable) amino acids (Winter, 1975). The following eight amino acids are essential for maintenance in adult human subjects: lysine, methionine, valine, leucine, isoleucine, threonine, phenylalanine, and tryptophan. Histidine, although unessential for adults, is required by the human infant and by the rat. Similarly, arginine can be shown to be an essential amino acid for the rat under conditions where growth is rapid.

The quality of a protein depends to some extent on its digestibility and absorbability but primarily on its indispensable amino acid composition. Both the specific amounts and the proportions of these amino acids are important. Protein deficiency manifests itself as lethargy, weakness, poor appetite, weight loss, reduced adult size, oedema, anaemia, and permanently lowered disease resistance, and can lead to severe liver damage and death.

The dispensable amino acids serve readily as energy sources. If a protein is consumed that provides more than the amino acid requirements, the residual is deaminated and the carbon chain is oxidized for energy. The excess nitrogen is excreted as urea. The rate of protein breakdown and synthesis depends on the nature of the tissue as well as on the existence and concentration of hormones, such as adrenaline and testosterone, which are influenced by the physiological conditions of the body (Winter, 1975).

Although many factors influence the protein requirements of an organism, the quality of the protein consumed is paramount. Factors impinging on protein requirements, other than the quality of the protein, include the animal's growth, body size, physiologic state, and level of energy intake. The influence of energy intake on the protein requirement is particularly important. Adequate energy intake fosters nitrogen retention, but as intake exceeds adequacy, retention decreases. Insufficient calories from carbohydrates and/or lipids, regardless of the quality or quantity of protein in the diet, mandate the oxidation of protein to supply energy needs (Groff and Gropper, 2000).

Dietary proteins, when ingested, are first subjected to the enzyme pepsin in the stomach, producing oligopeptides and polypeptides, which are then broken down into amino acids. Most of the amino acids are absorbed from the intestine into the blood, and transported to the plasma proteins, to the haemoglobin, to the muscles, to the kidney, and to the liver, which serves as a short-term protein reserve. They enter rapidly into biological equilibrium with the amino acids

of the body, becoming incorporated into newly formed proteins or entering into reactions that supply nitrogen for the synthesis of other amino acids or other nitrogenous constituents of the tissues (Winter, 1975). This is done at such a rate that the level of amino acids in the blood rises very little above normal, even after a protein rich meal. Tissues, except after fasting, have little capacity to store either amino acids or protein (Winter, 1975). The liver, intestinal mucosae, and kidneys have a high turnover rate of protein, while muscle protein is less labile. However, because of its great mass, muscle provides the greatest amount of protein in conditions of deprivation. The plasma proteins do not reflect a significant decrease until tissue protein has been depleted, with clinical evidence of wasting in starvation or protein deprivation (Davidson and Henry, 1974).

2. Protein Metabolism and Nitrogen Balance

The F.A.O./W.H.O./U.N.U.'s definition for the protein requirement of an individual/animal is defined as the lowest level of dietary protein intake that will balance the losses from the body in persons maintaining energy at modest levels of physical activity (F.A.O./W.H.O./U.N.U., 1985).

Since nitrogen is one element that is characteristic of protein, much information concerning protein metabolism has been gained from the study of nitrogen metabolism. The term "nitrogen balance" is often used to reflect the anabolic and catabolic phases of the dynamic state of protein metabolism. Dietary protein represents the majority of the nitrogen intake, and most of the nitrogenous excretory products are derived from protein catabolism. Protein metabolism is reflected in the balance between nitrogen intake from the diet and nitrogen output in the urine, faeces, and menses. For normal serum proteins, 6.54 g of protein yields 1 g of nitrogen. Since normal proteins contain 15.3 % nitrogen, this is equivalent to a nitrogen-to-

protein conversion factor of 6.54 (Davidson and Henry, 1974). The general factor 6.25 is used for most foods based on 16 % nitrogen (Pomeranz and Meloan, 1971).

Proteins in the body are synthesised from dietary amino acids, amino acids from protein breakdown, or amino acids formed by amination of the carbon skeleton from lipid and carbohydrate metabolism. The intimate relationship of carbohydrate, lipid, and protein metabolism is also reflected by protein catabolism, wherein the carbon skeleton generated by deamination of amino acids can be converted to fatty acids and carbohydrates. Protein depletion with a negative nitrogen balance occurs upon inadequate protein intake such as fasting, undernutrition, and malabsorption syndrome, upon excessive protein loss such as protein-losing gastroenteropathy and burns, and in states of accelerated catabolism of tissue protein such as infection, wasting diseases, fever, hyperthyroidism, and hyperadrenocorticism (Davidson and Henry, 1974).

The proteins of the body are continuously undergoing breakdown to, and re-synthesis from, their constituent amino acids. The specialised excretory products of protein metabolism are urea (N_2H_4CO) and ammonia (NH_3). The ornithine cycle, which is primarily concentrated in the liver, removes the toxic ammonia from the body in the form of urea (Davidson and Henry, 1974). Urea is the major waste product of protein catabolism. It constitutes about one-half of the total urinary solids, and comprises 80 % to 90 % of the total urinary nitrogen (Davidson and Henry, 1974). It, like other non-protein nitrogen constituents, is present in the blood and excreted in the urine.

Blood plasma amino acids may be considered to be in transit from one organ or tissue to another, either for synthesis or degradation (Davidson and Henry, 1974). Only traces of amino acids are normally found in the urine. Plasma and urinary amino acids are usually expressed as α-amino nitrogen (Davidson and Henry, 1974).

3. Evaluation of Protein Quality

Protein quality is most often assessed either by animal bioassays or by chemical scores. Over the last forty years, a large number of different biological procedures have been suggested for the measurement of protein quality. Results obtained by biological methods are a function of the limiting amino acid in the diet. They give little direct information about the supplementary value of the protein if given in combination with other proteins (Schultz and Anglemier, 1964). Methodologies for the determination of the quality of proteins, based on nitrogen balance studies or rat growth, were developed in the early 1900's. Methods for analysis of protein quality have included the officially recognised A.O.A.C. rat protein efficiency ratio, various *in vitro* enzymatic digestibility assays such as the pepsin digest residue and the pepsin-pancreatin digestion, and various calculated protein efficiency ratios, which take into account a protein's essential amino acid profile in comparison to a reference protein such as the chemical score method and the essential amino acid index.

Although the nutritional quality of a protein source is related to its amino acid composition, it cannot be reliably predicted from amino acid composition alone. Bioavailability, which consists of the digestibility, the absorbability, and the utilisability of amino acids, is one factor that must be considered since it can be affected by characteristics of the protein itself, including protein conformation and intramolecular cross-linking. The presence of inhibitors and the modification of amino acids by technological treatments can also affect amino acid bioavailability. Amino acids, as determined chemically, are not necessarily wholly available to animals. It is well known that heating causes a lowering of the nutritive value of protein. These losses are associated with the following factors: Maillard reactions in which amino groups react with an aldehyde group from a reducing sugar or other source, the breaking of disulphide cross-linkages in proteins to form more resistant bonds that gut enzymes are unable to split, and

absolute losses of amino acids by heat destruction. Methods combining enzymatic hydrolysis and amino acid analysis represent an attempt to take into account all of these characteristics. *In vitro* methods should reproduce the *in vivo* process with great precision so that the digestion products are similar in both cases.

The rate of growth of an animal under controlled conditions is related to the quality of the dietary protein, and has been used widely to evaluate proteins in foods. The validity of applying the results of animal growth studies to the evaluation of feeds for other animals is dependent upon the degree of similarity in protein metabolism between the two species.

4. Biological Methods

a. Protein Efficiency Ratio (P.E.R.)

It is widely recognised that studies that measure growth and/or other metabolic indicators, including nitrogen balance, provide the most accurate assessment of protein quality. Consequently, assay techniques designed to measure the effectiveness of a protein in promoting animal growth have been utilised. Since 1919, the protein efficiency ratio method, which measures the ability of a protein to support growth in young, rapidly growing rats, has been used in many countries (Food and Agriculture Organisation of the United Nations, 1991). Male rats are more sensitive to differences between proteins than female rats, which also tend to give maximal values at lower protein levels (Schultz and Anglemier, 1964).

The protein efficiency ratio is calculated as follows (Food and Agriculture Organisation of the United Nations, 1991):

$$\textbf{P.E.R.} = \frac{\textbf{weight gain of test group}}{\textbf{protein consumed by test group}}$$

Values are adjusted so that the weight gained per unit of protein ingested by animals fed the test diet is ranked against the same response criterion for a control group of animals fed a diet

containing casein. Values of the test protein are adjusted to that which would be expected if a P.E.R. of 2.5 were obtained for casein (Walker, 1983). The major criticisms of the P.E.R. method are that the results are positively correlated to food intake. As a P.E.R. is reduced by a low food intake, it does not properly credit protein used for maintenance purposes, and the results are not directly proportional to the quality of the protein. A protein source may not support growth and have a P.E.R. near zero, yet still be adequate for maintenance purposes, and because of this, the P.E.R. values of proteins of differing quality are not proportional in protein quality. A P.E.R. of 2.0 cannot be assumed to be twice as good as a P.E.R. of 1.0 (Food and Agriculture Organisation of the United Nations, 1991).

Rat bioassays for protein quality are essentially a measure of the amount of the limiting amino acid available to the test animal. The A.O.A.C. procedure for the biological evaluation of protein quality of a foodstuff requires that a number of nutritional factors be modified in order to standardise the reference casein and test diets. The type and amount of dietary lipids, the fibre level, the mineral composition, and the moisture level of the diet have relatively little influence on the P.E.R. of a food product. This is provided that the estimate was adjusted for the assay value determined for a reference casein protein source of a similar composition. The level of protein of the test diets is of critical importance and should be closely standardised (Hurt *et al.*, 1975).

b. Nitrogen Balance

The classical approach for determining protein biological value and net protein utilisation or for obtaining other quantitative estimates of the nutritional value of a protein directly in experimental animals and human subjects involves nitrogen balance studies.

The relation between the amount of nitrogen entering the body from the diet in the form of amino acids and the amount of nitrogen excreted from the body in the form of metabolic end-products is known as the nitrogen balance. The nitrogen balance is positive if intake exceeds output, negative if output exceeds intake, and if intake and output are essentially equal, there is nitrogen equilibrium and a balance of zero is the result (Oser, 1965).

Nitrogen balance may be defined by the equation:

$$\mathbf{B = I - (U + F)}$$

where B is the balance, I is the intake of nitrogen, and U + F is the nitrogen lost in the urine and faeces, respectively (Food and Agriculture Organisation of the United Nations, 1991).

Nitrogen balance studies can provide a direct measure of nitrogen retention, and hence, of the nutritional value of a protein. The balance represents the sum of the gains and losses of nitrogen by all body tissues and thus, gives an overall idea of nitrogen utilisation, but does not measure changes that may occur in the nitrogen content of various individual body compartments. The amount of nitrogen required to establish an equilibrium varies between proteins, and hence provides some information about the nutritive value of proteins and of protein requirements. The fact that animals can adapt to an altered nitrogen intake and achieve nitrogen balance at various levels of nitrogen intake however, makes interpretation of such data difficult (Schultz and Anglemier, 1964).

There is no real distinction between nitrogen balance and growth methods, both of which reflect the efficiency of retention of ingested nitrogen. Balance procedures directly measure the amount of nitrogen deposited in the tissues of growing animals, a value that is related directly to weight increases (Bender, 1958). This method tends to overestimate nitrogen intake and underestimate nitrogen excretion. It also ignores dermal nitrogen losses and losses due to bacterial fermentation of carbon skeletons.

The nitrogen balance methods have received concern due to the possibility of systematic overestimation of retention of nitrogen indicated by erroneous positive balances from improper adjustment periods before tests are performed, and the impact of varying levels of energy intake, stress, and physical activity. Despite these criticisms, if collection of losses and intake of nitrogen are properly performed, the nitrogen balance method is currently the only method allowing a balance to be measured over a period of one or more days.

c. Biological Value (B.V.)

Biological value is a measure of the nitrogen retained for growth and/or maintenance. It is expressed as a percentage of nitrogen absorbed. This procedure takes into consideration the fact that nitrogen-containing compounds are lost in the urine and faeces even if nitrogen-containing foods are not consumed. However, it does not consider digestibility.

The biological value of proteins is most often determined in experimental animals, but it can be determined in humans as well. The biological value is calculated as follows:

$$\textbf{B.V.} = \frac{[I - (F_N - F_K) - (U_N - U_K)]}{[I - (F_N - F_K)]} * 100$$

= (nitrogen retained / nitrogen absorbed) * 100

where I is intake of nitrogen, F_N is faecal nitrogen, F_K is endogenous faecal nitrogen, U_N is urinary nitrogen, and U_K is endogenous urinary nitrogen (Food and Agriculture Organisation of the United Nations, 1991).

Proteins exhibit a higher biological value when they are fed to the test animal at levels below the amount necessary for nitrogen equilibrium. As intake of the protein approaches or exceeds adequacy, retention decreases. An ideally balanced protein is given a biological value of 100. It may, however, require 2.4 times as much protein from a poor quality source such as wheat gluten to eliminate the negative nitrogen balance (Winter, 1975).

d. Net Protein Utilisation (N.P.U.)

Another biological measure of protein quality that includes an evaluation of protein digestibility as well as of the content of indispensable amino acids is net protein utilisation. This measures retention of food nitrogen rather than retention of absorbed nitrogen and is calculated as follows:

$$\textbf{N.P.U.} = \frac{\mathbf{I - (F_N - F_K) - (U_N - U_K)}}{\mathbf{I}}$$

where I is intake of nitrogen, F_N is faecal nitrogen, F_K is endogenous faecal nitrogen, U_N is urinary nitrogen, and U_K is endogenous urinary nitrogen (Food and Agriculture Organisation of the United Nations, 1991). In essence, it represents biological value multiplied by the digestibility of the protein.

Although net protein utilisation can be measured in humans, a more accurate measurement is made on experimental animals through direct nitrogen analysis of the animal carcasses. In either case, one experimental group is fed the test protein, while the other group receives an isocaloric, protein-free diet.

Net protein utilisation is not only a characteristic of the feed but also of animal's age, sex, body size, state of health, energy needs, and metabolism. Net protein utilisation declines with increasing levels of protein in the diet. Any dietary deficiency that leads to slow growth will lead to a reduction in the efficiency of protein utilisation (Winter, 1975).

5. Chemical Methods

a. Amino Acid Chemical Score

Procedures using chemical composition have been applied to the evaluation of proteins. Amino acid profiles of proteins can be compared relatively easily using a scoring pattern. The use of amino acid composition data for the evaluation of protein values of foods and feeds has

been widely used since the amino acid composition of egg (B.V. is approximately 100) was introduced as a standard by Block and Mitchell (1946) and adopted by F.A.O. in 1957. The amino acid scoring method is simple to use and allows for the identification of the limiting amino acid however, as a method of protein quality evaluation, no account is taken of amino acid availability or protein digestibility (Food and Agriculture Organisation of the United Nations, 1991).

The chemical score procedure involves expressing the amount of each indispensable amino acid in the test protein as a percent of that in the standard protein. The lowest score for any amino acid then becomes the chemical score for that foodstuff on the theory that the limiting amino acid determines the nutritional value for the whole protein. Lysine, threonine, tryptophan, and the sulphur amino acids are often the limiting amino acids in food proteins; therefore, the chemical scores of these amino acids are often sufficient to evaluate the nutritive value of proteins. This method appears to be a relatively good measure of feeding value in the young rat, and presumably in other monogastrics on a single food diet (Food and Agriculture Organisation of the United Nations, 1991). The amino acid chemical score is defined as follows (Food and Agriculture Organisation of the United Nations, 1991):

$$\textbf{Amino Acid Chemical Score} = \frac{\textbf{(mg of amino acid in 1 g of test protein)}}{\textbf{(mg of amino acid in 1 g of reference protein)}} * 100$$

An assumption underlying the chemical score is that all test proteins are fully or equally digestible and that all essential amino acids are fully absorbed. However, as this assumption is often violated, correlation between results from bioassays and chemical scores is often poor, yet the correlation can be improved when chemical scores are corrected for protein digestibility. The apparent digestibility of proteins can be rapidly determined *in vitro* using a combination of three or four enzymes, such as trypsin, chymotrypsin, peptidase, and protease (Food and Agriculture Organisation of the United Nations, 1991).

Another shortcoming of the chemical score is that it does not distinguish between D- and L-amino acids. Since only L-amino acids are usable in animals, the chemical score overestimates the nutritive value of a protein, especially in proteins exposed to high pH levels, which causes racemisation. The chemical score method does not account for the effect of anti-nutritional factors that might be present in the diet (Food and Agriculture Organisation of the United Nations, 1991). Despite these drawbacks, recent findings indicate that chemical scores, when corrected for protein digestibility, correlate well with biological assays for those proteins having biological values above 40 %. When the B.V. is below 40 %, the correlation is poor (Food and Agriculture Organisation of the United Nations, 1991).

b. In Vitro Assays

There are considerable advantages to using *in vitro* methods for protein quality evaluation. In general, *in vitro* methods are less time-consuming and require less of a sample, hence, more samples can be assayed at one time, but less information about the food material will be obtained. In particular, *in vitro* procedures do not detect the presence of unexpected toxic components of foods (Walker, 1983). Data from *in vitro* methods may be less variable than *in vivo* data, but caution is required in the interpretation of this, as precision may not necessarily be of biological significance. Therefore, it is necessary that *in vitro* methods are adequately correlated with rat assay methods or carried out with humans (Walker, 1983). Overall, the enzymatic methods tend to underestimate the true digestibility for humans of both plant and animal proteins (Food and Agriculture Organisation of the United Nations, 1991).

6. Protein Digestibility

Not all proteins are digested, absorbed, and utilised to the same extent. Differences in protein digestibility may arise from several different situations. These include: inherent differences in the nature of food protein such as protein configuration and amino acid bonding; the presence of non-protein constituents which modify digestion such as dietary fibre, tannins, and phytates; antiphysiological factors; and/or processing conditions that can alter the release of amino acids from proteins by enzymatic processes. In recognition of this fact, amino acid scores should be adjusted for "true" protein digestibility, in which case allowance is made for the faecal nitrogen loss on a protein-free diet or as "apparent" digestibility for which no such correction is made (Food and Agriculture Organisation of the United Nations, 1991). The classic procedure for determining digestibility has been the faecal index method, an *in vivo* procedure in which the nitrogen excreted in the faeces is subtracted from the amount of nitrogen ingested and the value is expressed as a percentage of intake. This gives an apparent digestibility value, which is calculated as follows:

$$\textbf{A.D.} = \frac{\textbf{(I - F)}}{\textbf{I}} * \textbf{100}$$

where I is intake nitrogen and F_N is faecal nitrogen (Food and Agriculture Organisation of the United Nations, 1991).

To determine true digestibility, it is necessary to correct for faecal nitrogen excreted when the subject is consuming either a protein-free diet, or a diet with just enough of a highly digestible protein to prevent excessive loss of body protein.

True digestibility is calculated as follows:

$$\textbf{T.D.} = \frac{\textbf{I - (F}_N \textbf{ - F}_K\textbf{)}}{\textbf{I}} * \textbf{100}$$

where I is intake nitrogen, F_N is faecal nitrogen, and F_K is metabolic or endogenous faecal nitrogen (Food and Agriculture Organisation of the United Nations, 1991).

True digestibility gives information on the percentage of nitrogen intake absorbed by the body. However, it does not provide information of how much of the absorbed nitrogen is actually retained or utilised by the body. Since true digestibility measurements take into account the metabolic faecal nitrogen, which is not of dietary origin, true digestibility of a food is always higher than the apparent digestibility. Apparent protein digestibility values increase with increasing protein intakes, whereas true digestibility values are independent of protein intake (Food and Agriculture Organisation of the United Nations, 1991).

7. Limiting Amino Acids

a. Lysine

Lysine ($NH_2(CH_2)_4CH(NH_2)COOH$), is reported to be the first essential amino acid rendered unavailable during heat processing or upon unfavourable storage conditions and therefore, its availability can be used to monitor processing damage. Lysine may easily deaminate, be oxidized, or undergo Maillard browning reactions (Eldred and Rodney, 1946; Henry *et al.*, 1948). Autoclaving the protein may render lysine less available to enzymatic digestion, possibly by promoting the Maillard reaction (Smith and Pena, 1977). It is rendered unavailable due to its epsilon (ε) amino group combining with other active groups under conditions of moist heat and forming a linkage that resists hydrolysis by enzymes, and therefore, its nutritive value is lost (Carpenter, 1958).

Lysine is easier to measure in its available, reactive form than other amino acids. The most satisfactory and best-established chemical procedure for lysine determination is the fluorodinitrobenzene (FDNB) method of Carpenter (1960). This method has been correlated

with the chick assay, although it does not correlate well with the rat assay. The presence of large quantities of carbohydrates in a food may interfere with the reaction of FDNB with lysine, leading to an underestimation of lysine availability of a protein (Carpenter and Booth, 1973). Another difficulty is that the Carpenter method does not always give satisfactory data for samples that have been processed under mild conditions, underestimating the extent of lysine availability (Hurrell and Carpenter, 1974). Heat applied during dehydration procedures does reduce the availability of lysine and partially destroys it (Holm, 1971).

More recently, dye-binding methods have been modified to estimate available lysine in foodstuffs. Various reagents have been used for this in the past such as ethyl chloroformate and trinitrobenzene sulphonic acid (Jones, 1974). The dye-binding methods have the disadvantage of requiring rigid standardisation of procedure and establishment of suitable conditions for each class of food material.

b. Sulphur Amino Acids

The sulphur amino acids, cysteine ($HSCH_2CH(NH_2)COOH$) and methionine ($CH_3SCH_2CH_2CH(NH_2)COOH$), are susceptible to oxidation-reduction, alkylation, photolysis, and other reactions. Hydrolysis introduces many problems, as the sulphur amino acids are partly destroyed by acid treatment at high temperatures and interact with other components commonly present (Schultz and Anglemier, 1964).

Another aspect of prime nutritional significance concerning the critical amino acids, aside from limiting amino acids, is their instability during processing, cooking, or even on prolonged storage under certain conditions (Schultz and Anglemier, 1964). It is known that there is a loss of availability of methionine due to oxidation or demethylation during processing - when

oxidized, methionine sulphoxides and/or methionine sulphone are formed (Schultz and Anglemier, 1964).

8. Protein in Marine Products

Seafoods, namely fish, crustaceans, and molluscs, are important sources of protein in human nutrition. Approximately 11 % (wb) to 27 % (wb) of seafoods consist of crude protein; however, contribution of non-protein nitrogenous (NPN) compounds to this value may reach up to a quarter of this amount.

Seafood proteins, like those of all other muscle foods, may be classified as sarcoplasmic, myofibrillar, and stroma. The sarcoplasmic proteins, mainly albumins, account for approximately 30 % of the total muscle proteins. The myofibrillar proteins in muscle are myosin, actin, actomyosin, and troponin and account for 40 % to 60 % of the total crude protein content of fish. The remainder of the muscle proteins, classified as stroma, are mainly collagenous matter from connective tissues.

F. Enzymes

1. Introduction

Enzymes are organic catalysts produced by living organisms. With the exception of a small group of catalytic RNA molecules, all enzymes are proteins. Enzymes, like other proteins, have molecular weights ranging from about 12 000 to over one million. They are generally soluble, colloidal substances characterised by great activity, specificity, and susceptibility to the influences of pH, temperature, and other environmental changes (Oser, 1965). Enzymes have the ability to bring about vast increases in the rates of reactions and commonly produce rate enhancements of 10^6 to 10^{12} (Solomons, 1992). Their catalytic activity depends upon the

integrity of their native protein conformation (Lehninger *et al.*, 1993). The energy used for enzymatic rate enhancements is derived from weak interactions (hydrogen bonds and van der Waals, hydrophobic, and ionic interactions) between the substrate and enzyme (Lehninger *et al.*, 1993). Enzymes are classified according to the type of reaction they catalyse. There are six classes: Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerases, and Ligases.

2. Enzymes and the Storage and Processing of Foods

When an organism is gathered for food, the enzymatic processes continue. Although the animal is dead, the enzyme systems continue to function until there is an exhaustion of substrate, or the pH has changed until it is unfavourable for enzyme action. The hydrolytic enzymes, such as the proteolytic enzymes, esterases, phosphatases, and glucosidases, continue to act on the cellular components long after the animal is dead. In fact, activities of these enzymes increase because of the gradual destruction of the cellular organisation and the denaturation of the proteins, acted on by proteolytic enzymes. Since there is a major cessation of anabolic reactions after death and a continuation and even acceleration of catabolic reactions, the net result is a deterioration of tissue. This is not necessarily bad since it may contribute to the flavour and the texture of the material (Whitaker, 1972).

An understanding of factors that influence enzyme activity can be a great asset in controlling and manipulating the activity of enzymes post-harvest. The variables most easily manipulated are temperature and substrate.

a. Temperature

Storage at lower temperatures slows the rate of enzyme activities and delays the time at which the quality of the product becomes unacceptable. It does not follow however, that for all

raw materials, the lower the temperature, the longer the product can be stored. Storage of some foods at, or just below 0°C, actually leads to an observed increase in enzymatic activity (Whitaker, 1972). This is a result of increasing accessibility of substrates to enzyme either as a result of concentration of the aqueous medium of the tissue and/or through damage to the cellular components by the large ice crystals formed by the slow freezing process (Whitaker, 1972). Storage should be either above or several degrees below 0°C, never at 0°C to -5°C unless there is a desire to increase enzymatic activity (Whitaker, 1972).

Freezing does not destroy enzyme activity. It only slows the activity thereby prolonging the storage life. When food has not received a short blanching treatment before freezing, there is a marked acceleration of enzyme activity immediately upon the thawing of the food (Whitaker, 1972).

Those enzymes which have molecular weights ranging from 12 000 M.W. to 50 000 M.W., are composed of single polypeptide chains, and/or have disulphide bonds, are quite resistant to heat treatment. The larger the enzyme and the more complex its structure, the more susceptible it is to high temperatures (Whitaker, 1972). In general, an enzyme is more stable to temperature in an intact tissue or in an homogenate, where its structure is protected by the presence of other colloidal material, than it is in a purified form (Whitaker, 1972).

Increasing the temperature of an enzyme reaction has two disproportionate effects; it increases the rate of the reaction proper, and it increases the rate of inactivation of the enzyme. As a rule of thumb, an increase of 10°C will cause the reaction rate to double, while the inactivation rate will increase sixty-four-fold. This effect depends upon time (Stauffer, 1989). The spontaneous, irreversible denaturation of enzymes at elevated temperatures is a process that is a first-order reaction with respect to the enzyme (Stauffer, 1989).

b. Moisture

Enzymes are generally sensitive to moist heat conditions, especially where temperatures range above the maximum for enzyme activity. Moist heat temperatures near the boiling point of water find enzymes nearly instantaneously inactivated. There are exceptions, but as a rule, one minute at 100°C, renders enzymes inactive (Desrosier, 1959). Enzymes require solvent to be active. Their activity is reduced with decreasing moisture, but a concentration of enzyme and substrate simultaneously occur. There is no activity at moisture levels below 1 % (Desrosier, 1959).

When exposed to dry heat at temperatures such as those used in dehydration, enzymes are notably insensitive to the effect of the energy. Short exposures to temperatures near 204°C have little effect on enzymes if the heating medium and the enzyme preparation are dry (Desrosier, 1959).

c. Perturbing Factors: Non-Simple Substrates

Many substrates for enzymes of interest to food scientists are not well-defined chemical species of known molecular weight, they are not soluble, so the kinetic analysis may not be made using molarity as a measure of the substrate's reactivity, and they are not stable, so the formation of product is not all due to enzymatic catalysis (Stauffer, 1989).

Inhibitors can retard enzyme reactions. Often these inhibitors are not added intentionally, but appear unintentionally. If their action is not recognised, they can lead to erroneous conclusions about the system and/or inaccurate assay reports.

Endogenous inhibitors may be present in crude extracts containing the enzyme being assayed. These usually low-affinity, reversible inhibitors are removed at some subsequent purification step, if enzyme purification is being performed. Should the enzyme study be carried

out on the crude extract, endogenous inhibition may be more of a problem. This is manifested by a marked non-linearity in the plot of the concentration of the product versus volume of extract.

3. Quantitative Estimation of Enzyme Action

The amount of enzyme present in any mixture is expressed in terms of its activity as defined in arbitrary units. Either the time required for a given amount of enzyme preparation to bring about a definite degree of conversion of the substrate, or the amount of preparation needed to bring about a definite degree of conversion in a specified time, may be made the basis of comparison. Inasmuch as enzyme action is greatly influenced by the pH of the solution, by the presence of inorganic salts and activators, and by temperature, it is important that the conditions in the reaction mixtures be controlled as closely as possible and that the presence of possible inhibitory components, especially in crude mixtures, be properly corrected for (Oser, 1965).

In quantitative studies of enzyme action, it is best to have a large excess of substrate and to keep the length of the experiments within the period when decomposition is proportional to time. To establish these limits an experiment must be conducted and a curve plotted showing the extent of decomposition at different time intervals. In certain cases, direct proportionality does not exist and thus, a standardisation curve relating the extent of reaction under defined conditions to the amount of enzyme added, is made the basis for comparison (Oser, 1965).

According to the recommendation of the International Union of Biochemistry (1961), an enzyme unit is defined as the amount of enzyme required to modify, under standardised conditions, 1 μmole of substrate per minute, or in the case of polysaccharides and proteins, 1 μequivalent per minute. The assay should be carried out, if possible, at 25°C, and at an optimum pH, and the substrate concentration should provide for enzyme saturation.

Measurements should be made in the range of initial velocity so that the kinetics follow a zero order reaction and substrate modifications are related in a linear manner to enzyme concentration. This requires short reaction times. In the case of lengthy measurements, the reaction velocity is extrapolated to zero time. Concentrations of enzymes should be expressed in units per cubic centimetre, and specific activities in units per milligram protein (International Union of Biochemistry, 1961).

4. Measurements

The most fundamental operation in any enzyme assay is the measurement of a concentration, either of product or of substrate. Measurement methods may be divided into two groups: direct and indirect. A direct measurement gives some reading, which is related to the concentration of product or substrate while the enzymatic reaction is proceeding, without the need for intermediate manipulations. Detection is by means of some instrument whose response changes as a solution property changes because of the reaction. Direct methods are generally the quickest, give continuous information about the reaction, and are convenient for initial rate assays. Indirect methods require a fixed incubation period followed by other manipulations before a reading related to product or substrate concentration is obtained. They are discontinuous, although by taking samples for analysis from a reaction mixture at frequent intervals an approximation of a continuous curve may be obtained. They allow a large number of assays to be performed concurrently, with the manipulations being carried out batch-wise after the enzyme runs are finished (Stauffer, 1989).

5. Proteases

a. General

The earliest enzyme studies were concerned with digestion and with brewing, and thus, there are numerous reports on the action of proteases (pepsin in stomach juices by Spallanzani in 1784) and amylases (diastases from malt by Berthelot in 1833) available. Given this early start, it is not surprising to note that studies of proteases and amylases have generated a greater variety of assays than any other group of enzymes (Stauffer, 1987).

For proteases, the reaction of interest is the hydrolysis of the peptide bond in a food protein: R-C=O-N(H)-R' + H_2O → R-C(=O)OH + H_2N-R'. From this comes the generic term "peptide hydrolases" or peptidolytic enzymes. The usual determinants of specificity are the side chains R and R' of the amino acids, although prior or successor amino acid members in the primary sequence of the protein chain being split may also be involved. In addition to the peptide bond, some peptide hydrolases will also catalyse hydrolysis of esters of compounds that are structurally related to the amino acid substrates (Stauffer, 1987).

The term "protease" is understood to apply to an enzyme having endo-peptidolytic activity (cleaves peptide bonds internal to a polypeptide protein chain). A "peptidase" is an enzyme having exo-peptidolytic activity (cleaves peptide bonds either at the N-terminal end of the polypeptide such as aminopeptidase or at the C-terminal end such as carboxypeptidase). In general, proteases will not hydrolyse a peptide bond if the R moiety has a free amino group or if the R' moiety has a free carboxyl group, whereas a peptidase requires that the appropriate group is freely ionisable (Stauffer, 1987).

Proteases may be broadly divided into four classes, based upon the mechanism of their enzymatic action: aspartic (or acidic), serine, sulfhydryl, and metallo (or neutral) (Stauffer, 1987). There are a couple examples of proteases such as calcium-activated proteases and low

molecular weight proteases, which do not fall into these four classes, but more than 95 % are one of these four types. With twenty naturally occurring amino acids, it is possible to have four hundred different side chain configurations around the peptide bond being hydrolysed by a protease. The specificity of a protease consists of both a binding constant and a rate factor (Stauffer, 1987). Substrate proteins also vary in the frequency and distribution of the different possible peptide bonds (Stauffer, 1987). Haemoglobin and casein are respectively the first and second most commonly employed substrate proteins for protease assays. This fact leads to the conclusion that, while protein-based assays are most generally applicable to all proteases, they are of severely limited value in comparing two different protease preparations being considered for a given application (Stauffer, 1987).

b. Krill Proteases

Seafood quality is becoming increasingly important with developments and expansions in aquacultural production and processing, and international marketing. One of the obstacles to the utilisation of krill protein is its quick autolysis by strong proteases (Jiang *et al.*, 1991). Proteases are also responsible for significant quality changes in fresh shrimp and prawns, (Baranowski *et al.*, 1984). Rapid post-mortem degradation of Antarctic krill has been attributed to muscle and visceral proteases (Chen *et al.*, 1978). Muscle protein readily decomposes into low molecular weight compounds because of these enzymes. The effects of the proteases on actomyosin are the most pronounced in *E. pacifica*, followed by *E. superba.*

Destructive enzyme reactions take place within a few hours at temperatures above freezing, especially when the oxygen content around the krill is high. There may be a loss of protein due to enzyme-catalysed hydrolysis. Vacuum conditions, elevated temperatures during heating, and rapid drying rates substantially reduce or prevent these reactions. During cold

storage (0°C), proteolytic activity increases due to the diffusion of enzymes from the intestines to the muscular tissue, and the raw material becomes unfit for processing into food products. The shelf-life of frozen krill at -18°C is only three months (Food and Agriculture Organisation, 1985).

The activity of krill proteases depends on sex, age (size), physiological condition, and feeding conditions. The activity is particularly high in immature specimens, which is explained by the intensive metabolism connected with the growth of the animal. Seki *et al.* (1977) isolated three types of krill proteases - serine, metallo, and sulfhydryl. These enzymes were stable at a weak alkali or a neutral pH. All three enzymes decomposed krill actomyosin and caused the autolysis of the krill meat. Konagaya (1980) reported that the activity of krill proteases at pH 6 showed fluctuations of ± 30 % between each individual krill body and attributed this to the breakability of the krill body due to the weak structure of the cephalothorax. This implies that once the viscera is destroyed during handling, visceral enzymes will cause additional digestive rupture of the organs themselves and of the surrounding tissue (Suzuki, 1981). From the above mentioned studies it is clear that the autolysis of krill is influenced by protein decomposing enzymes in the viscera, and that removal of viscera can prevent rapid autolysis (Suzuki, 1981). It has been found that over 95 % of protease activity in the krill body takes place in the cephalothorax while the remaining 5 % is in the tail portion (Suzuki, 1981). Removing the contents of the cephalothorax (by centrifugal action or pressing) considerably limits autolytic processes in the raw material.

G. Lipids

1. Introduction

The name lipid comes from the Greek word *lipos*, for fat. Lipids are a group of naturally occurring substances characterised by their insolubility in water and their solubility in solvents such as ether, chloroform, boiling alcohol, and benzene. Chemically, lipids are either esters of fatty acids or substances capable of forming such esters (Oser, 1965). They are widespread in nature, being found in all vegetable and animal matter. Some members of this group, such as the phosphatides and sterols, are found in all living cells where, with proteins and carbohydrates, they form an essential part of the colloidal complex of cytoplasm. Complex lipids are also found in large quantities in brain and nervous tissues, thus, indicating the important role these substances play in the living organism. Other lipids, such as the fats and oils, represent the chief form in which excess nutrients are stored in the animal body. They arise from ingested lipids and from the metabolism of carbohydrates and proteins, and are stored in fat deposits. Lipids act as heat insulators and as reserve supplies of energy (Oser, 1965).

The simple lipids are esters of fatty acids with certain alcohols. They are usually further classified according to the nature of the alcohols. This is done as follows: fats and oils are esters of fatty acids and glycerol; and waxes are esters of fatty acids with long-chain aliphatic alcohols or with cyclic alcohols. These may then be subdivided into true waxes, cholesterol esters, vitamin A and its carotenol esters, and vitamin D esters (Oser, 1965).

The compound lipids are esters of fatty acids that, on hydrolysis, yield other substances in addition to fatty acids and an alcohol. Some important members of this group are: phospholipids such as phosphatidic acids, lecithins, cephalins, plasmalogens, sphingomyelins; glycolipids such as cerebrosides, gangliosides, cytolipins; lipoproteins, consisting mainly of cholesterol esters and phospholipids with principally stearic, palmitic, and oleic acids; and sulfolipids (Oser, 1965).

Derived lipids are substances formed in the hydrolysis of simple or compound lipids that retain the properties of this class of compound. They include fatty acids, alcohols, hydrocarbons, and vitamins D, E, and K (Oser, 1965).

2. The Oxidative Deterioration of Food Lipids

Food lipids undergo extensive deterioration on prolonged exposure to atmospheric oxygen. This process of oxidative rancidity, which is known as autoxidation, results in the development of unpleasant odours and flavours that render the food unpalatable and sometimes even toxic. In lipids containing unsaturated linkages, it has been found that rancidity is primarily due to oxygen attack at, or anywhere along the hydrocarbon chain. It is widely accepted that hydroperoxides are the primary products of autoxidation. Secondary degradation products are formed largely from hydroperoxide decomposition (Ingold, 1962). Some of the routes of decomposition of the hydroperoxides are as follows: polymerisation to dimers and higher polymers; fission to aldehydes, alcohols and then to acids; dehydration to keto-glycerides; further oxidation to diperoxides and then to polymers; and oxidation of double bonds in other molecules to epoxides and OH-glycerides (Lea, 1962).

Lipid autoxidation is accelerated by high temperatures, UV and blue light, ionising radiation, peroxides, lipoxidase enzyme, organic iron catalysts such as haemoglobin, and trace metal catalysts such as copper and iron (Lea, 1962).

3. Digestion and Absorption of Autoxidized Lipids

Lipids entering the small intestine are finely emulsified by bile until enough surface is available for attack by pancreatic lipase. This enzyme, being specific for the α positions on the glycerol, rapidly hydrolyses the fatty acyl groups from these positions and, after the slow

isomerisation of the β-acyl groups to the α position, then starts to attack these groups. The result is a mixture containing mainly fatty acids, some β-monoglycerides, and small quantities of di- and triglycerides, as well as other lipid-soluble materials originally present in the ingested lipid (Mead, 1962).

There are two main types of compounds produced from the autoxidation of lipids. The peroxides and the polymeric materials, including cyclised acids. Peroxides in the intestine destroy antioxidants and vitamins (especially A and E) and deficiency symptoms can be produced in this manner (Mead, 1962). It appears that polymeric lipid products containing C-C or C-O-C bonds cannot be readily digested or absorbed. Monomeric, cyclic, or oxygenated acids may be split from glycerides and then appear in the lymph. During the process of absorption, but not digestion, peroxide groups are reduced, and the resulting oxygenated acids are absorbed. Toxicity of cyclic monomers appears to be exerted systemically while that of the peroxides must occur within the intestinal lumen or cells (Mead, 1962).

4. Lipid Oxidation in Marine Products

Marine products are more rapidly oxidized and reactions are more complicated than those of non-marine foods. This is mainly because of the types of fatty acids present, which are more highly unsaturated and more numerous (Hilditch, 1956). Fish oil fatty acids are distributed among the various triglycerides, phospholipids, and other lipid derivatives, providing an array of individual chemical compounds. Highly unsaturated fatty acid derivatives are oxidized much in the same fashion as those of lower unsaturation. The oxidation is characterised by an induction period, followed by a rapidly increasing rate of oxygen absorption during which rancidity occurs, and it then culminates in the formation of viscous polymers. The reaction is typical of a free radical chain reaction in which hydroperoxides are the first relatively stable intermediates. The

thermal or catalysed decomposition of the hydroperoxides leads to the formation of new radicals. Eventually, low molecular weight carbonyls (ketones and aldehydes) and other breakdown products accumulate. These are responsible for the tastes and odours of rancidity. The high molecular weight derivatives are responsible for the increase in viscosity and the decrease in solubility (Evans, 1961).

The oxidation of fish products is accelerated by light (or other irradiation), heavy metal salts, including haeme substances, oxidative enzymes, and free radicals from any source. It is inhibited by antioxidants. Those fish products that contain larger amounts of unsaturated fat or those with more haeme pigments are the ones more likely to be readily attacked.

In most cases, the oxidation of fresh seafood is not considered a matter of great importance, as the single most important factor involved in deterioration is microbiological spoilage. Stored frozen marine products are subject to oxidative deterioration or rancidity. Deterioration can be delayed by preventing the access of oxygen to the surface of the product (Tarr, 1948).

5. Lipid Oxidation in Dehydrated Foodstuffs

The problems of lipid oxidation in dehydrated foods can be divided into four areas: the relationship of moisture content to oxidative stability; special phenomena associated with the oxidation of lipids in dehydrated food systems; the relationship of enzyme action to oxidative deterioration; and possible interactions between products of lipid oxidation and other food constituents such as proteins (Olcott, 1962).

The water molecules of the monolayer may be regarded as a discontinuous phase, bound by functional groups of proteins and carbohydrates, but having influence over the entire surface of the food. Water attached at these sites, probably by hydrogen bonding, should protect them

from reaction with oxygen, possibly by excluding adsorption of oxygen directly, and/or possibly by co-ordinating trace metals and reducing their catalytic effect (Olcott, 1962). Salwin (1959) demonstrated that for almost every food item the moisture content that was specified for good stability agreed very closely with the amount that represented a monomolecular layer of adsorbed water. Evidence was obtained that the monolayer amount of water does protect against adsorption of atmospheric oxygen. However, oxygen already adsorbed, was not displaced by moisture (Olcott, 1962). Thus, it appears that water, at least in part, exerts its influence by inhibiting the adsorption of oxygen.

Lea *et al.* (1958; 1960) have published the results of an extensive series of tests with stored herring meal. Adsorption of oxygen was found to be a function of moisture content. Meal containing 6 % water was oxidized more readily than meal containing 12 % water. Oxidation was accompanied by a marked drop in iodine value and by a loss in lipid extractability.

The stability of lipids in dehydrated foods depends not only on moisture content but upon a number of factors, many of them process-induced. These may include the rupture of lipid micelles, the smearing of lipids upon new surfaces, thus, giving a greater surface to volume ratio, the relocation of enzymes, and the exposure of an enlarged surface area to air (Togashi *et al.*, 1961).

Many highly reactive intermediates are formed during autoxidation of unsaturated lipids. These are capable of entering reactions with one or more of the functional groups within the protein molecule (Putnam *et al.*, 1953). Consequently, a variety of complex interactions occur between proteins and autoxidizing lipids under suitable reaction conditions. Such interactions produce changes in protein solubility and cause cross-linking of protein molecules resulting in alterations in the physical properties of the dehydrated food.

MATERIALS AND METHODS

III. MATERIALS AND METHODS

All reagents were reagent grade from Fisher Scientific, (Nepean, ON.) unless otherwise stated.

All weighing was done using a Sartorius BP 3105 scale, (Germany) unless otherwise stated.

All pH measurements were done using a Fisher Scientific, (Nepean, ON.) Accumet pH meter model 420 digital pH/ion meter.

All vortexing was done using a Fisher Scientific, (Nepean, ON.) Vortex Genie 2.

A. Acquisition of *Euphausia pacifica*

1. Frozen *Euphausia pacifica*

Protein Plus Inc., (Vancouver, B.C.) generously donated the frozen krill that was utilised in this research project. The shipment of *Euphausia pacifica* received by Food Science, U.B.C., (Vancouver, B.C.) was, upon ocean-harvest, expeditiously deposited into a contracted trawler's 4°C storage unit. The catch was then maintained in the same 4°C receptacle for the remainder of the expedition (another approximately twelve hours). Directly upon the craft's return to port, the haul was unloaded and promptly transported to a processing plant. Once at the plant, the krill was immediately frozen and stockpiled. No further details were released by the company.

The supply of frozen *Euphausia pacifica* was delivered frozen to Food Science, U.B.C. several months before the start of this thesis. It was in the form of six 10 kg, rectangular slabs (approximately 8 cm by 40 cm) that were individually packaged in a plastic wrap. Once received, the blocks were, without delay, placed in a -34°C freezer (Food Science, U.B.C., Vancouver, B.C.).

2. Hydrolysed, Freeze-Dried *Euphausia pacifica* (HZ)

Biozyme Systems Inc., (Vancouver, B.C.) generously donated the hydrolysed, freeze-dried krill that was utilised in this research project. The processed *Euphausia pacifica* was transported to Food Science, U.B.C. in a large plastic bucket that had been lined with a plastic bag. Once received, the 10 kg container of krill was directly placed in the -34°C freezer. It was stored for several months before the start of this thesis. The company would not release any information regarding the specific techniques that were applied in the preparation of this product.

B. Dehydration of *Euphausia pacifica*

1. Freeze-Dried *Euphausia pacifica* (FD) *(Lyophilisation)*

One-third of the supply of the donated frozen krill was removed from the -34°C freezer and allowed to partially thaw overnight in a 2°C walk-in cold room (Food Science, U.B.C., Vancouver, B.C.). The next day, the slabs were sawed into small sections, and the pieces were placed in circular aluminium trays (225 cm (diameter) by 30 cm (depth) (Safeway, Richmond, B.C.)). Once the pans were three-quarters full, they were covered with aluminium foil, which had been punctured (using a pencil) with several 5 mm holes, and left overnight in the -34°C freezer. The following day, the krill was placed in a freeze-dryer (Virtis Research Equipment, Gardiner, N.Y.) and processed at a gauge pressure of 1.6 ± 0.1 mm Hg, with a shelf temperature of 21.0 ± 0.5°C and a condenser temperature of -50.0 ± 0.5°C. Subsequent to the lyophilisation, which required approximately two weeks per batch to complete, the processed krill was transferred from the trays to several polyethylene, 0.8 to 1.0 mil thick, resin bags (55.9 cm (length) by 35.6 cm (width), 13 pounds per bag) (Unisource, New Westminster, B.C.). These were then vacuum packed at settings: vacuum 10; impulse 3.5; and gas 0.0) (Multivac type AG5,

Germany) and stored in the -34°C freezer, until other experimental preparations necessary to the research project had been executed, a period of approximately three months.

2. *Euphausia pacifica* Vacuum Microwaved at a Low Temperature (VL)

a. Vacuum Microwaving

One-third of the supply of the frozen krill was removed from the -34°C freezer and allowed to partially thaw overnight in a 2°C walk-in cold room. The following day, the slabs were sawed into sections, which were subsequently divided into 4 kg (ISHIDA MTC-20 Scale, The Scale Shop, Vancouver, B.C.) portions. The allotments, as required, were then halved and each portion was evenly distributed by hand in a clean, cheesecloth-lined (to prevent product seepage) compartment belonging to a vacuum microwave basket (15.9 cm radius) (model 4B (4 kW batch), Dehydration Research Incorporated (D.R.I.), Vancouver, B.C.). For basket details, refer to Appendix I.

Once prepared, the basket was transferred to a clean, 4 kW, 2 450 MHz, cylindrical vacuum microwave dehydrator (VMD) (pilot plant scale, model 4B, external measurements: 58.4 cm (depth), 38.1 cm (radius); internal measurements: 50.8 cm (depth), 34.3 cm (radius) (D.R.I., Vancouver, B.C.)). For vacuum microwave dehydrator details refer to Appendix II.

Each batch of *Euphausia pacifica* was subjected to 60 ± 2 minutes of vacuum microwave processing under controlled power, temperature, and rotational conditions. The dryer, linked to a vacuum pump (to lower the pressure in the chamber), achieved a pressure of 32 ± 1 mm Hg. The power setting was adjusted to attain a nominal energy level of 4 kW. Four, 1 kW magnetrons supplied microwaves to the unit. Microwave power was measured by the IMPI 2L test (Buffler, 1993). The magnetron was powered at 3.2 kW. The boiling point of water was maintained at a temperature of 30.0 ± 0.5°C. The internal temperature of the processed krill however, exceeded

this temperature by approximately 20°C. Chamber pressure was adjusted with an air flow valve (Air Flow Meter, Dwyer Instruments Inc., Michigan City, IN.), which, to minimise condensation within the chamber (Durance, 2000), bled small amounts of ambient air (3 L/min) into the drying chamber in opposition to the vacuum pump. Finally, the speed controller (Dayton® DC Speed Control, Dayton Electric Inc., Niles, IL.) to the dryer's rotatable metal shaft (responsible for turning the basket) was adjusted to allow the basket to rotate at a rate of 3 rpm. The moisture content of the *E. pacifica* at the end of the vacuum microwave treatment at a low temperature was 28 % by weight. The krill was then air-dried.

The vacuum microwave dehydration procedure was monitored, timed, and when appropriate, terminated, by a data logger/computer (Data Taker, Field Logger DT 100F (Data Electronics Pty. Ltd., Australia)/UBC*pro* Computer and programme Decipher) unit that was connected to the dryer.

b. Air-Drying

Following the vacuum microwave treatment, the krill was thinly, and as evenly as possible, distributed by hand on several air-dryer trays (hand-assembled trays, Food Science, U.B.C., Vancouver, B.C.). For air-dryer tray details, refer to Appendix III. These were then uniformly stacked in the air-dryer (hand-assembled wooden air-dryer, Food Science, U.B.C., Vancouver, B.C.). For air-dryer details, refer to Appendix III. The krill was air-dried for 48 ± 2 hours at 30°C (Super Furnace, Super Furnace Electrical Co., Toronto, ON.). During the procedure, the airflow through the tray screens was 1.1 ± 0.1 m^3/s (Turbometer Wind Speed Indicator, Davis Institute, Hayward, CA.) and the relative humidity was 10 %. The moisture content of the *E. pacifica* at the end of the air-drying treatment was 8 % by weight. On completion of the process, the krill was transferred into several plastic bags, vacuum packed as

previously described (refer to **III.B.**1.) and stored in the -34°C freezer until other experimental preparations necessary to the research project had been executed, a period of approximately three months.

3. *Euphausia pacifica* Vacuum Microwave Blanched at a High Temperature and Air-Dried (VH)

a. Vacuum Microwaving

One-third of the supply of the frozen krill was vacuum microwave blanched at a high temperature by Mrs. Linda Wang, D.R.I., (Vancouver, B.C.) one year before the start of this research. The processed *Euphausia pacifica* was prepared, in several batches, by the same methods applied when vacuum microwave dehydrating the krill at a low temperature (refer to **III.B.**2.*a.*), but with the following exceptions. Each batch of *Euphausia pacifica* was subjected to 15 ± 1 minutes of vacuum microwave processing under controlled power, temperature, and rotational conditions. The dryer achieved a pressure of 290 ± 1 mm Hg. The boiling point of water was maintained at a temperature of 75.0 ± 0.5°C. The internal temperature of the processed krill however, did exceed this temperature by approximately 20°C. The moisture content of the *E. pacifica* at the end of the vacuum microwave treatment at a high temperature was 78 % by weight. The krill was then air-dried.

b. Air-Drying

Following the vacuum microwave treatment, the krill was air-dried by the same methods applied when air-drying the krill at a low temperature (refer to **III.B.**2.*b.*). The moisture content of the *E. pacifica* at the end of the air-drying treatment was 14 % by weight.

C. Preparation of the Dehydrated *Euphausia pacifica* (for the Animal Study)

1. Water Activity

The water activities (a_w) of 1 g samples of the four dehydrated krill preparations (as previously described, refer to **III.A.**2. and **III.B.**1. to **III.B.**3.) were individually measured (Aqualab CX-2, Decagon Devices, Inc., Pullman, WA.) at 26.6 ± 0.1°C. Each of the four krill products was evaluated in triplicate.

2. Grinding

The four processed krill preparations (previously described, refer to **III.A.**2. and **III.B.**1. to **III.B.**3.) were individually ground, using a 0.12 mm mesh, in an ultracentrifugal mill (Retsch, ZM 100, Clifton, N.J.) set at 12 000 rpm. The mill was cleaned with a vacuum cleaner between each dehydrated product to prevent cross-contamination.

3. Packaging

Once the krill preparations were ground, they were transferred to separate plastic bags, vacuum packed as previously described (refer to **III.B.**1.), and stored in the -34°C freezer until other experimental preparations necessary to the animal study had been executed, a period of several weeks.

D. Proximate Determination

Proximate determinations, separate measurements allowing one to ascertain the moisture, crude protein, crude lipid, ash, and crude carbohydrate contents of a product, were completed on the frozen *Euphausia pacifica*, the four dehydrated *Euphausia pacifica* preparations (VH, VL, FD, and HZ), and the five animal feeds (refer to **III.E.**1.).

1. Moisture

Moisture analyses were done by measuring approximately 5.0 g, weighed to the nearest 0.0001 g, of each of the samples into new, pre-weighed, aluminium weighing dishes and heating them in a vacuum oven (model 5850-5, serial CAS) (National Appliance Co., Portland, Oregon) at 70.0 ± 0.5°C for approximately fifteen hours. Following this, the tins were placed in a glass desiccator with silica gel until they reached room temperature, at which point they were then re-weighed. On completion of the moisture analyses, the samples were once again placed in a desiccator with silica gel, at room temperature, where they were maintained for future ash analyses. Each sample was evaluated in triplicate.

Percent moisture was calculated as follows:

$$\% \text{ Moisture (wb)} = \frac{\text{(initial weight of sample - final weight of sample)}}{\text{(initial weight of sample)}} * 100\ \%$$

$$\% \text{ Moisture (db)} = \frac{\text{weight of water}}{\text{dry weight of sample}} * 100\ \%.$$

2. Crude Protein

The amount of total organic nitrogen present was determined by The Department of Animal Science, U.B.C., (Vancouver, B.C.) using the Leco Method as described by A.O.A.C. 992.15 (A.O.A.C., 1995). The approximately 0.25 g, weighed to the nearest 0.0001 g, dehydrated samples were placed in an automated Leco FP-328 Nitrogen Analyser (Leco Corporation, Joseph, Michigan, U.S.A.), where they were combusted. The analysis of each sample required approximately ten minutes. The standard for calibration was EDTA (9.58 % nitrogen). The results, which were read directly from a digital display, were multiplied by 6.25 to obtain the crude protein values.

3. Crude Lipid

a. Preparation

Day 1

Lipid analyses were done using Folch's Double Phase Method (Folch *et al.*, 1957). Samples of approximately 0.50 g, weighed to the nearest 0.0001 g, were weighed into desiccated, pre-weighed, 125 mL Erlenmeyer flasks. The remainder of this analysis was then conducted in a fume hood. To each flask, 50 mL of Folch's solution (2:1 chloroform ($CHCl_3$): methanol (CH_3OH)) was added and briefly stirred with a glass rod for even distribution. The flasks were then sealed with parafilm (Parafilm M®, American National, Aurora, IL.) and left in a shaker (Blue M Electric Co., Blue Island, IL.) at room temperature overnight for the lipid extraction.

Day 2

The samples were filtered through fluted Whatman® Number 1 filter paper (5.0 cm radius) (Whatman Ltd., Whatman, England) into lipid-free (cleaned overnight with Extran (diluted 1:50 with ddH_2O) (VWR, Mississauga, ON.)) 100 mL glass-stoppered graduated cylinders. The Erlenmeyer flasks were then rinsed with 10 mL to 20 mL of Folch's solution, which was also filtered into the graduated cylinders. Next, a volume of 0.88 % sodium chloride (NaCl) solution 0.25 times that of the final volume of the solution in each graduated cylinder was added to all the cylinders. These were then stoppered and gently tilted twice to mix the solutions. This was the first wash. The cylinders were left standing overnight for phase separation.

Day 3

The top CH_3OH layer containing aqueous soluble compounds was siphoned off each graduated cylinder, using tap suction, and discarded. Care was taken so as not to remove any samples' bottom $CHCl_3$ lipid layer. To each graduated cylinder, 10.0 mL of 3:47:48 ($CHCl_3$: CH_3OH:ddH_2O) solution was then added. This was the second wash. The cylinders were stoppered, gently tilted twice to mix the solutions, and again left standing overnight (for phase separation).

Day 4

The final volume of the $CHCl_3$ lipid layer in each cylinder was recorded, and then the CH_3OH layer was siphoned off and discarded. The total lipids from each of the original samples were dissolved in the saved chloroform layer. This layer, from all the cylinders, was used in the analyses of both the total crude lipids and the fatty acids. Each sample was evaluated in triplicate.

b. Total Crude Lipids

New aluminium weighing dishes were dried overnight in an oven. The next day they were then desiccated, labelled, and pre-weighed. In a fume hood, aliquots of 5.0 mL of the chloroform layers as previously discussed (refer to **III.D.**3.*a. Day 1* to *Day 4*) were pipetted into the respectively labelled weighing dish. These were placed, using tongs, on a hot plate set at 2, as hot as a hand could stand, and evaporated to dryness. The dishes were then transferred, using tongs, to a desiccator with silica gel and left to cool overnight. The following day, they were weighed and the extract weights were determined. Each sample was evaluated in triplicate.

The percent of total crude lipids was calculated as follows:

$$\% \text{ TCL} = \frac{(V_f)(\text{extract / aliquot})}{(\text{sample})} * 100\ \%$$

V_f = final volume of chloroform in the graduated cylinder

Extract = lipid extract weight (g)

Aliquot = aliquot volume (mL)

Sample = original sample weight (g).

c. Fatty Acids

This experiment was conducted in a fume hood. First, 10.0 mL of each of the chloroform layers as previously discussed (refer to **III.D.**3.*a. Day 1* to *Day 4*) were transferred into respectively labelled 20 mL screw cap test tubes. Next, the tubes were placed in a rack in a 35.0 ± 0.5°C waterbath (Blue M Electric Co., Blue Island, IL.), evaporated to dryness under nitrogen gas (Praxair, Vancouver, B.C.), and then cooled to room temperature. Once cool, 2.5 mL of 0.5 M methanol-potassium hydroxide was pipetted into each of the test tubes, which were then capped and vigorously shaken until the lipids were completely dissolved. Following this, the tubes were placed in a rack in a 50.0 ± 0.5°C waterbath for 60 ± 1 minutes. Once removed and cooled to room temperature, 1.0 mL of petroleum ether (37.8°C to 53.5°C) was added to each of the test tubes. They were tilted twice to aid mixing, and then allowed to stand for approximately twenty minutes for phase separation. The upper layer of ether and non-saponifiables was then siphoned off and discarded. Next, the tubes were grouped together in 500 mL beakers, and to each tube were added, two boiling chips, followed by one to two drops of 0.4 M hydrochloric acid (HCl), and then 5.0 mL of boron trifluoride (Sigma Chemical Co., St. Louis, MO.). Caps were only loosely placed on the test tubes to allow gas to escape. Slowly and carefully, 100 mL of boiling water was added to the beakers. Over an approximately twenty-

minute period, cooling boiled water was slowly added, to fill the beakers. Water was added only after the reaction (bubbling in the test tubes) slowed. Once the test tubes cooled to room temperature, a period of approximately sixty minutes, to each test tube, two drops of ddH_2O, and then 2.5 mL of hexane were added. They were gently tilted twice to aid mixing, and left to stand until the phases separated. The fatty acid methyl esters were dissolved in the upper layer. The upper layer (hexane) was then carefully transferred to 1.5 mL graduated Eppendorf tubes (Rose Scientist Ltd., Edmonton, AB.) containing a thin layer of drying agent (4:1 sodium sulphate: sodium hydrogen carbonate) on the bottom. Analyses were carried out using a Varian 3700 Gas Chromatograph (GC-17A, Shimadzu, Scientific Instruments Inc., Columbia, MD.) equipped with a flame ionisation detector and an AOC 1400 autoinjector, (Shimadzu, Scientific Instruments Inc., Columbia, MD). A volume of 1.0 mL of each of the prepared samples was individually injected into a silicone fused OmegawaxTM 320, 30 m x 0.32 mm ID capillary column (Supelco Inc., Bellefonte, PA.) with a 0.25 mm film thickness. Helium was used as the carrier gas. The injector temperature was 200 ± 1°C, the detector temperature was 220 ± 1°C, and the column temperature was 200 ± 1°C. The column flow rate was set at 1.9 mL/minute. Each sample was evaluated in triplicate. A mixed standard of short-chain, medium-chain, and long-chain saturated, mono-unsaturated, and poly-unsaturated fatty acids (Sigma Chemical Co., St. Louis, MO.) were run for comparisons.

4. Crude Ash

Crude ash contents were determined by weighing approximately 2.0 g, weighed to the nearest 0.0001 g, of the dehydrated samples, which had been saved from the moisture analyses, into cleaned, oven dried, desiccated, and pre-weighed porcelain crucibles with lids. The covered

crucibles were then placed in a Muffle Furnace (Lab-Heat box type with solid state, vari-watt power level control, Blue M Electric Co., Blue Island, IL.) and heated at 550 ± 5°C for 32 ± 1 hours. On completion of the ashing process when samples were a light grey powder, the covered crucibles were transferred to a glass desiccator with silica gel until cooled to room temperature, and then were re-weighed. Each sample was evaluated in triplicate.

The ash content was calculated as follows:

$$\text{\% Ash (db) (for dry samples)} = \frac{\text{(weight of crucible after ashing – tare weight of crucible)}}{\text{(initial weight of dry sample)}} * 100\ \%$$

$$\text{\% Ash (db) (for wet samples)} = \frac{\text{(weight of crucible after ashing – tare weight of crucible)}}{\text{(initial weight of sample) * dry matter coefficient}} * 100\ \%$$

dry matter coefficient = % total solids/100.

5. Crude Carbohydrates

Carbohydrate contents were determined upon completion of the moisture, crude protein, crude lipid, and ash analyses. The evaluations were done by transposing the results of the moisture, crude protein, crude lipid, and ash analyses into the appropriate variables of the following calculation:

% Crude carbohydrates =

100 % - (% moisture + % crude protein + % crude lipid + % ash).

E. Animal Study

1. Experimental Feeds

The five rat feeds were formulated based on standard protein feeds for rats or mice as described by ICN Biochemicals, (Cleveland, OH.). The five feeds all contained 19.0 % corn starch (Challenge Canada, 100 % pure Original Corn Starch), 11.0 % lipid from the added krill

plus vegetable oil (100 % Pure Canola Oil (Q.S.P. 'Quality Services Partnership', (B.C.)), 4.0 % mineral mixture U.S.P. XIV (ICN Biochemicals, Cleveland, OH.), 39.0 % sucrose (Rogers White Granulated Sugar), 1.0 % vitamin diet fortification mixture AIN (76) (ICN Biochemicals, Cleveland, OH.), and 25.7 % to 26.1 % protein. There were five different protein sources (one per feed). These were lactose free casein from bovine milk, (Sigma-Aldrich Co., Oakville, ON.) which was the control feed (CA), and the four previously described (refer to **III.A.**2. and **III.B.**1. to **III.B.**3.) ground, dehydrated krill preparations (VH, VL, FD, HZ) (Table 3.1). The dry materials were all added to an industrial cake mixer (bucket: 21.6 cm (radius); 40.6 cm (depth) (Hobart Model), followed by the oil. Mixing was initiated at low power to prevent loss of dry matter, and then, once the dry materials were moistened with the oil, the mixer was set to high power and the ingredients were blended until homogenous, a period of approximately ten minutes. This was done in several batches.

2. Bomb Calorimetry

Samples of the five rat feeds were submitted to The Department of Animal Science, U.B.C., for Bomb Calorimetry analysis by the Parr Method (Parr, 2001). The approximately 1.0 g, weighed to the nearest 0.0001 g, samples were individually placed in a strong bomb calorimeter container, which was then sealed with excess oxygen and ignited electrically in the bomb calorimeter (Parr Instrument Co., Moline, Illinois, U.S.A.). The heats of combustion of the samples (calorific values of food and fuels) at a constant volume were then calculated from the resulting rise in temperature.

Table 3.1. Composition of the experimental feeds fed to the rats.

	Feed[1] (%)				
	CA	**VH**	**VL**	**FD**	**HZ**
Dietary Components					
Casein	25.7	-	-	-	-
VH Dehydrated *Euphausia pacifica*	-	25.9	-	-	-
VL Dehydrated *Euphausia pacifica*	-	-	26.1	-	-
FD Dehydrated *Euphausia pacifica*	-	-	-	26.1	-
HZ Dehydrated *Euphausia pacifica*	-	-	-	-	25.9
Sucrose	39.0	39.0	39.0	39.0	39.0
Corn Starch	19.0	19.0	19.0	19.0	19.0
Canola Oil + Krill Lipid	11.3[2]	11.1	10.9	10.9	11.1
Mineral Mixture	4.0	4.0	4.0	4.0	4.0
Vitamin Mixture	1.0	1.0	1.0	1.0	1.0

[1] CA = casein; VH = vacuum microwave blanched at a high temperature and air-dried; VL = vacuum microwave dehydrated at a low temperature; FD = freeze-dried; HZ =hydrolysed, freeze-dried.
[2] No krill lipid was present.
- = not added.

3. Amino Acid Analysis

Samples of the five rat feeds were couriered to The Department of Animal Science, University of Manitoba, (Winnipeg, MN.) for a complete amino acid analysis. There, 2.0 g, weighed to the nearest 0.0001 g, of the individual approximately samples were refluxed with 6.0 M HCl for approximately twenty-four hours at 110 ± 1°C under vacuum in order to hydrolyse the amide linkages and therefore, produce a mixture of amino acids. To obtain cysteine, the samples were first oxidized with performic acid and then hydrolysed. To obtain tryptophan, the samples were hydrolysed with 4.2 M sodium hydroxide (NaOH) for approximately sixteen hours at 121 ± 1°C under vacuum. The solutions were then analysed using an automatic amino acid analyser (Pharmacia BiaCore 20) with a cation-exchange column and ninhydrin detection. The eluate was read at 440 nm (for proline) and 570 nm.

4. Metabolic Study

Once a week, for five consecutive weeks, ten three-week old, male, Wistar, albino rats (a total of fifty male rats) were purchased from Charles River (Montreal, P.Q.). They were then couriered to Food Science, U.B.C., (Vancouver, B.C.), where they were secured in an animal laboratory. The weights of the fifty weanling rats ranged from 33.9 g to 66.9 g. Upon each of the five arrivals, the animals, for a one-week adjustment period, were maintained in two groups of five, in 38.1 cm (length) by 17.8 cm (width) by 17.8 cm (height) aluminium cages. This was done under controlled temperature (21°C to 24°C) and lighting conditions (12 hours:12 hours, day:night cycle). The rats were provided with ddH_2O *ad libitum* and trained to meal-feed on a rat diet (5012 Lab Rat Diet, PMI Feeds Inc., Richmond, B.C.) that had been ground in an ultracentrifugal mill (Retsch, ZM 100, Clifton, N.J.) with a 0.12 mm mesh, at a speed of

12 000 rpm. Following the one-week adjustment period, the animals were randomly segregated into the five dietary groups with respect to the dietary protein (refer to **III.E.**1.). They were then, for a twenty-eight day test period, individually housed in the aluminium cages under the same controlled temperature and lighting conditions. Each day, the animals were meal-fed over an eight-hour duration. Fresh feed was provided daily and ddH_2O was made available to the rats *ad libitum*. Daily feed intakes and daily body weights were recorded.

The feed dishes and water bottles were cleaned every evening. This was done in a machine washer (Heinicke Instruments, Hollywood, FL.) with hot water and Sparkleen 2 (Fisherbrand, Fisher Scientific, Pittsburgh, PA.). The dishes were washed for approximately five minutes, rinsed with hot water for approximately two minutes, and then dried overnight in a glassware dryer set at $70.0 \pm 0.5^{\circ}C$.

Upon conclusion of the feeding studies, the P.E.R. value for each rat was calculated (refer to **II.E.**4.). This was based on the rodent's weight gain and feed consumption (for which protein (N * 6.25) had previously been determined) (refer to **III.D.**2.).

5. Nitrogen Balance Study

A seven-day nitrogen balance study was performed when the animals were eight weeks of age. Each week, for five weeks, eight of the ten eight-week old rats from four of the five dietary groups (alternating each week: (i.e.) (VH, VL, FD, HZ); (CA, VL, FD, HZ); (CA, VH, FD, HZ); (CA, VH, VL, HZ); (CA, VH, VL, FD)) were selected and individually placed in metabolic cages. The metabolic cages (Nalgene, Rochester, N.Y.) were equipped with collection funnels and separation cones in order to separate the faeces and the urine, thus, eliminating urine washover and the subsequent contamination of the faeces. Separation of the excreta was immediate and complete using this apparatus. The urine and faecal samples were collected in

plastic containers, weighed, and then stored in the -34°C freezer in preparation for further use for this thesis. Each day, the animals were meal-fed over an eight-hour duration. Fresh feed was provided daily, and ddH_2O was made available to the rats *ad libitum*. Each animal's feed intake and water consumption was measured daily. Rat weights were recorded at the beginning and at the end of the seven-day study.

Upon completion of the nitrogen balance study, and following a night of fasting, the rats (one at a time) were anaesthetised using a vapour mixture of oxygen and 4 % fluorothane (Animal Care, South Campus, U.B.C., Vancouver, B.C.) at a flow rate of 4 L/minute. Once anaesthetised, the rats were exsanguinated. Blood was collected in test tubes and separated by centrifugation at $1085 \cdot a_g$, 4°C (Sorvall® RC 5B Plus, Mandel Scientific Co., (Dupont), Norwalk, CT.). The partitioned plasma (top layer) was then transferred to graduated Eppendorf tubes, frozen in liquid nitrogen, and stored in the -34°C freezer. The animals' organs and right quadriceps were removed, weighed, placed in plastic petri plates, frozen in liquid nitrogen (Physics Stores, U.B.C., Vancouver, B.C.), and stored in the -34°C freezer.

6. Final Preparation of the Animal Samples

a. Faeces

The specimens of frozen faeces were all freeze-dried at a gauge pressure of 1.6 ± 0.1 mm Hg, a shelf temperature of 21.0 ± 0.5°C, and a condenser temperature of -50.0 ± 0.5°C. The lyophilisation took approximately four days. Once completed, the forty processed faecal collections were, one by one, machine ground (Wiley Mill, General Electric, Philadelphia, PA.) using a 20 mm mesh, with the grinder being vacuum-cleaned between each to prevent cross contamination, and re-collected into forty new Ziploc® plastic bags.

Next, the faecal samples were pooled into five groups representative of the five different animal feed groups (CA, VH, VL, FD, and HZ). This was done by transferring approximately 0.25 g, weighed to the nearest 0.01 g of the ground faeces from each of the eight rats in the five feed groups, to five Ziploc® plastic bags. These were then thoroughly hand-shaken to obtain an even distribution. The pooled samples were delivered for fluoride analysis (refer to **III.J.**). The remaining faecal matter (in the forty Ziploc® plastic bags) was delivered for nitrogen analysis (refer to **III.D.**2.).

b. Urine

The forty specimens of frozen urine were thawed for approximately five hours in a 2°C walk-in cold room and pooled into five groups representative of the five different animal feed groups. This was done by transferring 1.0 mL of urine from each of the eight rats in the five feed groups, to five respectively labelled containers. These were then thoroughly hand-shaken and delivered for fluoride analysis (refer to **III.J.**). Individual 5.0 mL urine samples from each of the rats were delivered for nitrogen analysis (refer to **III.D.**2.).

c. Muscle

The frozen leg muscles from the fifty rats were thawed in a 2°C walk-in cold room and then approximately 1.0 g, weighed to the nearest 0.1 g, samples were removed, placed into fifty petri dishes, and delivered for nitrogen analysis (refer to **III.D.**2.).

d. Plasma

Plasma (refer to **III.E.**5.) from each of the fifty rats was collected and pooled into five groups representative of the five different animal feed groups. This was done by transferring

200 μL of plasma from each of the eight rats in the five feed groups, to five respectively labelled containers. These were then thoroughly hand-shaken and delivered for fluoride analysis (refer to **III.J.**).

F. Available Lysine Determination

1. Procedure

The method used for the available lysine determination was that as described by Kakade and Liener (1969). To begin, 10.0 ± 0.1 mg of each of the finely ground experimental feeds was added to screw cap test tubes. Next, 1.0 mL of 4.0 % sodium hydrogen carbonate (pH 8.5) was added to the tubes. All the mixtures were then incubated together in a 40.0 ± 0.5°C shaking waterbath, placed in a fume hood, for 10 ± 1 minutes. Next, 1.0 mL of 1.0 % TNBS (Sigma Chemical Co., St. Louis, MO.) was added and the reaction was allowed to proceed, in a darkened environment, for 120 ± 2 minutes in the 40.0 ± 0.5°C waterbath. Following this, 3.0 mL of concentrated HCl was added to each test tube, and the tubes were then all autoclaved (Barnstead Co., Boston, MA.) at 121.0 ± 0.0°C, 1.1-1.2 kg/cm^2 (15-17 psi) for 60 ± 1 minutes. Once cooled to room temperature, 5.0 mL of ddH_2O was added to each tube and the contents were filtered through fluted Whatman® Number 2 filter paper (5.0 cm radius) and then transferred to glass stoppered lipid extraction flasks and extracted twice with 10.0 mL of ethoxy ethane (diethyl ether), being allowed to stand for 10 ± 1 minutes each time to separate. Next, in a fume hood, the flasks were heated in a 70.0 ± 0.5°C waterbath for 5 ± 1 minutes to evaporate any residual ethoxy ethane. Finally, the aqueous layer of the solutions was read in the Shimadzu spectrophotometer at 346 nm.

The blank was prepared in the same manner as the samples except that concentrated HCl was added to the protein solution before the addition of the TNBS reagent. A second blank with

no added protein was also prepared. A standard curve was prepared using 0 μg, 10. μg, 20. μg, 50. μg, 100. μg, 150. μg, and 200. μg of L-lysine-HCl (Sigma Chemical Co., St. Louis, MO.). The assay was performed in triplicate.

2. Calculations

The following are known:

a.) Lysine molecular weight = 146.2 g/mol

b.) Lysine extinction coefficient = 1.56×10^4 L/mol·cm

c.) Concentration = $\frac{\text{absorbance}}{\text{extinction coefficient}}$

G. Plasma α-Amino Nitrogen Determination

1. Preparation and Procedure

The protocol described by Goodwin (1968) for the determination of plasma α-amino nitrogen was used in this experiment. Stock 1-fluoro-2, 4-dinitrobenzene (DNFB) (reagent grade, Sigma Chemical Co., St. Louis, MO.), was prepared by mixing 650 μL DNFB with 2-propanone in a 50 mL volumetric flask. A 5.0 % sodium tetraborate solution was also made. The sodium tetraborate was allowed to stand overnight prior to use. The working DNFB solution was prepared fresh the day of use, at room temperature, by mixing one part of stock DNFB with nine parts of 5.0 % sodium tetraborate. A protein precipitant was prepared fresh the day of use, by mixing one part 13.3 % w/v sodium tungstate (99.3 %, Sigma Chemical Co., St. Louis, MO.) with nine parts of 0.11 M HCl. A stock amino acid solution was prepared by dissolving 1.050 ± 0.001 g of L-glutamic acid (monosodium salt, reagent grade, Sigma Chemical Co., St. Louis, MO.) and 0.540 ± 0.001 g of glycine (reagent grade, Sigma Chemical Co., St.

Louis, MO.) together in ddH_2O in a 100 mL volumetric flask. This was then added to a 1 L volumetric flask, mixed with 2.000 ± 0.005 g of sodium benzoate, and 700 mL of 1.0 M HCl, and diluted to volume with ddH_2O.

The plasma α-amino nitrogen was determined from 0.5 mL samples of rat plasma collected from all fifty rats used in the feeding experiment. The procedure involved adding 0.5 mL of ddH_2O and 4.0 mL of protein precipitant to 0.5 mL of rat plasma. A reagent blank was carried through the procedure by substituting ddH_2O for the plasma. The three components were lightly mixed and then centrifuged (IEC Centra-7R Centrifuge, Needham Heights, MA.) for 10 ± 1 minutes at 16 a_g, 4 ± 1°C, and the supernatant was filtered through fluted Whatman® Number 514 filter paper (6.2 cm radius) and saved in test tubes. To 1.0 mL of the supernatant, 1.0 mL of working DNFB solution was added. The supernatant and working DNFB solution were then vortexed and the pH was monitored to ensure the pH was between 9 and 10. The tubes were then incubated for 15 ± 1 minutes in a 70.0 ± 0.5°C waterbath. Once removed from the bath, the tubes were cooled and, in the fume hood, 5.0 mL of acidified dioxane (concentrated HCl in 1,4-dioxane (in assay 99.0 %, BDH, Toronto, ON.) mixed 1:50) were added. The absorbance of each sample was then determined at 420 nm with the Shimadzu spectrophotometer. The instrument was zeroed using the reagent blank. The samples were evaluated in triplicate.

2. Quantitation

A standard curve was prepared by taking 0.5 mL volumes of diluted amino acid standard solutions and analysing them as stated above. The diluted amino acid solutions were made by adding 0 mL, 1.0 mL, 2.0 mL, 3.0 mL, 4.0 mL, 5.0 mL, 6.0 mL, and 8.0 mL of stock amino acid solution to separate 10 mL volumetric flasks and diluting them to volume with ddH_2O. These

solutions then represented 0 mg, 2.0 mg, 4.0 mg, 6.0 mg, 8.0 mg, 10. mg, 12 mg, and 16 mg of α-amino nitrogen per 100. mL respectively.

H. Protein Digestibility

1. *In Vitro* Protein Digestion

The *in vitro* enzymatic digestion method described by Iwami *et al.* (1986) was used. First, 100 mL of ddH_2O was added to clean, dry, 250 mL Erlenmeyer flasks. Next, 0.500 ± 0.005 g, from each of the five experimental feed proteins (the casein meal and the four dehydrated krill meals) was suspended in the respectively labelled flask. The pH's were then adjusted to 1.9 with 0.11 M HCl. Following this, the Erlenmeyer flasks were incubated in a 37°C shaking waterbath until the contents reached 37.0 ± 0.5°C, a period of approximately ten minutes. Once at the desired temperature, 15.0 ± 0.1 mg of porcine pepsin (Porcine Stomach Mucous 1:10 000, specific activity of 1 750 units/mg protein at 37 °C and pH 2.0, Sigma Chemical Co., St. Louis, MO.) was added to each flask to initiate the hydrolysis. After 30 ± 1 minutes of pepsin digestion, 40 mL from each of the protein suspensions was removed and transferred to a clean, pre-warmed, 100 mL Erlenmeyer flask; the remaining contents were discarded. The pepsin in the transferred volumes was inactivated by raising the pH to 8.0 with 4.0 M NaOH. The flasks were then placed in the 37.0 ± 0.5°C shaking waterbath, and, to each one, 20.0 ± 0.1 mg of porcine pancreatin (Porcine Pancreas Grade II, activity at least equivalent to NF grade, Sigma Chemical Co., St. Louis, MO.) was added. At intervals of approximately one minute, two minutes, three minutes, four minutes, five minutes, six minutes, eight minutes, ten minutes, twelve minutes, sixteen minutes, twenty minutes, and thirty minutes, 1.0 mL aliquots from each flask were removed, transferred to 1.5 mL graduated Eppendorf tubes and deproteinised by the addition of 1.0 mL of 20.0 % trichloroacetic acid (TCA). The tubes were

then centrifuged (Eppendorf Centrifuge 5402) at 9880 a_g and 4 ± 1°C for 15 ± 1 minutes and the TCA-soluble fraction in each was transferred to a new centrifuge tube.

The Trinitrobenzenesulfonic Acid method described by Kwan *et al.* (1983) was used to determine the protein digestion rates of the casein meal and the four dehydrated krill meals. This experiment was conducted in a fume hood. From each TCA-soluble fraction (previously described) in the five sets of centrifuge tubes, 100 μL was removed and pipetted into a new test tube (disposable, 13 mm by 100 mm, Borosilicate Glass, Fisher Scientific, Nepean, ON.) to which 2.0 mL of 0.2 M sodium borate (pH 9.2) had already been added. Next, under darkened conditions, 1.0 mL of 4.0 mM 2,4,6-trinitrobenzenesulfonic acid (TNBS) (Sigma Chemical Co., St. Louis, MO.) was added to each tube. After 30 ± 1 minutes of incubation at room temperature in the dark (the tubes were covered by aluminium foil), 1.0 mL of 2.0 M sodium dihydrogen phosphate (reagent grade, BDH Chemicals, Toronto, ON.) containing 18 mM sodium sulfite was added to each sample. The absorbance of each solution was measured at 420 nm with a Shimadzu UV-160 recording spectrophotometer (Hadley, Tekscience, Oakville, ON.). Duplicates of three trials were conducted.

2. Initial Rate Method

The initial rate method used was a modified version of the procedure described by Maga *et al.* (1973). The absorbance 420 nm data from the first ten minutes of the *in vitro* protein digestion of each of the samples was subjected to linear regression analysis. The initial proteolysis rates among the five feeds were then compared.

I. Protein Digestibility with Chitinase

1. *In Vitro* Protein Digestion

The protocol followed for this experiment, with the exception of the chitinase addition to the protein samples, was identical to that described in 'protein digestibility, *in vitro* protein digestion' (refer to **III.H.**1. and **III.H.**2.). Duplicates of two trials were conducted.

2. Chitinase Addition

The chitinase solution was prepared based on calculations where it was assumed that the krill used in this experiment contained approximately 2 % to 10 % chitin dry basis (Suzuki, 1981; Pastuszewska *et al.*, 1983). A 50 mM citrate buffer at pH 6.0 at 25.0 ± 0.5°C was prepared and 110 mL of the buffer was then transferred to 250 mL Erlenmeyer flasks. Next, 1.0 mL of a prepared chitinase solution (twenty units of stock chitinase in 20.0 mL of 50 mM citrate buffer); (Stock Chitinase) (Sigma Chemical Co., St. Louis, MO.) (one unit of stock chitinase liberated 1.0 mg of *N*-acetyl-β-D-glucosamine (NAG) from chitin/hour at pH 6.0 at 25°C in a two step reaction) was added to all the flasks. This was followed by 0.500 ± 0.005 g of one of each of the five rat feeds, and finally, to prevent bacterial growth, 10 μL of 2.0 % sodium azide (minimum 99.0 %, Sigma Chemical Co., St. Louis, MO.). The protein samples were digested for approximately nine hours, at which point, another 1.0 mL of chitinase solution was pipetted into the flasks. The digestion was then allowed to continue for approximately five more hours. Once this time had elapsed, the *in vitro* digestion of the samples was completed.

J. Fluoride Analyses

Homogenised powdered, approximately 5.0 g, weighed to the nearest 0.1 g, samples from the five rat feeds, as well as samples from each of the five pooled groups of the rats' faeces,

urine, and plasma (refer to **III.E.**6.*a.* to *d.*), were couriered to SGS Canada Inc. General Testing Laboratories Division, (Vancouver, B.C.) and analysed for their fluoride contents as described by A.O.A.C. 984.37 (A.O.A.C., 1965). The method was as follows.

1. Preparation

First, a total ionic strength adjustment buffer (TISAB) (Orion Research Inc.) was prepared by dissolving 57 mL of ethanoic acid, 58.0 g of NaCl, and 4.0 g cyclohexylene dinitrilo tetraacetic acid (CDTA) in 500 mL of ddH_2O in a 1 L beaker. The solution was cooled and adjusted to pH 5.0 to pH 5.5 with 5 M NaOH. Once it had again cooled to room temperature, it was transferred to a 1 L volumetric flask, and diluted to volume with ddH_2O.

Next, fluoride standard solutions were prepared. A 209.9 mg sample of previously dried (for approximately four hours at 150 ± 1°C) sodium fluoride reference standard was transferred to a 500 mL volumetric flask, dissolved, and then in 250 mL volumetric flasks, diluted serially with ddH_2O to prepare the 0.01 M, 0.001 M, and 0.0001 M standards.

2. Procedure

First, the samples (containing 1 mg to 2 mg of fluoride) were added to 100 mL volumetric flasks, diluted to volume with ddH_2O, and mixed. The mixture was not left in the glass container for more than sixty minutes. Next, 20.0 mL from each standard and from each sample preparation was transferred to another separate, plastic beaker; followed by the addition of 20.0 mL of TISAB. A selective ion meter (a fluoride-combination electrode filled with an internal filling solution and a potentiometer equipped with a fluoride-selective electrode) was then immersed into each of the prepared solutions. These were stirred at a constant rate on a

magnetic stirrer with an asbestos pad placed on top to reduce heat transfer during the readings. Millivolt measurements were taken when the potential stabilised to within ± 0.1 mV.

3. Standard Curve

Two-cycle semi-logarithmic graph paper was used to prepare the standard curve, which was plotted with the molar fluoride standard concentrations on the log axis and the mV potential on the linear axis. The molar fluoride concentration (C_i) of each of the sample preparations was then determined from the standard curve. Separate millivolt measurements for each of the standard solutions as well as the standard curve were prepared.

4. Calculations

The milligrams of fluoride were calculated as follows:

mg F^- / aliquot = C_i * (19 mg F^- / mL)(100 mL) = 1900C_i

where C_i = the molar concentration in the sample preparation as determined from the standard curve.

K. Protease Assay

This assay is based on the casein assay described by Kunitz (1947). For this assay, 0.100 ± 0.005 g of the casein and each of the four dehydrated krill samples, as well as the frozen krill, were all individually used as both the substrate and the protease provider. The samples were each individually added to test tubes individually containing 10.0 mL of one of three buffers. The buffers used were 0.1 M citrate pH 4.0 [0.1 M citric acid (food grade, Fisher Scientific, Nepean, ON.) in solution with 0.1 M sodium citrate (buffer grade, Fisher Scientific, Nepean, ON.)], 0.1 M potassium phosphate pH 7.0 [0.1 M potassium phosphate monobasic

(buffer grade, BDH Inc., Toronto, ON.) in solution with 0.1 M potassium phosphate dibasic (buffer grade, BDH Inc., Toronto, ON.)], and 0.1 M tris (hydroxymethyl) aminomethane (Tris) pH 9.0 [0.2 M Tris (buffer grade, Sigma Chemical Co., St. Louis, MO.) dissolved in 0.2 M HCl]. Next, 10 μL of 2.0 % sodium azide (minimum 99.0 %, Sigma Chemical Co., St. Louis, MO.) was added to all the tubes to prevent bacterial growth. The tubes were then vortexed and incubated in a 37.0 ± 0.5°C waterbath. At the end of each incubation period, 1.0 mL of 5.0 % TCA was added to every tube, the tubes were vortexed and allowed to stand one hour for precipitation. The blanks were prepared by adding to each tube, 10.0 mL of the appropriate buffer, followed by 1.0 mL of 5.0 % TCA, and finally 10 μL of 2.0 % sodium azide. The tubes were then vortexed and incubated in a 37°C waterbath. At approximately one hour, twenty hours, forty-one hours, forty-eight hours, and sixty-six hours, 1.0 mL from each of the samples and each of the blanks was removed and centrifuged (IEC Centra-7R Centrifuge, Needham Heights, MA.) at 16 a_g, 4 ± 1°C, for 5 ± 1 minutes to separate the supernatant from the precipitate. The absorbance of each of the supernatants was then read in the Shimadzu spectrophotometer at 280 nm using quartz cuvettes and zeroing the spectrophotometer with the appropriate blank.

L. Statistical Analyses

Data were analysed using one-way analysis of variance (ANOVA), two-way ANOVA, or by regression or correlation. To satisfy statistical considerations concerning normality and homogeneity of variances, data were transformed when necessary. Lilliefors (Conover, 1980) method was used to test data for normality of distribution and Levene's (Snedecor and Cochran, 1980) method was employed to test for the homogeneity of variances. Differences among mean values were established using Tukey's honestly significant difference test (Petersen, 1985). All

of the statistical evaluations were performed at the significance level $\alpha = 0.05$. Statistical analyses were performed using SYSTAT version 8.0 (SPSS Inc., Chicago, Il., U.S.A.).

RESULTS AND DISCUSSION

IV. RESULTS AND DISCUSSION

A. Physical Characteristics of the Dehydrated *Euphausia pacifica*

The dehydrated *Euphausia pacifica* samples were distinguishable based on visual assessment of colour. The VH samples were pale red to brown in colour, the VL samples were dark-red in colour, the FD samples were yellow-orange in colour, and the powdered HZ samples were a deep red colour. The brown appearance of the VH samples was due to their exposure to heat during drying. The FD samples were pale in colour, as compared to the other krill samples, as with sublimation drying, the red pigments are not brought to the surface of the product to the same extent that they are with vacuum microwave dehydration and with air-drying. It might also be expected that the VH, VL, and HZ samples would appear deeper in colour than the FD samples since, assumingly, the densities of these were greater than the FD product, thus, yielding a higher concentration of pigment per volume of tissue (Lin *et al.*, 1998a). The yellow-orange and red colours of the krill can be attributed to the presence of carotenoids. The level of astaxanthin present in the fresh krill may have varied, thus, also affecting the colour of the dehydrated product.

The texture, prior to grinding, of the VH and VL samples appeared to be harder than that of the FD samples, which appeared to exhibit excellent structure retention. Since the frozen sample remained rigid when moisture was sublimed, removal of water molecules resulted in numerous voids within the structure, thus, no shrinkage occurred (Lin *et al.*, 1998a). The texture of the VH samples appeared to be harder than the VL samples. The hardness of a product's surface may partially be an indicator of the extent of case hardening that has occurred during drying (Kim and Toledo, 1987). However, puffing can reduce hardness. The VH samples were air-dried for a longer period than the VL samples, and the VL samples were more puffed than the

VH samples. The VH, VL, and FD treatments all produced samples with whole and individual krill bodies.

Although no formal organoleptic testing of the dehydrated *Euphausia pacifica* samples was performed, it was evident that the intensity of odour in the VL and HZ samples was stronger in comparison with the VH and FD samples. The VL and HZ samples had a strong wholesome fish aroma, while the VH samples had a mild fishy odour. The FD samples initially had a mild aroma but over time did take on a fishy odour.

B. Proximate Determination

1. *Euphausia pacifica*

The proximate constituents of the frozen and dehydrated (VH, VL, FD, HZ) *Euphausia pacifica* samples, are given in Table 4.1, and are consistent with results of other studies for *E. pacifica* and *E. superba* (Brown, 1959; Suyama *et al.*, 1965; Vinogradova, 1960; Yamada, 1964; and Pierce *et al.*, 1969). The chemical composition of raw freeze-dried krill (db) as determined by Argent Chemical Laboratories (1996) was 1.3 % moisture, 66.9 % crude protein, 10.7 % crude lipid, 12.6 % ash, and 8.5 % crude carbohydrates.

The significant differences ($p \leq 0.05$) noted in the moisture, crude lipid, and ash determinations between this author's samples of *E. pacifica* may be due to the fact that the krill for the HZ samples were caught at a different time than the krill for the VH, VL, and FD samples. The disparities are consistent with the known seasonal fluctuations that take place in euphausiids (Pierce *et al.*, 1969). Differences may also possibly be due to the location of the catch (Littlepage, 1962). These explanations also hold true for the variation seen between this

Table 4.1. Proximate determinations[1] and water activity (a_w) of the dehydrated and the frozen *Euphausia pacifica*.

Treatment[2]	Moisture (%)	Crude Protein[3] (%)	Crude Lipid (%)	Crude Ash (%)	Crude Carbohydrate (%)	a_w[4]
VH	13.84 ± 0.34^{b}	74.45	9.23 ± 0.03^{d}	12.85 ± 0.10^{a}	3.47	0.453 ± 0.001^{a}
VL	7.88 ± 0.23^{d}	63.97	14.10 ± 0.19^{b}	10.37 ± 0.14^{c}	11.56	0.334 ± 0.001^{c}
FD	8.85 ± 0.23^{c}	68.96	15.93 ± 0.26^{a}	12.50 ± 0.32^{ab}	2.61	0.357 ± 0.002^{b}
HZ	4.95 ± 0.15^{e}	67.41	16.23 ± 0.03^{a}	11.45 ± 0.31^{b}	4.91	0.244 ± 0.001^{d}
RW	$74.04 \pm 1.61^{5\,a}$	66.37	13.29 ± 0.04^{c}	12.19 ± 0.19^{ab}	8.15	N/A

[1] Data are expressed as mean percentage (dry basis) ± SEM (3 measurements taken).
[2] VH = krill vacuum microwave blanched at a high temperature and air-dried; VL = krill vacuum microwave dehydrated at a low temperature; FD = freeze-dried krill; HZ = hydrolysed, freeze-dried krill; RW = raw krill.
[3] Only a single protein value was obtained for each feed.
[4] Readings were completed at a temperature of 26.6 ± 0.1°C.
[5] Data are expressed as mean percentage (wet basis) ± SEM (3 measurements taken).
N/A = not available.

Means with different superscript letters within a column are significantly different at $p \leq 0.05$.

author's values and the literature values. As well, differences in the VH, VL, FD, and HZ samples' moisture and crude lipid contents may also be reflective of the dehydration method used. Differences in moisture may have been due to the amount of case hardening that occurred because of the nature of the drying process. Air-drying generally results in a product that has a moisture content greater than 12 % (Durance and Liu, 1996a). This is seen in the VH samples. The VH treatment was akin to a vacuum microwave blanching followed by air-drying. Differences in crude lipid contents may have been due to varying degrees of oxidation and of volatile losses.

According to Suyama *et al.* (1965), the soluble non-protein nitrogen components of crustacea are composed of amino acids, particularly alanine, glycine, and proline, in addition to taurine, betaines, and trimethylamine oxide. From the data of Suyama *et al.* (1965) and Suzuki (1981), these amount to 17 % of the total nitrogen. This percentage is consistent with the difference in the values for total nitrogen and protein nitrogen for *E. pacifica* (68.4 % and 53.8 % (biuret), respectively) and *E. superba* (61.8 % and 44.8 % respectively) reported previously by Pierce *et al.* (1969). With the assumption that the non-protein nitrogen in this author's values is also 17 %, the protein nitrogen of this author's samples ranges from 47 % to 58 %. This range is consistent with literature values (Ellingsen and Mohr, 1979; Yanase, 1974; Suyama *et al.*, 1965; Pierce *et al.*, 1969). Differences seen in the carbohydrate contents were proportional to the amounts of crude protein, crude lipid, and ash present in the samples.

2. Experimental Feeds

The rat chow was fed to the weanling rats upon their delivery for a one-week adjustment period. The proximate constituents of the chow were determined but the chow was not considered during the remainder of this study.

The proximate determinations and the gross energies of the experimental feeds are shown in Table 4.2. The crude lipid and ash contents of the experimental krill meal feeds showed no significant ($p \geq 0.05$) differences between treatments. The ash content of the CA feed was significantly lower ($p \leq 0.05$) than the krill meal feeds. The moisture content of the CA feed was significantly lower ($p \leq 0.05$) than the moisture contents of the VH, VL, FD, and HZ feeds, and the moisture content of the VL feed was significantly higher ($p \leq 0.05$) than the moisture contents of the VH, FD, and HZ feeds. The VH and FD feeds' moisture contents were not statistically different ($p \geq 0.05$), nor were the moisture contents of the FD and HZ feeds. The feeds were not isonitrogenous as the crude protein contents ranged from 25.7 % for the control and HZ feeds to 26.1 % for the VL and FD feeds. The crude protein contents of the four krill meal feeds however, were very close, differing by only 0.2 %. The differences were incurred during the preparation of the feeds.

Krill meal contains approximately 12.6 kJ/g to 18.8 kJ/g (Krasnodebska *et al.*, 1979). This author's krill meals contained between 19.1 kJ/g and 20.6 kJ/g and were considered isocaloric.

Fatty acid analyses of the total lipid extracts of the experimental feeds fed the rats are given in Table 4.3. Literature values for the fatty acid composition of *E. pacifica* and of canola oil, which was also added to this author's experimental feeds, are given in Table 2.2. Upon comparison of the data, the fatty acids present in this author's feeds, with the exception of lauric acid, are all equal to or less than the fatty acid literature values for *E. pacifica* plus canola oil. The CA feed had results comparable to the canola oil. This indicates that some lipid oxidation of the krill meal feeds occurred, but limited lipid oxidation of the casein control meal. Saturated fatty acids in this author's krill, assuming 20:5 at 18 %, 22:6 at 11 % and 22:16 at 11 % (Argent

Table 4.2. Proximate determinations and gross energies of the experimental rat feeds[1].

Feed[2]	Moisture (%)	Crude Protein[3] (%)	Crude Lipid (%)	Crude Ash (%)	Crude Carbohydrate (%)	Gross Energy[4] (kJ/g)
CH	9.77 ± 0.08^{a}	29.36	5.07 ± 0.15^{b}	7.02 ± 0.08^{a}	58.55	20.9
CA	5.69 ± 0.04^{e}	25.65	11.32 ± 0.28^{a}	3.49 ± 0.01^{b}	59.54	20.2
VH	7.66 ± 0.11^{c}	25.94	11.14 ± 0.18^{a}	6.62 ± 0.15^{a}	56.30	20.2
VL	8.97 ± 0.19^{b}	26.08	10.90 ± 0.29^{a}	6.98 ± 0.07^{a}	56.04	20.5
FD	7.56 ± 0.21^{cd}	26.14	10.94 ± 0.32^{a}	6.86 ± 0.09^{a}	56.06	20.6
HZ	6.86 ± 0.17^{d}	25.94	11.10 ± 0.26^{a}	6.75 ± 0.02^{a}	56.21	19.1

[1] Data are expressed as mean percentage (dry basis) ± SEM (3 measurements taken).
[2] CH = rat chow; CA = casein; VH = krill vacuum microwave blanched at a high temperature and air-dried; VL = krill vacuum microwave dehydrated at a low temperature; FD = freeze-dried krill; HZ = hydrolysed, freeze-dried krill.
[3] Only a single protein value was obtained for each feed.
[4] Only a single bomb calorimetry value was obtained for each feed.

Means with different superscript letters within a column are significantly different at $p \leq 0.05$.

Table 4.3. Percent fatty acids (%) present in the experimental rat feeds[1].

Feed[2]	12:0	14:0	16:0	16:1ω7	18:0	18:1ω9	18:2ω6	18:3ω3
CH	0.04 ± 0.00^{a}	1.22 ± 0.01^{c}	16.06 ± 0.05^{b}	1.70 ± 0.01^{b}	4.25 ± 0.06^{a}	21.97 ± 0.17^{e}	50.83 ± 0.20^{a}	3.93 ± 0.04^{d}
CA	0.03 ± 0.00^{b}	0.13 ± 0.00^{d}	4.70 ± 0.05^{d}	0.26 ± 0.01^{c}	2.39 ± 0.01^{d}	61.59 ± 0.33^{a}	21.57 ± 0.16^{b}	9.03 ± 0.08^{a}
VH	0.02 ± 0.00^{d}	1.19 ± 0.02^{c}	11.17 ± 0.14^{c}	1.80 ± 0.04^{b}	2.70 ± 0.08^{bc}	54.85 ± 0.48^{b}	19.43 ± 0.12^{c}	8.21 ± 0.21^{bc}
VL	0.04 ± 0.00^{c}	2.41 ± 0.07^{b}	16.80 ± 0.32^{b}	3.97 ± 0.10^{a}	2.64 ± 0.04^{c}	49.08 ± 0.44^{c}	17.40 ± 0.36^{d}	8.33 ± 0.13^{b}
FD	0.03 ± 0.00^{bc}	2.50 ± 0.07^{b}	18.20 ± 0.06^{a}	4.29 ± 0.11^{a}	2.84 ± 0.01^{b}	47.64 ± 0.34^{cd}	16.06 ± 0.08^{e}	7.81 ± 0.00^{bc}
HZ	0.03 ± 0.01^{bc}	3.05 ± 0.09^{a}	18.90 ± 0.29^{a}	4.36 ± 0.13^{a}	2.84 ± 0.01^{b}	47.24 ± 0.07^{d}	16.57 ± 0.09^{d}	7.64 ± 0.09^{c}

[1] Data are expressed as mean percentage ± SEM (3 measurements taken).
[2] CH = rat chow; CA = casein; VH = krill vacuum microwave blanched at a high temperature and air-dried; VL = krill vacuum microwave dehydrated at a low temperature; FD = freeze-dried krill; HZ = hydrolysed, freeze-dried krill.
12:0 = lauric acid; 14:0 = myristic acid; 16:0 = palmitic acid; 16:1ω7 = palmitoleic acid; 18:0 = stearic acid; 18:1ω9 = oleic acid; 18:2ω6 = linoleic acid; 18:3ω3 = linolenic acid.

Means with different superscript letters within a column are significantly different at $p \leq 0.05$.

Chemical Laboratories, 1999), account for approximately 30 % of the total fatty acids. This is comparable to the value calculated by Pierce *et al.* (1969).

C. Nutritional Evaluation

One of the objectives of this author's study was to determine if there was a nutritional difference in the protein utilisation of *Euphausia pacifica* vacuum microwave blanched at a high temperature and air-dried, by VMD at a low temperature, by freeze-drying, and by hydrolysis plus freeze-drying. This was evaluated by conducting P.E.R. and nitrogen balance studies. The protein quality was further evaluated by α-amino nitrogen analyses, amino acid analyses, and *in vitro* digestibility studies.

1. Protein Efficiency Ratio

Body weights, feed intakes, and protein efficiency ratios of the rats fed the five experimental feeds are given in Table 4.4. The results show that the inclusion of the dehydrated *Euphausia pacifica* (VH, VL, FD, and HZ) as the only source of protein in the feeds, resulted in significantly reduced ($p \leq 0.05$) body weight gains, feed intakes, and P.E.R.s of the VH, VL, FD, and HZ groups as compared to the CA control group. Significant differences ($p \leq 0.05$) in body weight gains and feed intakes were also noted amongst the VH, VL, FD, and HZ fed rats. The VL and FD fed rats had significantly lower ($p \leq 0.05$) weight gains than the VH and HZ fed rats. There were no significant differences ($p \geq 0.05$) between the P.E.R.s of the krill meal fed rats. The groups fed (eight hours daily/four weeks in the P.E.R. study) the dehydrated krill meals gained, on average, 80.2 g (VH, HZ) and 45.1 g (VL, FD) as compared to 187.8 g for the CA control group. The feed intakes were 36.9 % (VH, HZ) and 50.8 % (VL, FD) lower for the

Table 4.4. Protein efficiency ratios (P.E.R.s) of the rats fed the experimental feeds[1].

Feed[2]	Initial body Weight[3] (g)	Final body Weight[4] (g)	Dry Matter Intake (g)	P.E.R.[5]
CA	65.4 ± 4.2^{a}	253.2 ± 7.1^{a}	550.9 ± 24.5^{a}	2.5 ± 0.1^{a}
VH	65.5 ± 3.2^{a}	140.2 ± 10.5^{b}	335.1 ± 24.0^{b}	1.6 ± 0.2^{b}
VL	62.4 ± 3.1^{a}	109.4 ± 8.3^{c}	270.4 ± 15.0^{c}	1.3 ± 0.2^{b}
FD	68.0 ± 3.8^{a}	111.1 ± 9.9^{c}	272.1 ± 17.0^{c}	1.1 ± 0.2^{b}
HZ	68.1 ± 4.0^{a}	153.7 ± 9.2^{b}	360.2 ± 10.7^{b}	1.7 ± 0.2^{b}

[1] Data are expressed as mean ± SEM (n = 10).
[2] CA = casein; VH = krill vacuum microwave blanched at a high temperature and air-dried; VL = krill vacuum microwave dehydrated at a low temperature; FD = freeze-dried krill; HZ = hydrolysed, freeze-dried krill.
[3] Weights at four weeks of age.
[4] Weights at eight weeks of age.
[5] Values have been adjusted by a factor of 1.7 to give the casein meal a P.E.R. of 2.5.

Means with different superscript letters within a column are significantly different at $p \leq 0.05$.

animals fed the experimental krill meals than for those fed the casein meal. The average adjusted P.E.R. for the rats fed krill meal was 1.4 (adjusted) as compared to 2.5 (adjusted) for the rats fed the casein meal. Thus, the protein efficiency of the dehydrated *Euphausia pacifica* was, on average, 44 % less than that of casein. Different genetic strains of rats have been reported to differ as much as 20 % in P.E.R.s and body weight gains when fed a casein meal feed (Rebeca *et al.*, 1991). The reduction observed for the P.E.R. in the VH, VL, FD, and HZ meals can be associated with the lower feed intakes and thus, it does not properly credit protein used for maintenance purposes and the results are not directly proportional to the quality of the protein (McLaughlan, 1979).

The initial weights of the animals were, on average, 65.9 g. All the control animals exhibited a good eating behaviour on the meal-fed program, however, the rats in the VH, VL, FD, and HZ groups all had altered patterns of feed consumption. These animals continuously nibbled the feed throughout the day. No indications of diarrhoea were observed in any of the animals. Visual observations of the rats were made throughout the P.E.R. study. The rats fed the four krill meal feeds were all notably smaller, more lethargic, and more excitable than the casein meal fed rats. The rats in the VL and FD groups appeared to be anorexic. The animals in the FD group all had severe eczema and hair loss and showed signs of wasting and distress. One animal in the FD group rapidly lost a great amount of weight and was in extreme distress by the end of the experiment.

2. Nitrogen Balance, Biological Value, Digestibility, and Net Protein Utilisation

Feed intakes, feed to weight gain ratios, water intakes, and amounts of urine and faeces excreted for the rats fed the experimental feeds for the seven-day nitrogen balance study (fed eight hours daily/seven days) are given in Table 4.5. The results show that the feed intake of the

Table 4.5. Feed intakes, feed to gain ratios, and urinary and faecal excretion for the rats fed the experimental feeds over the seven-day nutritive study.

Feed[1]	Weight Gain[2] (g)	Dry Matter Intake[2] (g)	Feed:Gain Ratio[2,3]	Water Intake (mL)[2]	Urinary Output[2] (mL)	Faecal Output[2] (g)
CA	$48.3 \pm 3.4^{4\,a}$	$106.6 \pm 6.8^{4\,a}$	$2.48 \pm 0.20^{4\,a}$	$247.8 \pm 28.0^{4\,a}$	$105.7 \pm 13.6^{4\,ab}$	$4.4 \pm 0.6^{4\,c}$
VH	29.8 ± 3.2^{bc}	76.0 ± 6.4^{ab}	2.65 ± 0.22^{a}	193.9 ± 14.7^{ab}	110.2 ± 8.1^{ab}	9.4 ± 1.2^{ab}
VL	27.0 ± 3.4^{bc}	58.8 ± 2.8^{bc}	2.48 ± 0.34^{a}	146.5 ± 15.3^{c}	86.1 ± 10.2^{b}	5.6 ± 0.7^{c}
FD	23.3 ± 3.0^{c}	51.7 ± 4.7^{c}	2.34 ± 0.18^{a}	157.4 ± 21.0^{bc}	95.6 ± 12.9^{ab}	4.8 ± 0.7^{c}
HZ	38.0 ± 4.6^{ab}	78.7 ± 3.3^{ab}	2.28 ± 0.27^{a}	224.5 ± 16.1^{a}	139.7 ± 11.7^{a}	6.7 ± 0.5^{bc}

[1] CA = casein; VH = krill vacuum microwave blanched at a high temperature and air-dried; VL = krill vacuum microwave dehydrated at a low temperature; FD = freeze-dried krill; HZ = hydrolysed, freeze-dried krill.
[2] Data are expressed as mean ± SEM (n = 8).
[3] Data are calculated by dry matter intake (g) / weight gain (g).
[4] Data are expressed as mean ± SEM (n = 6).

Means with different superscript letters within a column are significantly different at $p \leq 0.05$.

FD group, 51.7 g, was significantly reduced ($p \leq 0.05$) from the feed intakes of the CA, VH, and HZ groups, 87.1 g on average. This is consistent with the poor health of the animals in the FD group. The feed intake of the VL group, 58.8 g, was significantly lower ($p \leq 0.05$) than that of the CA control group, 106.6 g. There was no significant ($p \geq 0.05$) difference in feed intake between the VL and the FD groups. The body weights of the animals in these groups were not statistically different ($p \geq 0.05$). This is in agreement with the visual observations of the rats. The feed to gain ratios of the five groups showed no significant differences ($p \geq 0.05$). This indicated that the protein conversion efficiency was not a contributing factor to the poor health of the animals. It is of interest to note that in this nitrogen balance study, the feed intakes for the VH and HZ groups were not statistically different ($p \geq 0.05$) from that of the CA group. However, the feed intakes of these two groups were significantly lower ($p \leq 0.05$) than the control group in the P.E.R. study. As well, in the nitrogen balance study, the feed intake for the VL group was not statistically different ($p \geq 0.05$) from the feed intakes for the VH, FD, and HZ groups. In the P.E.R. study, the VL group only showed no statistical difference ($p \geq 0.05$) from the FD group. These discrepancies may have been due to the rats adjusting to their respective feeds by the time the nitrogen balance study was conducted. Declines in dietary intake by rats have often been noted in toxic or nutritionally inadequate diets, but may be a temporary response, as the feed intake has been reported to return to near normal levels after a period of reduced consumption (Zebrowski and Suttie, 1966).

The daily water intake of the VL group, 20.9 mL, was significantly lower ($p \leq 0.05$) than the daily water intakes of the CA, VH, and HZ groups, 31.7 mL on average, and the daily water intake of the FD group, 22.5 mL, was significantly lower ($p \leq 0.05$) than the CA and HZ groups, 33.8 mL on average. However, the volume water intake/g body weight/day for each of the five

groups, averaging 0.1 ± 0.0 mL water intake/g body weight/day, showed no significant differences ($p \geq 0.05$). The daily urinary output of the VL group, 12.3 mL, was significantly lower ($p \leq 0.05$) than that of the HZ group at 20.0 mL, but there was no statistical difference ($p \geq 0.05$) between any of the groups and the control. The volume of urine excreted/g body weight/day for each of the five groups, averaging 0.54 ± 0.1 mL urine excreted/g body weight/day, showed no significant differences ($p \geq 0.05$). The daily faecal output of the VH group, 1.3 g was significantly higher ($p \leq 0.05$) than the daily outputs of the CA, VL, and FD groups, 0.4 g on average. However, the grams of faeces excreted/g body weight/day for each of the five groups, averaging 0.03 ± 0.01 g faeces excreted/g body weight/day, showed no significant differences ($p \geq 0.05$). The apparent digestibility of the VH feed, 84.6 %, was significantly lower ($p \leq 0.05$) than the apparent digestibilities of the CA, VL, FD, and HZ feeds, 91.7 % on average, which exhibited no significant differences ($p \geq 0.05$). These results indicate that feed intake was the main factor that varied amongst the experimental groups.

Nitrogen balances, biological values, apparent digestibilities, and apparent net protein utilisations are given in Table 4.6. The nitrogen balances (nitrogen retained) of the rats in the VH, VL, FD, and HZ groups, 123.0 g % nitrogen on average, were all significantly lower ($p \leq 0.05$) than the nitrogen balance of the CA control group at 224.4 g % nitrogen. The nitrogen balance of the HZ group, 161.1 g % nitrogen, was significantly higher ($p \leq 0.05$) than that of the VL and FD groups, 97.9 g % nitrogen on average. The HZ meal had been pre-digested, perhaps aiding its utilisation. The differences in nitrogen retention indicate that the animals fed the krill meals were not utilising the same amount of ingested nitrogen as the animals in the casein meal control group. The chitin present in the exoskeletons of the krill used in the krill meals may have been responsible for the lower values. The apparent digestibilities of the CA, VL, FD, and HZ

Table 4.6. Seven-day nutritive and biological values for the rats fed the experimental feeds.

Feed[1]	Nitrogen Balance[2] (g % N)	Apparent B.V.[2] (%)	Apparent Digestibility[2] (%)	Apparent Urinary Nitrogen Loss[2] (%)	Apparent N.P.U.[2] (%)
CA	$224.4 \pm 13.1^{3\,a}$	$58.4 \pm 1.8^{3\,a}$	$94.9 \pm 0.5^{3\,a}$	$60.5 \pm 1.8^{3\,a}$	$55.4 \pm 1.6^{3\,a}$
VH	135.2 ± 16.0^{bc}	53.3 ± 2.8^{a}	84.8 ± 1.4^{b}	60.5 ± 2.1^{a}	45.3 ± 2.8^{a}
VL	99.3 ± 3.9^{c}	49.4 ± 2.8^{a}	90.4 ± 1.0^{a}	54.5 ± 2.1^{a}	44.8 ± 2.9^{a}
FD	96.5 ± 9.4^{c}	52.1 ± 1.5^{a}	90.7 ± 1.0^{a}	56.6 ± 1.3^{a}	47.3 ± 1.6^{a}
HZ	161.1 ± 8.3^{b}	57.8 ± 1.6^{a}	91.0 ± 0.7^{a}	61.7 ± 1.3^{a}	52.7 ± 1.8^{a}

[1] CA = casein; VH = krill vacuum microwave blanched at a high temperature and air-dried; VL = krill vacuum microwave dehydrated at a low temperature; FD = freeze-dried krill; HZ = hydrolysed, freeze-dried krill.
[2] Data are expressed as mean ± SEM (n = 8).
[3] Data are expressed as mean ± SEM (n = 6).
N = Nitrogen.
B.V. = Biological Value (% Nitrogen Absorbed).
N.P.U. = Net Protein Utilisation.

Means with different superscript letters within a column are significantly different at $p \leq 0.05$.

feeds, which averaged 91.7 %, were not statistically different ($p \geq 0.05$). The VH feed had an apparent digestibility of 84.8 %, which was significantly lower ($p \leq 0.05$) than the apparent digestibilities of the other four experimental feeds. The pepsin digestibility of krill meal has been reported to range from 76 % to 90 % (Rehbein, 1981).

It is possible that the more severe heat treatment received by the *Euphausia pacifica* that was added as the protein source to the VH feed, may have caused the krill proteins to form new bonds that were resistant the rats' gut enzymes, thus, decreasing the VH meal's digestibility.

The apparent digestibilities of the four krill meals were all less than the apparent digestibility of the casein meal. The differences noted, again, may have been due to the presence of chitin in the krill meals. No significant differences ($p \geq 0.05$) amongst the five experimental groups were noted for the apparent B.V.s, average 54.2 %, for the apparent N.P.U.s, average 49.1 %, or for the apparent urinary nitrogen losses, average 58.7 %. Thus, the proportions of absorbed nitrogen that were retained for maintenance and growth, the proportions of feed nitrogen that were retained, and the proportions of nitrogen losses in the urine, were each equivalent across the five experimental groups.

There were no significant differences ($p \geq 0.05$) in the percent protein of the leg muscles of the sacrificed animals. The average percent protein of the leg muscles of the CA, VH, VL, FD, and HZ groups were 22.69 % protein, 21.80 % protein, 21.79 % protein, 21.50 % protein, and 21.57 % protein, respectively. These values are comparable to literature values for the protein content of leg muscles of mammals (U.S.D.A., 2001). However, one animal in the FD group had minimal muscle remaining upon sacrifice, and therefore, no muscle from that animal was analysed. This data indicates that the protein content of muscle was not affected by the weight of the animals.

Protein deprivation with a negative nitrogen balance occurs when there is inadequate protein intake, excessive protein loss, and/or there is a state of accelerated catabolism of tissue proteins resulting in clinical evidence of starvation and wasting (Davidson and Henry, 1974).

As the nitrogen balances of the animals on the CA, VH, VL, FD, and HZ feeds were all positive, and as the apparent B.V.s, the apparent N.P.U.s, and the nitrogen losses in the urine showed no statistical differences ($p \geq 0.05$), it can be reasoned that there was no protein deprivation and protein quality was not responsible for the negative effects of krill meal on the feed intakes and the health of the animals.

3. Amino Acid Profiles

The amino acid profiles of the experimental feeds are given in Table 4.7. The profiles of the four experimental *E. pacifica* meals were similar to amino acid literature values for *E. superba*. The FD feed had the lowest lysine and tryptophan contents of the five experimental feeds, however, it had the highest total sulphur-containing amino acids content. The HZ feed had the lowest total sulphur-containing amino acids content of the five experimental feeds, but the highest lysine and tryptophan contents.

The work of Lea *et al.* (1958; 1960) indicated that amino compounds were not much involved in the oxidative reactions in fishmeal, as the loss of available lysine was only 8 % after twelve months of storage. The conclusion is that the nutritive value of fishmeal is not damaged by oxidation.

Amino acid scores of the VH, VL, FD, and HZ feeds are presented in Table 4.8. The reference patterns for lysine, tryptophan, and the total sulphur-containing (cysteine plus methionine) amino acids were selected as lysine is reported to be the first essential amino acid rendered unavailable during heat processing (Smith and Pena, 1977), and tryptophan is the first

Table 4.7. Amino acids present in the experimental rat feeds[1].

	Feed[2] (mg amino acid/100 mg protein)				
	CA	**VH**	**VL**	**FD**	**HZ**
Amino Acid					
Alanine	4.41	6.86	7.04	6.65	6.91
Arginine	4.98	8.08	7.61	7.09	3.89
Aspartic Acid	10.98	13.05	13.03	11.80	12.22
Cysteine	1.73	1.71	1.88	2.05	1.68
Glutamic Acid	35.69	18.44	18.04	16.25	16.88
Glycine	2.74	6.82	8.36	7.88	6.88
Histidine	3.81	2.25	2.36	2.16	2.13
Isoleucine	6.24	4.65	4.87	4.64	4.43
Leucine	13.69	9.04	9.00	8.27	8.51
Lysine	11.45	9.87	10.67	9.46	11.89
Methionine	3.14	3.46	3.04	3.34	2.84
Phenylalanine	7.12	4.76	4.91	4.47	4.60
Proline	15.39	3.99	5.64	5.89	4.82
Serine	9.02	5.15	4.89	4.57	3.76
Threonine	6.26	5.26	5.36	4.97	4.70
Tryptophan	1.46	1.48	1.20	0.91	1.78
Tyrosine	6.18	3.76	3.91	3.41	2.99
Valine	7.88	4.95	5.24	4.88	4.74
Amide Nitrogen	2.66	1.71	1.70	1.69	1.59

[1] Data expressed as mg amino acid/100 mg protein.
[2] CA = casein; VH = krill vacuum microwave blanched at a high temperature and air-dried; VL = krill vacuum microwave dehydrated at a low temperature; FD = freeze-dried krill; HZ = hydrolysed, freeze-dried krill.

Table 4.8. Amino acid scores for the experimental rat feeds obtained using the reference patterns for lysine, tryptophan and the total sulphur-containing amino acids (cysteine and methionine)[1].

	Feed[2]			
	VH	**VL**	**FD**	**HZ**
Reference Pattern[3] (% of reference feed)				
Lysine	86.19	93.18	82.59	103.88
Tryptophan	101.28	82.05	62.39	121.79
Cysteine + Methionine	100.36	95.75	104.86	87.85

[1] Data expressed as a percentage of:
mg amino acid in test protein/mg amino acid in reference protein;
reference protein = casein.

[2] CA = casein; VH = krill vacuum microwave blanched at a high temperature and air-dried; VL = krill vacuum microwave dehydrated at a low temperature; FD = freeze-dried krill; HZ = hydrolysed, freeze-dried krill.

[3] The amino acid pattern of the casein meal was used as the reference standard.

limiting amino acid in crustaceans (Bender, 1958). As well, it has been reported, that it is necessary only to analyse proteins for lysine, tryptophan, and the total sulphur-containing amino acids (Harper, 1981). The reasoning for this is that if the requirements for these are met by a feed protein, it is unlikely that the requirements for the other amino acids will not be met (Harper, 1981).

The scores for each of the four krill meals for the three reference patterns (in comparison to the CA feed) all varied from one another. The scores for the lysine and the cysteine and methionine reference patterns were all high and within a general agreement, ranging from 82.59 % to 103.88 %; and 87.85 % to 104.86 %, respectively. The scores for the VH, VL, and HZ feeds for the tryptophan reference pattern were also all high and within a general agreement, ranging from 82.05 % to 121.79 %, however, the score for the FD feed was markedly lower at 62.39 %. The correlation for the P.E.R. on the amino acid score for lysine for the krill meals was $r = 0.63$, ($p \geq 0.05$); for P.E.R. on tryptophan $r = 0.97$, ($p \leq 0.05$); and for P.E.R. on the total sulphur amino acids $r = 0.68$, ($p \geq 0.05$). Thus, tryptophan in the FD feed was limiting, and partially accounted for the low values reported for the P.E.R. and nitrogen balance studies.

4. Available Lysine

Testing was conducted to determine if there was a difference between the available lysine values of the dehydrated *Euphausia pacifica* meals fed to young rats and the available lysine values of the control meal fed to young rats; and to determine if there was a difference between the available lysine values of the dehydrated *Euphausia pacifica* meals fed to young rats.

Table 4.9 gives the results obtained for the determination of available lysine in the five experimental feeds. The average available lysine value for the casein meal, 7.6 mg lysine/100 mg protein, was in excellent agreement with literature values for casein, 7.8 mg lysine/100 mg

Table 4.9. Available and total lysine contents of the experimental feeds and percent available lysine of total lysine in the experimental feeds[1].

Feed[2]	Available Lysine (mg lysine/100 mg protein)	Total Lysine (mg lysine/100 mg protein)	% Available Lysine of Total Lysine[3] (%)
CA	7.6 ± 2.6^{a}	11.5	66.3
VH	5.6 ± 2.7^{b}	9.9	56.8
VL	8.5 ± 4.7^{a}	10.7	79.4
FD	8.5 ± 4.2^{a}	9.5	89.6
HZ	8.9 ± 3.3^{a}	11.9	74.9

[1] Data expressed as mg lysine/100 mg protein ± SEM (3 measurements taken).
[2] CA = casein; VH = krill vacuum microwave blanched at a high temperature and air-dried; VL = krill vacuum microwave dehydrated at a low temperature; FD = freeze-dried krill; HZ = hydrolysed, freeze-dried krill.
[3] Total lysine values were obtained from Table 4.7.

Means with different superscript letters within a column are significantly different at $p \leq 0.05$.

protein (dry sample) (Kakade and Liener, 1969). The available lysine content of the VH feed, 5.6 mg lysine/100 mg protein, was significantly lower ($p \leq 0.05$) than the available lysine contents of the CA, VL, FD, and HZ feeds, which averaged 8.4 mg lysine/100 mg protein. This may be indicative of processing damage by Maillard reactions. The available lysine contents in the VL, FD, and HZ feeds were all higher than the available lysine content in the CA feed.

The average available lysine present in fishmeal is 4.3 mg lysine/100 mg protein and in fish protein is 8.0 mg lysine/100 mg protein (Kakade and Liener, 1969). The values determined for dehydrated *Euphausia pacifica* fall within this range.

The available lysine contents did not account for the low P.E.R. values seen in the VL, FD, and HZ groups. However, it is possible that the low P.E.R. value obtained for the VH group was due, in part, to the reduced amount of available lysine in the feed. The lysine may have been rendered unavailable by Maillard reactions during the dehydration of the *Euphausia pacifica*, and therefore, some of its nutritive value lost (Carpenter, 1958). The *E. pacifica* that was added as the protein source in the VH feed received, of the four dehydrated krill samples, the most severe heat treatment.

5. Plasma α-Amino Nitrogen

It has been suggested that post-prandial plasma amino acid levels may be useful in detecting decreases in the digestive release of amino acids such as lysine and methionine, which may occur due to the processing of the protein sources (Vaughan *et al.*, 1974). Testing was conducted to determine if there was a difference between the plasma α-amino nitrogen levels of the young rats fed the dehydrated *Euphausia pacifica* meals and the plasma α-amino nitrogen levels of the young rats fed the control meal; and to determine if there was a difference between the plasma α-amino nitrogen levels of the young rats fed the dehydrated *Euphausia pacifica*

meals.

Results from this author's plasma α-amino nitrogen analyses are given in Table 4.10. The data shows that only the FD group had a plasma α-amino nitrogen level, 13.1 mg nitrogen/100 mL plasma, significantly lower ($p \leq 0.05$) than that of the CA control group at 17.5 mg nitrogen/100 mL plasma. The FD group however, was not statistically different ($p \geq 0.05$) from the VH, VL, and HZ groups, which had an average plasma α-amino nitrogen concentration of 15.2 mg nitrogen/100 mL plasma.

Plasma proteins do not reflect a significant decrease in levels until tissue proteins have been depleted (Davidson and Henry, 1974). As the rats in the FD group had lower plasma α-amino nitrogen levels than the other groups, and as they were in poor physical condition, it is possible that their tissue proteins had been or were nearly, depleted.

6. *In Vitro* Digestibility

Testing was conducted to determine if there was a difference between the *in vitro* digestibilities, with and without chitinase, of the dehydrated *Euphausia pacifica* meals and the *in vitro* digestibility of the control meal; and to determine if there was a difference between the *in vitro* digestibilities of the dehydrated *Euphausia pacifica* meals, with and without chitinase.

The initial time courses of the pepsin-pancreatin digestion and the pepsin-pancreatin digestion with chitinase of the five experimental feeds are shown in Figure 4.1 and, in Appendix IV, in Figures 7.4a and 7.4b. No attempt was made to remove the products of digestion, which are known to accumulate and ultimately inhibit the process of enzymatic digestion (Robbins, 1978). However, the use of the initial slope method overcame this disadvantage.

Significantly slower ($p \leq 0.05$) rates of proteolysis of the feeds were observed for the VH,

Table 4.10. Plasma α-amino nitrogen values of the rats fed the experimental feeds.

Feed[1]	Plasma α-Amino Nitrogen[2] (mg/100 mL)	(range)[3] (mg/100 mL)
CA	17.5 ± 0.4^{a}	(12.7 - 24.8)
VH	15.9 ± 0.3^{ab}	(12.01- 21.3)
VL	14.4 ± 0.3^{ab}	(6.1 - 20.2)
FD	13.1 ± 0.3^{b}	(8.0 - 16.3)
HZ	15.3 ± 0.4^{ab}	(12.8 - 19.9)

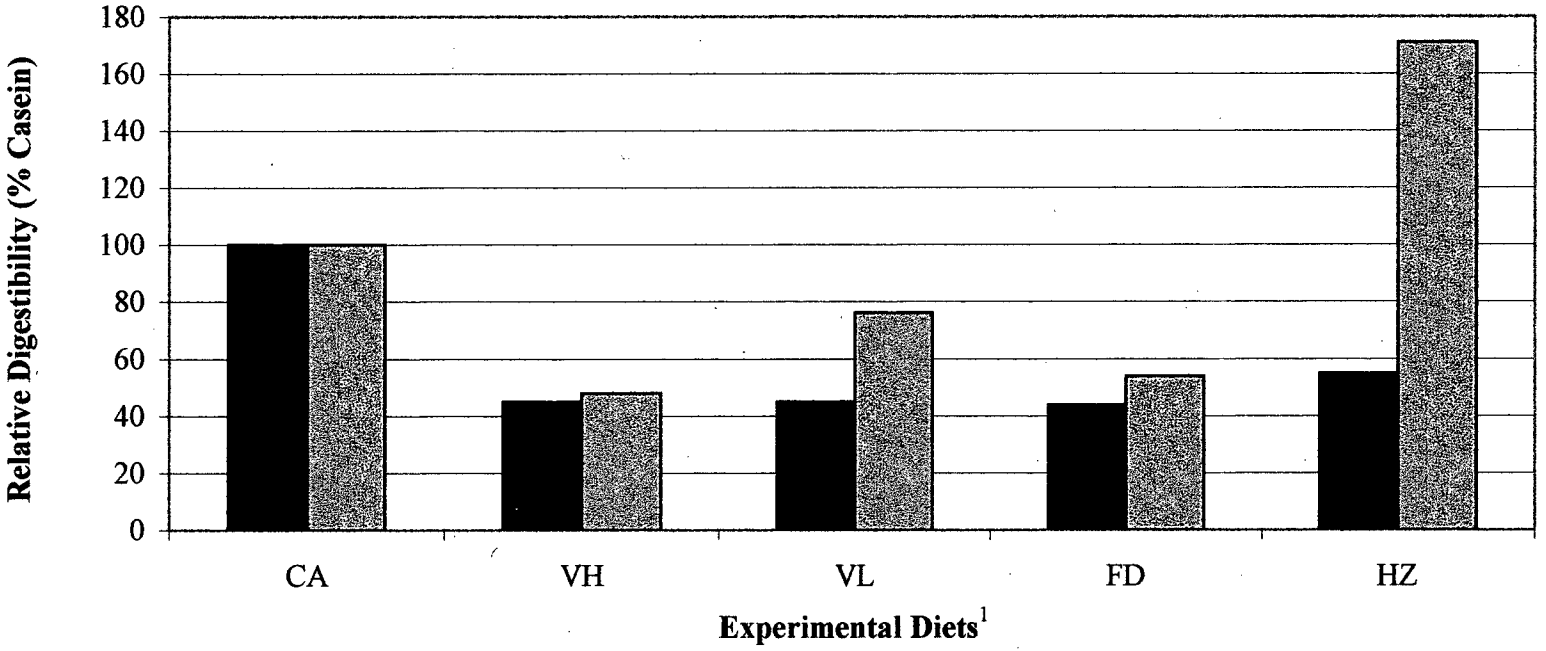
[1] CA = casein; VH = krill vacuum microwave blanched at a high temperature and air-dried; VL = krill vacuum microwave dehydrated at a low temperature; FD = freeze-dried krill; HZ = hydrolysed, freeze-dried krill.

[2] Data are expressed as mean ± SEM (n = 30). Plasma from each of the ten rats fed the experimental feeds was evaluated for plasma α-amino-nitrogen in triplicate.

[3] Data are expressed as mean ranges (n = 30). Plasma from each of the ten rats fed the experimental feeds was evaluated for plasma α-amino-nitrogen in triplicate.

Means with different superscript letters within a column are significantly different at $p \leq 0.05$.

Figure 4.1. Pepsin-pancreatin digestion estimates of the experimental rat feeds calculated from the initial reaction rates



(0 minutes to 10 minutes). Respective slopes for the untreated feeds were: CA = 9.1 x 10^{-3}; VH = 4.1 x 10^{-3}; VL = 4.1 x 10^{-3}; FD = 4.0 x 10^{-3}; HZ = 5.0 x 10^{-3}; regression range = $0.62 \geq r^2 \leq 0.98$.
Respective slopes for the chitinase treated feeds were: CA = 9.1 x 10^{-3}; VH = 4.4 x 10^{-3}; VL = 6.9 x 10^{-3}; FD = 4.9 x 10^{-3}; HZ = 14.9 x 10^{-3}; regression range = $0.81 \geq r^2 \leq 0.98$.

[1] CA = casein; VH = krill vacuum microwave blanched at a high temperature and air-dried; VL = krill vacuum microwave dehydrated at a low temperature; FD = freeze-dried krill; HZ = hydrolysed, freeze-dried krill.

(■) = no treatment

(▩) = with chitinase treatment.

VL, FD, and HZ feeds as compared to the CA feed as shown in Figure 4.1. The differences in the proteolysis rates between the casein meal and the four krill meals could be explained based on protein structure. The protein structure of casein is highly randomised and thus, enzymatic treatment results in a rapid rate of hydrolysis (Maga *et al.*, 1973). Maillard reactions may have occurred in the VH and VL krill meals during processing thus, decreasing these meals' digestibilities. As well, some of the protein in the four krill meals may have been bound to chitin, and thus, been resistant to enzymatic digestion. Significantly slower ($p \leq 0.05$) rates of proteolysis of the feeds with added chitinase were observed for the VH, VL, and FD feeds as compared to the CA feed, however, the HZ feed was found to have a significantly higher ($p \leq 0.05$) rate of proteolysis as compared to the CA feed. The protein in the HZ feed had been hydrolysed, and thus, it was not surprising that it was rapidly digested in this assay. That the rate of proteolysis for the HZ feed digested with chitinase was significantly higher ($p \leq 0.05$) than the rate of proteolysis for the CA feed, confirms that some of the protein in HZ krill meal was bound to chitin, and thus, was resistant to enzymatic digestion.

The rates of proteolysis of the VL, FD, and HZ feeds with added chitinase were all significantly faster ($p \leq 0.05$), and the VH feed showed a trend towards being faster, than the rates of proteolysis of these feeds without the added chitinase. This indicates that chitin, at least in part, was responsible for the decreased *in vitro* digestibilities, and possibly for the decreased *in vivo* digestibilities (as compared to the casein meal) of the four experimental krill meals.

In the *in vitro* assay of the feeds without added chitinase, significantly lower ($p \leq 0.05$) digestibilities were observed for the VH, VL, FD, and HZ feeds as compared to the CA feed; however, in the *in vivo* assay, the apparent digestibilities of the VL, FD, and HZ meals were not significantly lower ($p \geq 0.05$) than the CA meal. Therefore, it is possible that the rats were able

to digest some of the chitin present in the krill meal feeds. According to Kühl *et al.* (1978), krill chitin was partly digested by rats and its nitrogen absorbed. It was suggested that the decomposition of chitin takes place mainly in the caecum due to bacterial activity. This was proposed since the weights of the caecae in animals given feeds containing krill shells were greatly increased.

There may be problems in the interpretation of *in vitro* digestibility data when heat processed samples are analysed (Walker, 1983). Pronczuk *et al.* (1973) found that the *in vitro* digestibility (with papain and pancreatin) of casein autoclaved with glucose for various time intervals was more closely associated with N.P.U. than with *in vivo* digestibility as determined with rats. It was noted that the longer the sample was autoclaved, the greater the resistance to *in vitro* digestion; however, this was not reflected as a decrease in the *in vivo* digestibility, even after two hours and fifteen minutes of autoclaving (Pronczuk *et al.*, 1973). They found that urine from rats fed heat-damaged casein contained protein and high molecular weight peptides, which were not found in the urine of rats that were fed non-heat-damaged casein. Ford and Shorrock (1971) obtained similar results for other types of heated foods. These reports indicate that large heat-damaged peptides are absorbed by the rat, but, being unsuitable for utilisation in the body, are excreted in the urine (Walker, 1983). Urinary nitrogen is particularly useful as a direct predictor of protein quality (Bodwell *et al.*, 1979).

D. Fluoride Evaluation

The objectives of this study were to first determine the levels of fluoride present in the experimental feeds, then to assess the potential toxicity of fluoride in the dehydrated *Euphausia pacifica* meals and to determine if the different dehydration methods influenced the overall toxicity of fluoride in *Euphausia pacifica.* Testing was conducted to determine if there was a

difference between the fluoride balances of the young rats fed the dehydrated *Euphausia pacifica* meals and the fluoride balance of the young rats fed the control meal; and to determine if there was a difference between the fluoride balances of the young rats fed the dehydrated *Euphausia pacifica* meals.

The effects of the ingestion, by animals, of excessive amounts of dietary fluoride at levels of 200 mg F^-/kg dry diet to 600 mg F^-/kg dry diet, have been extensively studied. One of the major physiological responses repeatedly noted is a decline in growth rate. This decline is the result of a marked decrease in feed consumption by the animals (Simon and Suttie, 1968). Some studies have specifically indicated that the degree to which the consumption is decreased is lessened as the period of exposure is extended (Leemann *et al.*, 1967). It has also been shown that the ingestion of a high fluoride feed by the rat results in an increase in the plasma fluoride content (Shearer and Suttie, 1967), and that the decline in feed consumption, at least in short-term experiments, is inversely correlated with this increased plasma fluoride level (Simon and Suttie, 1968).

Dietary, urinary, faecal, and plasma fluoride concentrations from this author's study are presented in Table 4.11. The results show that the four feeds containing krill (VH, VL, FD, and HZ) all had markedly higher levels of fluoride, 299 mg F^-/kg dry diet on average, than the control (CA) at 34 mg F^-/kg dry diet. The data indicates that for the VH, VL, FD, and HZ groups there was a sharp drop in feed intake as compared to the CA control group, and an immediate effect on growth. These were both statistically ($p \leq 0.05$) related to the amount of fluoride present in the feed. The regression of feed intake on dietary fluoride concentration for the five experimental feeds produced an $r^2 = 0.36$, ($p \leq 0.05$), and the regression of weight gain on dietary fluoride concentration produced an $r^2 = 0.69$, ($p \leq 0.05$). Weight gain was statistically

Table 4.11. Fluoride concentrations found in the experimental rat feeds, in the rat excrement, and in the rat plasma of the rats fed the experimental feeds.

Feed[1]	Dietary F^{-2} (mg F^-/kg dry diet)	Urinary F^{-2} (μg F^-/mL urine)	Faecal F^{-2} (μg F^-/g dry faeces)	Plasma F^{-2} (μg F^-/mL plasma)
CA	34	164	262	ND
VH	312	220	362	ND
VL	276	590	337	6
FD	291	490	298	10
HZ	315	500	379	ND

[1] CA = casein; VH = krill vacuum microwave blanched at a high temperature and air-dried; VL = krill vacuum microwave dehydrated at a low temperature; FD = freeze-dried krill; HZ = hydrolysed, freeze-dried krill.
[2] Data obtained from ten pooled samples.
F^- = Fluoride.
ND = Not Detectable.

($p \leq 0.05$) related to feed intake. The regression of weight gain on feed intake produced an $r^2 = 0.42$, ($p \leq 0.05$) (calculated from the nitrogen balance study data). The consumption of feed by the rats fed the casein meal was almost twice as much as that of the rats fed the krill meals. The feed intake of the control group at the start of the P.E.R. experiment averaged 14.6 g/day (9.5 g/100 g body weight) and 16.5 g/day (6.5 g/100 g body weight) at the end, while the feed intake of the VH, VL, FD, and HZ groups at the start of the P.E.R. experiment averaged 7.1 g/day (4.6 g/100 g body weight) and 9.7 g/day (7.5 g/100 g body weight) at the end. These results are similar to those of other authors (Simon and Suttie, 1968; Shearer and Suttie, 1967).

The data from similar experiments indicates that weanling rats appear to be more severely affected by the fluoride than adult rats (Simon and Suttie, 1968). In weanling animals, the feed intake of those fed 600 mg F^-/kg dry diet (as NaF) did not recover to the extent that it did in the older animals (Simon and Suttie, 1968).

As previous experiments have indicated (Simon and Suttie, 1968; Shearer and Suttie, 1967), there is a close relationship between decreased feed intake and plasma fluoride concentration. In a study by Simon and Suttie (1968), the plasma fluoride concentrations of 260 g rats receiving 300 mg F^-/kg dry diet for a period of forty-two days were higher than the controls. The plasma fluoride concentrations of their rats tended to average about 2 μg F^-/mL for the entire experiment. Similar results were obtained for this author's study. Feed intakes and weight gains of the experimental groups were statistically ($p \leq 0.05$) related to the fluoride concentration in the rats' plasma. The regression of feed intake on plasma fluoride concentration produced an $r^2 = 0.38$ (negatively correlated), ($p \leq 0.05$), and the regression of weight gain on plasma fluoride concentration produced an $r^2 = 0.26$, ($p \leq 0.05$). The VL and FD groups had plasma fluoride concentrations markedly higher, 8 μg F^-/mL on average, than the CA control

group, which had non-detectable levels of plasma fluoride. The VH and HZ groups also had non-detectable levels of fluoride in their plasma.

The elimination rate of fluoride from plasma exceeds the absorption rate after the bulk of the fluoride has been absorbed (Whitford, 1989). This may explain why the VH and HZ groups had non-detectable levels of plasma fluoride. Regulatory mechanisms maintain the plasma fluoride content within narrow limits, but these mechanisms can be overridden by sustained, increased intakes. This has been observed with rats during periods of high fluoride intake, or during long periods of feed deprivation (Singer and Armstrong, 1964). As well, starvation has been proven to increase fluoride permeability in yeast cells (Malm, 1947). As the rats in the VL and FD groups had significantly lower ($p \leq 0.05$) feed intakes and final body weights (in the P.E.R. study) than the other experimental groups, it is possible that there was a higher degree of fluoride permeation in the cells of the VL and FD groups, and that the regulatory mechanisms of the skeletal and renal tissues were overridden. These explanations may account for the high plasma fluoride concentrations noted in these two groups.

The sharp decline in dietary intake, which has often been noted in fluoride toxicity, may be a temporary response as feed intake has been reported to return to near normal levels after a period of reduced consumption (Zebrowski and Suttie, 1966). A response such as this may account for the increases seen in this author's results in the feed intakes of the VH, VL, and HZ fed rats between the P.E.R. study and the nitrogen balance study. The rats' feed intake responses to the inclusion of this toxic agent (fluoride) in the feed are very similar to those which have been observed in amino acid imbalances in which animals adapt to the nutritional disorder and gradually increase their intake of the feed as they continue to ingest it (Simon and Suttie, 1968). The ability of the rat to increase its consumption of the high fluoride feed following an initial drop in consumption suggests some type of adaptation to the elevated plasma and soft tissue

fluoride concentrations (Simon and Suttie, 1968). It is postulated that the relationship between feed intake and plasma fluoride is an indirect one, mediated through an effect on tissue enzyme activity which results in a shift in the concentration of some metabolites, and that this altered balance of tissue metabolites, rather than the increase in fluoride concentration, is the signal for the depressed dietary intake (Simon and Suttie, 1968). The ability of an animal to increase its dietary intake in the presence of a continued high tissue fluoride concentration could be explained by an alteration of metabolic pathways, resulting in a return of metabolite concentrations to near normal. That alterations, in at least some metabolic pathways, do occur is evident from changes in carbohydrate (Zebrowski and Suttie, 1966) and lipid metabolism as demonstrated in rats fed fluoride (Simon and Suttie, 1968).

Urinary fluoride analysis can be a useful diagnostic aid as urinary fluoride levels are correlated to some extent with dietary fluoride intake. The urinary fluoride level however, is affected by a number of important variables, including the duration of fluoride ingestion, the time of day the sampling is conducted, and the total urinary output (Shupe *et al.*, 1963b).

Healthy animals that are consuming nourishing diets, and are not exposed to unusual fluoride intakes from their feed, water, or atmosphere, excrete 80 % or more of their ingested fluoride in their urine (Machle *et al.*, 1942). With the less soluble or more slowly soluble forms of fluoride added to the diet, a lower percentage appears in the urine because of decreased absorption (Underwood, 1971). In sheep and cattle not exposed to excess fluoride, daily urinary fluoride concentrations rarely exceed 10 μg F^-/mL and are usually closer to 5 μg F^-/mL. With elevated fluoride intakes, the daily urinary fluoride concentrations rise quickly to between 15 μg F^-/mL and 30 μg F^-/mL and may reach 80 μg F^-/mL (Underwood, 1971). Higher values are occasionally observed, and great variation can exist among animals consuming the same amounts of fluoride, as well as among samples from the same animal taken on different days or

at different times on the same day (Underwood, 1971). Borderline toxicity ranges from 20 μg F^-/mL to 30 μg F^-/mL, and systemic toxicity is reached at 35 μg F^-/mL (Underwood, 1971).

The urinary fluoride concentrations of this author's experimental animals were varied. All the krill meal fed groups had higher urinary fluoride concentrations than the control group; however, the CA group had an unexpectedly high fluoride concentration in the urine. After accounting for (by subtraction) the urinary fluoride in the control group, the VH group had the lowest daily urinary fluoride concentration of the krill meal fed groups at 8 μg F^-/mL. The VL, FD, and HZ groups had daily urinary fluoride concentrations of 61 μg F^-/mL, 47 μg F^-/mL, and 48 μg F^-/mL, respectively. The VL, FD, and HZ groups all surpassed the levels of systemic toxicity. Urinary fluoride levels of the five experimental groups were statistically ($p \leq 0.05$) related to the dietary fluoride intakes. The regression of urinary fluoride on dietary fluoride consumed produced an $r^2 = 0.21$, ($p \leq 0.05$).

The clearance of fluoride by the kidney increases with urine volume (Whitford *et al.*, 1976). Eagers (1969) found that the consumption of water by sheep and swine tended to increase with an increase in the concentration of fluoride in the diet. Studies on the physiologic responses of the rat to a large fluoride challenge provide evidence that rats can control plasma fluoride and dispose of fluoride by urinary excretion (Yeh *et al.*, 1970). This author's results are in agreement with these findings. The consumption of water by the rats in the five experimental groups was statistically ($p \leq 0.05$) related to the dietary fluoride concentration. The regression of water consumption on dietary fluoride concentration produced an $r^2 = 0.11$, ($p \leq 0.05$). The volume of urine produced by the rats was statistically ($p \leq 0.05$) related to the plasma fluoride concentration, to the volume of water ingested, and to the amount of feed consumed. The

regression of urine excreted on plasma fluoride concentration produced an $r^2 = 0.25$, ($p \leq 0.05$), the regression of urine production on water consumption produced an $r^2 = 0.61$, ($p \leq 0.05$), and the regression of urine production on feed intake produced an $r^2 = 0.26$, ($p \leq 0.05$). These were as expected.

The faecal fluoride concentrations of this author's experimental animals were varied. All the krill meal fed groups had higher faecal fluoride concentrations than the control group; however, the CA group had an unexpectedly high fluoride concentration in the faeces. After accounting for (by subtraction) the faecal fluoride in the control group, the FD group had the lowest daily faecal fluoride concentration of the krill meal fed groups, at 5 μg F^-/g. The VH, VL, and HZ groups had daily faecal fluoride concentrations of 14 μg F^-/g, 11 μg F^-/g, and 17 μg F^-/g, respectively. The amount of faeces excreted by the rats in the five experimental groups was statistically ($p \leq 0.05$) related to the dietary fluoride concentration. The regression of faeces excreted on dietary fluoride concentration produced an $r^2 = 0.12$, ($p \leq 0.05$). The level of fluoride in the faeces was statistically ($p \leq 0.05$) related to the amount of fluoride in the consumed diet. The regression of faecal fluoride on consumed fluoride produced an $r^2 = 0.58$, ($p \leq 0.05$). The high levels of faecal fluoride were possibly due to fluoride binding with calcium in the diets, forming insoluble complexes, and thus being eliminated in the faeces.

The fluoride balances for the experimental groups are presented in Table 4.12. The fluoride balances of the rats in the VH and FD groups were not significantly different ($p \geq 0.05$) from the balances of the rats in the CA group. The rats in the VL and HZ groups had fluoride balances significantly different ($p \leq 0.05$) from the balances of the rats in the CA and the VH groups. The balances of the VL, FD, and HZ groups were not significantly different ($p \leq 0.05$). All the balances were negative. The balance of fluoride can be negative. It depends on the

Table 4.12. Seven-day fluoride balances and fluoride absorption values for the rats fed the experimental feeds.

Feed[1]	Fluoride Balance[2] (mg F^-)	Fluoride Absorbed[2] (%)
CA	-14.9 ± 2.4[3 ab]	68.7 ± 3.2[c]
VH	-3.9 ± 0.9[a]	85.8 ± 1.4[b]
VL	-36.5 ± 5.5[c]	88.6 ± 1.1[ab]
FD	-33.2 ± 6.2[bc]	90.8 ± 0.9[a]
HZ	-47.6 ± 6.0[c]	89.7 ± 0.7[a]

[1] CA = casein; VH = krill vacuum microwave blanched at a high temperature and air-dried; VL = krill vacuum microwave dehydrated at a low temperature; FD = freeze-dried krill; HZ = hydrolysed, freeze-dried krill.
[2] Data are expressed as mean ± SEM (n = 8).
[3] Data are expressed as mean ± SEM (n = 6).
F^- = Fluoride

Means with different superscript letters within a column are significantly different at $p \leq 0.05$.

blood-bone fluoride steady-state. A negative balance occurs when chronic fluoride intake is reduced sufficiently to allow the plasma fluoride concentration to fall, which promotes the mobilisation of the fluoride ion from calcified tissues, or when a steady-state had not yet been achieved. Negative balances have been seen with porotic lyses of fluorotic bones (Mansfield, 1999).

Studies of rats with elevated plasma fluoride concentrations showed that a diet high in calcium increased faecal fluoride excretion by forming insoluble complexes with fluoride and reducing fluoride absorption from the gastrointestinal tract, such that fluoride losses could equal or exceed fluoride intake (Whitford, 1994). The faecal fluoride concentrations in this author's five experimental groups all exceeded their respective dietary fluoride concentrations. Casein contains calcium, and ash derived from krill has been reported to be high in calcium (Kinumaki, 1980). Fluoride present in faeces results from two sources: the ingested fluoride that was not absorbed and the absorbed fluoride that was re-excreted into the gastrointestinal tract (World Health Organisation, 1984). This author found that, for the CA group, 31 % of the ingested fluoride was excreted in the faeces; this was 14.2 % for the VH group; 11.4 % for the VL group; 9.2 % for the FD group; and 10.3 % for the HZ group.

It has been demonstrated in other experiments that 80 % to nearly 100 % (Whitford, 1989; Eagers, 1969) of fluoride is absorbed from the gut. From this author's results, only 68.7 % of the fluoride ingested by the casein meal fed group, was absorbed. This was significantly lower ($p \leq 0.05$) than the fluoride absorptions for the four experimental krill meal fed groups, 88.7 %, on average. The fluoride absorption for the VH group, 85.8 %, was significantly lower ($p \leq 0.05$) than the fluoride absorptions for the VL, FD, and HZ groups, 89.7 %, on average.

The availability of fluoride in solid foods is generally reduced from that of soluble fluorides (Groff and Gropper, 2000). However, fluoride absorption is enhanced by a high

protein diet, which is known to increase gastric acidity (Groff and Gropper, 2000). Low pH (lumenal concentration of HF) facilitates the gastric absorption of fluoride. The extent of fluoride absorption does not appear to be influenced by plasma levels except at very high concentrations.

Absorbed fluoride leaves the bloodstream very quickly and is distributed rapidly throughout the body, particularly to the hard tissues. As the amount of absorbed fluoride increases, so does the quantity taken up by the hard tissues. However, the percentage retained at high absorption rates diminishes because urinary excretion increases (Groff and Gropper, 2000). Excretion of fluoride takes place rapidly via the urine, which accounts for approximately 80 % to 90 % of the total excretion (Groff and Gropper, 2000). This author's results are in agreement with this statement. For the CA group, 93.2 % of the excreted fluoride was removed via the urine; this was 87.7 % for the VH group; 96.4 % for the VL group; 96.9 % for the FD group; and 96.4 % for the HZ group.

Overall, fluoride appeared to be present in the dehydrated *Euphausia pacifica* samples at toxic concentrations. This most likely reduced the rats' dietary intake of the krill meal feeds.

E. Protease Determination

It is currently unknown if young fish fed feeds containing digestive enzymes experience increased protein utilisation. A potential source of digestive enzymes in fish feed could be derived from krill (*Euphausia pacifica*). However, one of the obstacles to the utilisation of krill protein is its quick autolysis by strong visceral proteases (Jiang *et al.*, 1991); therefore, the objective of this experiment was to determine if there was protease activity in the dehydrated *Euphausia pacifica* samples that may have enhanced or decreased protein utilisation by the young rats. Testing was conducted to determine if there was a difference between the protease

activities of a raw *Euphausia pacifica* sample and the dehydrated *Euphausia pacifica* samples, and the protease activity of the casein control; to determine if there was a difference between the protease activities of the dehydrated *Euphausia pacifica* samples; and to determine if there was a difference between the protease activities of the dehydrated *Euphausia pacifica* samples and the protease activity of the raw *Euphausia pacifica* sample.

Graphic results of the trends at pH 4.0, pH 7.0, and pH 9.0 of the protease activities in the four dehydrated and one raw krill samples versus casein are shown in Figure 4.2a, Figure 4.2b, and Figure 4.2c. The assays were run for sixty-six hours, as destructive enzyme reactions take place within this time frame at temperatures above freezing. Casein had no activity. At forty-eight hours, the trends of the FD and RW samples at pH 7.0 exhibited dramatic, unexpected increases in protease activity. It is possible that, at this point, these samples became contaminated, and therefore, gave false positives. No significant ($p \geq 0.05$) activity was present in any of the samples at pH 4.0 or at pH 7.0 (up to forty-eight hours). However, the activity trends of the VL and FD samples were higher at pH 7.0 than at pH 9.0. This indicates the possible presence of different types of krill proteases. Three types of krill proteases have been characterised (Seki *et al.*, 1977) - serine, metallo, and sulfhydryl. These enzymes are stable at a neutral or at a weak alkali pH. Significant ($p \leq 0.05$) activity was detected in the RW sample at pH 9.0. No activity was found in the FD sample. The VL, HZ, and RW samples all showed activity at one hour, which continued as such for the HZ and RW samples until twenty hours, when their activity increased. The VL sample showed a slow decrease in activity until this same time, at which point, no activity remained. At forty-eight hours the activity in the HZ sample sharply dropped. The VH sample showed minimal activity until forty-one hours, when there was an increase. At forty-eight hours, the activity in this sample began to decrease. The activity present in the raw krill sample was constantly higher than the activities of the other samples.

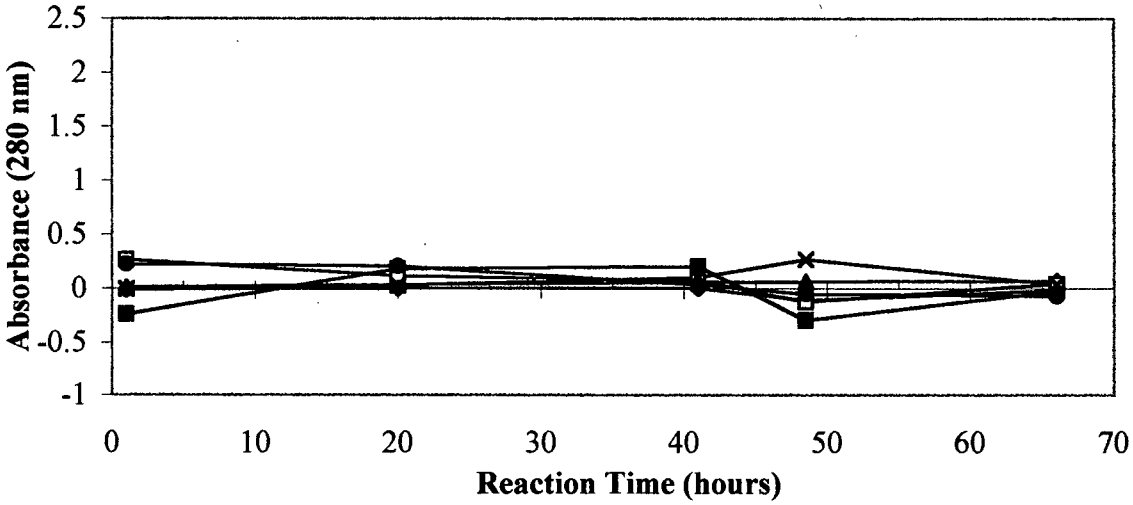


Figure 4.2a. Trends of proteolytic activity (absorbance 280 nm versus reaction time in hours) for the frozen and dehydrated *Euphausia pacifica* samples versus casein at pH 4.0.

CA = casein (♦); VH = krill vacuum microwave blanched at a high temperature and air-dried (■); VL = krill vacuum microwave dehydrated at a low temperature (▲); FD = freeze-dried krill (□); HZ = hydrolysed, freeze-dried krill (●); RW = raw krill (x).

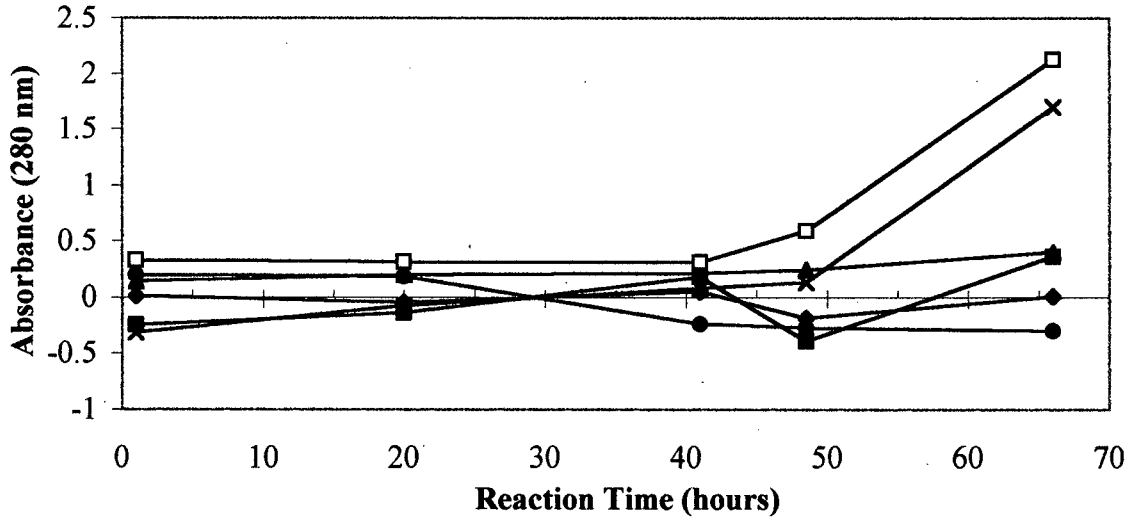


Figure 4.2b. Trends of proteolytic activity (absorbance 280 nm versus reaction time in hours) for the frozen and dehydrated *Euphausia pacifica* samples versus casein at pH 7.0.

CA = casein (♦); VH = krill vacuum microwave blanched at a high temperature and air-dried (■); VL = krill vacuum microwave dehydrated at a low temperature (▲); FD = freeze-dried krill (□); HZ = hydrolysed, freeze-dried krill (●); RW = raw krill (x).

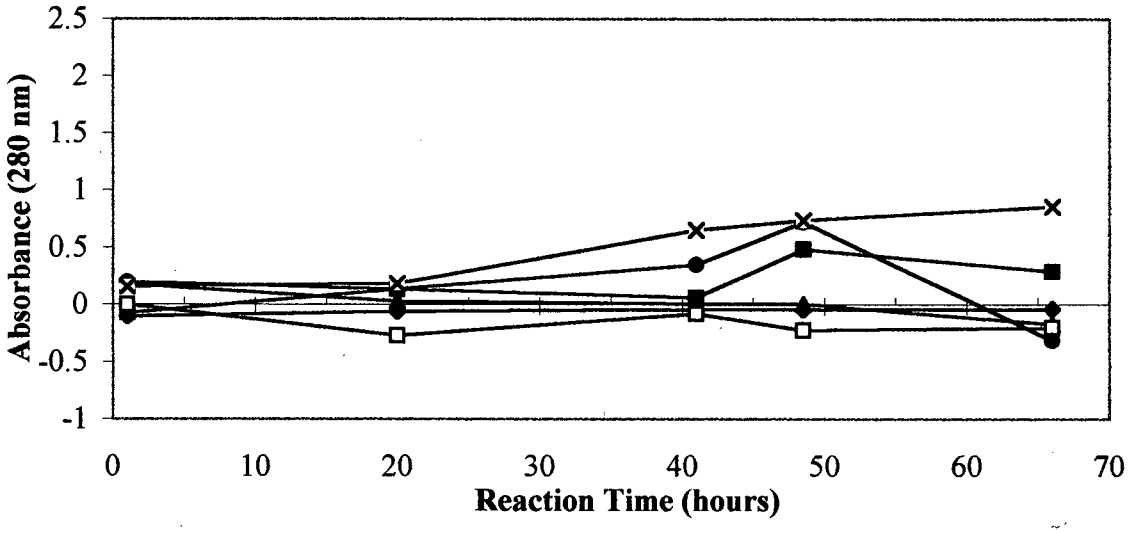


Figure 4.2c. Trends of proteolytic activity (absorbance 280 nm versus reaction time in hours) for the frozen and dehydrated *Euphausia pacifica* samples versus casein at pH 9.0.

CA = casein (♦); VH = krill vacuum microwave blanched at a high temperature and air-dried (■); VL = krill vacuum microwave dehydrated at a low temperature (▲); FD = freeze-dried krill (□); HZ = hydrolysed, freeze-dried krill (●); RW = raw krill (x).

From these analyses, it appears that drying *Euphausia pacifica* by vacuum microwave blanching at a high temperature and air-drying, by vacuum microwave dehydration at a low temperature, and by freeze-drying, all significantly reduce ($p \leq 0.05$) or destroy protease activity. Upon comparison of the protease activity trends at pH 9.0 of the hydrolysed, freeze-dried (HZ) sample, which showed some activity, (it was not statistically different ($p \geq 0.05$) from the raw krill sample), and the freeze-dried (FD) sample, which showed no activity, it is likely that the HZ krill sample was hydrolysed with proteases.

Overall, very limited protease activity remained in the krill samples. It can be surmised that protein quality and utilisation of the dehydrated krill meal feeds were not affected by proteases inherent to *Euphausia pacifica*.

CONCLUSIONS AND FUTURE DIRECTIONS

V. CONCLUSIONS AND FUTURE DIRECTIONS

The proximate constituents of the frozen and dehydrated (VH, VL, FD, HZ) *Euphausia pacifica* samples were consistent with results of other studies for *E. pacifica* and *E. superba* (Brown, 1959; Suyama *et al.*, 1965; Vinogradova, 1960; Yamada, 1964; and Pierce *et al.*, 1969). The chemical composition of the experimental *E. pacifica* (db) was, on average, 8.9 % moisture, 68.2 % crude protein, 13.8 % crude lipid, 11.9 % ash, and 6.1 % crude carbohydrates.

The protein efficiency ratios of the dehydrated *Euphausia pacifica* meals, 1.4 on average, were statistically lower ($p \leq 0.05$), 44 % on average, than the P.E.R. of the casein meal at 2.5. The reduction observed for the P.E.R. in the VH, VL, FD, and HZ meals can be associated with the lower feed intakes and thus, it does not properly credit protein used for maintenance purposes and the results are not directly proportional to the quality of the protein (McLaughlan, 1979). There were no statistical differences ($p \geq 0.05$) amongst the krill meal feeds.

The nitrogen balances of the animals on the CA, VH, VL, FD, and HZ feeds were all positive. The nitrogen balances of the krill meal feeds, 123.0 g % N on average, were all statistically lower ($p \leq 0.05$) than that of the casein meal at 224.4 g % N. The nitrogen balance of the HZ feed, 161.1 g % N, was statistically higher ($p \leq 0.05$) than the nitrogen balances of the VL and FD feeds, 97.9 g % N, on average. The apparent B.V.s, the apparent N.P.U.s, and the nitrogen losses in the urine showed no statistical differences ($p \geq 0.05$). Therefore, it can be reasoned that there was no protein deprivation and protein quality was not responsible for the negative effects of krill meal on the feed intakes and the health of the young animals.

The correlation for the P.E.R. on the amino acid score for lysine for the krill meals was

r = 0.63, ($p \geq 0.05$); for P.E.R. on tryptophan r = 0.97, ($p \leq 0.05$); and for P.E.R. on the total sulphur amino acids r = 0.68, ($p \geq 0.05$). Thus, tryptophan in the FD feed was limiting, and partially accounted for the low values reported for the P.E.R. and nitrogen balance studies.

The available lysine contents did not account for the low P.E.R. values seen in the VL, FD, and HZ groups. However, it is possible that the low P.E.R. value obtained for the VH group was due, in part, to the reduced amount of available lysine in the feed. The available lysine content of the VH feed, 5.6 mg lysine/100 mg protein, was significantly lower ($p \leq 0.05$) than the available lysine contents of the CA, VL, FD, and HZ feeds, which averaged 8.4 mg lysine/100 mg protein. Some of the lysine may have been rendered unavailable by Maillard reactions during the dehydration of the *Euphausia pacifica*, and therefore, some of its nutritive value lost (Carpenter, 1958). The *E. pacifica* that was added as the protein source in the VH feed received, of the four dehydrated krill samples, the most severe heat treatment.

Only the FD group had a plasma α-amino nitrogen level, 13.1 mg nitrogen/100 mL plasma, significantly lower ($p \leq 0.05$) than that of the CA control group at 17.5 mg nitrogen/100 mL plasma. The FD group however, was not statistically different ($p \geq 0.05$) from the VH, VL, and HZ groups, which had an average plasma α-amino nitrogen concentration of 15.2 mg nitrogen/100 mL plasma. Plasma proteins do not reflect a significant decrease ($p \leq 0.05$) in levels until tissue proteins have been depleted (Davidson and Henry, 1974). As the rats in the FD group had noticeably lower plasma α-amino nitrogen levels than the other groups, and as they were in poor physical condition, it is possible that their tissue proteins were nearly depleted.

The rates of proteolysis of the VL, FD, and HZ feeds with added chitinase were all significantly faster ($p \leq 0.05$), and the VH feed showed a trend towards being faster, than the rates of proteolysis of these feeds without the added chitinase. This indicates that chitin, at least

in part, was responsible for the decreased *in vitro* digestibilities, and possibly for the decreased *in vivo* digestibilities (as compared to the casein meal) of the four experimental krill meals.

For the *in vitro* assay of the feeds without added chitinase, significantly lower ($p \leq 0.05$) digestibilities were observed for the VH, VL, FD, and HZ feeds as compared to the CA feed; however, in the *in vivo* assay, the apparent digestibilities of the VL, FD, and HZ meals were not significantly lower ($p \geq 0.05$) than the CA meal. Therefore, it is possible that the rats were able to digest some of the chitin present in the krill meal feeds.

The four feeds containing krill (VH, VL, FD, and HZ) all had markedly higher levels of fluoride, 299 mg F^-/kg dry diet on average, than the control (CA) at 34 mg F^-/kg dry diet. The data indicates that for the VH, VL, FD, and HZ groups there was a sharp drop in feed intake as compared to the CA control group, and an immediate effect on growth. These were both statistically ($p \leq 0.05$) related to the amount of fluoride present in the feed. Overall, fluoride appeared to be present in the dehydrated *Euphausia pacifica* samples at toxic concentrations. This most likely reduced the rats' dietary intake of the krill meal feeds.

Very limited alkaline protease activity remained in the dehydrated krill samples. Protein quality and utilisation of the dehydrated krill meal feeds were not affected by proteases inherent to *Euphausia pacifica*.

Overall, fluoride present in the krill meals was correlated to a decrease ($p \leq 0.05$) in the rats' feed intake, which affected the values of the biological methods used for assessing protein quality and utilisation. The method used to dehydrate *Euphausia pacifica* did not statistically ($p \geq 0.05$) affect protein utilisation in the rat model.

Krill meal and frozen krill have been shown to be excellent feeds for salmonids (Koops, 1979; and Trzebiatowski *et al.*, 1979). Three-year-long investigations of salmon and trout, fed exclusively with krill, showed that the level of fluoride in their muscle was lower than that in

Baltic fish from the same area (Grave, 1981). Grave (1981) believes that there are no contra-indications to feeding krill to salmonids. Therefore, future studies could possibly be designed where young fish would be the model used to assess protein quality and utilisation of *Euphausia pacifica* that has been dried using vacuum microwave blanching at a high temperature and air-drying (VH), vacuum microwave dehydration at a low temperature (VL), freeze-drying (FD), and hydrolysis plus freeze-drying (HZ).

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APPENDICES

VII. APPENDIX I

The vacuum microwave basket was divided into four different components, all of which, except for one, a metal shaft, were manufactured from a 1.0 cm thick, machine-perforated, heat-resistant plastic. The material had been punched with numerous holes with a 0.5 cm radius, to maximise the basket's heat penetrability. The pieces included: two curved cylindrical surfaces, 12.0 cm in height, two detachable 1.0 cm thick O-ring endplates with an inner radius of 1.0 cm and an outer radius of 15.9 cm, one 2.0 cm thick O-ring cogwheel with an inner radius of 1.0 cm and an outer radius of 16.5 cm, and one 40.0 cm long metal shaft with a radius of 1.0 cm. The parts were assembled in the following manner. First, to form one basket compartment, one of the two O-ring endplates, acting as a basket floor, was fastened to a rim of one of the two cylindrical surfaces. The section was then filled with krill. Once this had been accomplished, the O-ring cogwheel, acting as a lid, was placed on the cylindrical surface's remaining edge. Following this, the second cylindrical surface was stacked on top of the O-ring cogwheel, with the cogwheel acting as a floor, thus, forming the second basket compartment. This too, was then filled with krill, before the second O-ring endplate, acting as a lid, was fastened to the remaining rim. Finally, the metal shaft (axle) was incorporated into the structure at its axis. It traversed the two basket compartments by being manipulated through the apertures of the three aligned O-ring plates. Approximately 6 cm of the shaft extended beyond both the top and the bottom endplates.

APPENDIX II

The basket's axle was cradled horizontally in a branched (two branches), bracketed stand that had been affixed to the centre of the bottom surface of the drying chamber. There was a distance of 5.1 cm from the VMD floor to the branch of the stand, and each branch had a height of 17.8 cm. There was a length of 33.0 cm between the branches. This suspended the basket above the bottom surface of the chamber, allowing it to be rotated throughout the drying process. Tumbling ensured even exposure of the krill to the microwave field and it reduced clumping (Durance, 2000). Next, the basket's cogwheel was meshed, in Spur gear fashion, with a smaller, but complementary 1.0 cm thick O-ring cogwheel with an inner radius of 0.5 cm and an outer radius of 3.8 cm (Food Science, U.B.C., Vancouver, B.C.). This second cogwheel was attached to the end of a 24.2 cm long rotatable metal shaft with a radius of 0.5 cm that projected out of the vacuum microwave dehydrator's back wall 43.2 cm above the bottom surface of the drying chamber.

Microwaves were fed into the vacuum chamber through waveguides fitted with microwave transparent "windows". The waveguide windows allowed microwaves to pass but prevented leakage of air into the chamber (Durance, 2000).

APPENDIX III

The hand-assembled air-dryer trays were 36.1 cm by 42.9 cm metal, mesh screens supported by 1.5 cm thick and 3.8 cm wide wooden frames (Food Science, U.B.C., Vancouver, B.C.). These were, through a 43.2 cm (length) by 38.1 cm (width), front-loading, bottom-hinged door, uniformly stacked. There was a space of 4.6 cm between each frame in the air-dryer. The external measurements of the hand-assembled wooden air-dryer were as follows: 44.5 cm (width) by 61.0 cm (depth) by 61.7 cm (height). The internal measurements of the air-dryer were as follows: 43.1 cm (width) by 59.7 cm (depth) by 60.5 cm (height)) (Food Science, U.B.C., Vancouver, B.C.). The heater (Super Furnace, Super Furnace Electrical Co., Toronto, ON.) was tightly fitted into a 19.1 cm (length) by 15.2 cm (width) sawed-out opening situated in the bottom centre of the front panel of the dryer. This was for fan circulation. The top of the 'Super Furnace' was a distance of 10.2 cm from the bottom of the first tray.

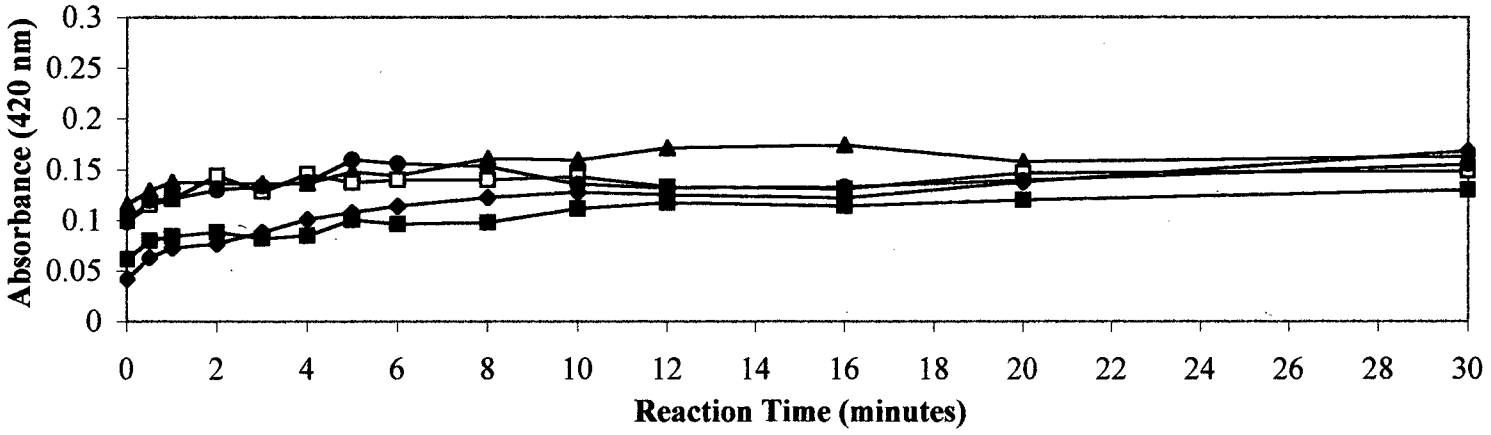

Figure 7.4a. Initial time course (absorbance 420 nm versus reaction time in minutes) of the pepsin-pancreatin digestion of the five experimental rat feeds. All points represent duplicate measurements of three incubations.
SEM (6 measurements taken) range for CA: ± 0.00 to ± 0.03; VH: ± 0.00 to ± 0.02; VL: ± 0.00 to ± 0.02; FD: ± 0.00 to ± 0.01; HZ: ± 0.00 to ± 0.02.
CA = casein (♦); VH = krill vacuum microwave blanched at a high temperature and air-dried (■); VL = krill vacuum microwave dehydrated at a low temperature (▲); FD = freeze-dried krill (□); HZ = hydrolysed, freeze-dried krill (●).
SEM = standard error of the mean.

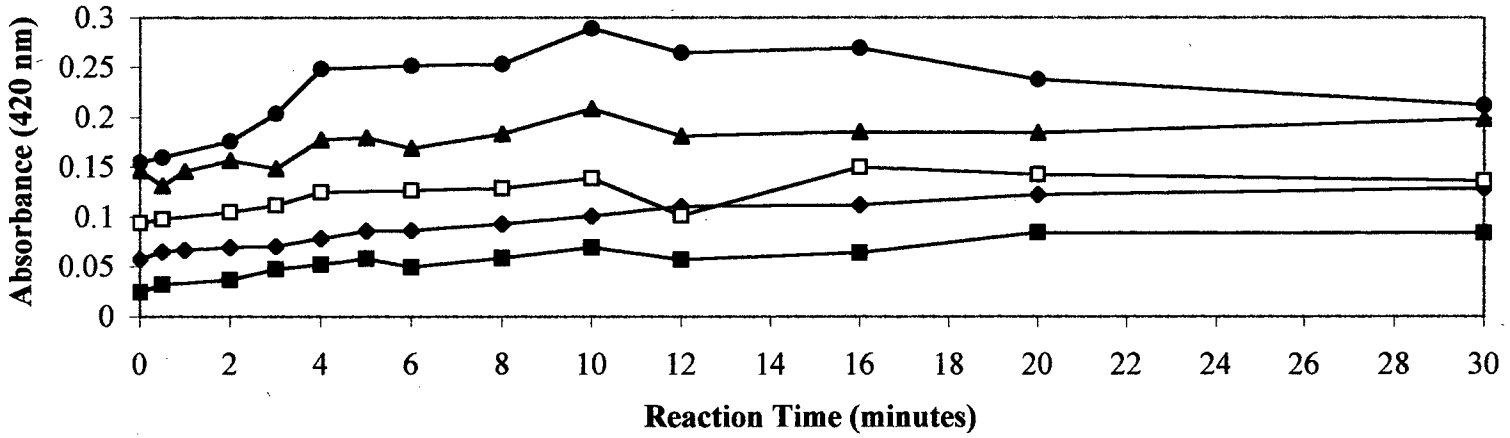

Figure 7.4b. Initial time course (absorbance 420 nm versus reaction time in minutes) of the pepsin-pancreatin digestion with chitinase of the five experimental rat feeds. All points represent duplicate measurements of two incubations.

SEM (4 measurements taken) range for CA: ± 0.00 to ± 0.01; VH: ± 0.01 to ± 0.03; VL: ± 0.01 to ± 0.04; FD: ± 0.01 to ± 0.04; HZ: ± 0.00 to ± 0.03.

CA = casein (♦); VH = krill vacuum microwave blanched at a high temperature and air-dried (■); VL = krill vacuum microwave dehydrated at a low temperature (▲); FD = freeze-dried krill (□); HZ = hydrolysed, freeze-dried krill (●). SEM = standard error of the mean.

GLOSSARY

VIII. GLOSSARY

Arc: A luminous, electrical gas discharge characterised by high current density and low potential gradient (Pitt, 1977).

Bomb Calorimeter: A device used for measuring the heat evolved by the combustion of a fuel, (the calorific values of fuels and foods). It consists of a strong container in which the sample is sealed with excess oxygen and ignited electrically. The heat of combustion at constant volume can be calculated from the resulting rise in temperature (Daintith, 1985).

Casein: The protein precipitate that results from treating skim milk with acid or with rennet.

Cephalothorax: Anterior part of body, composed of fused cephalon (head) and thorax (Abercrombie *et al.*, 1980).

Dielectric: A substance that is capable of sustaining an electrical stress, for example, an insulator (Pitt, 1977).

Fluoride/Fluorine: The terms "fluorine" and "fluoride" are used interchangeably in most literature as generic terms. This document uses the term "fluoride" as a general term everywhere, where an exact gaseous and particulate form is uncertain or unnecessary. The term covers all combined forms of the element, regardless of the chemical form, unless there is a specific reason to stress the gaseous elemental form F_2, in which case the term "fluorine" is used (World Health Organisation, 1984).

Gross Energy: The amount of heat that is released when a substance is completely oxidized in a bomb calorimeter containing 25 atmospheres to 30 atmospheres.

Hydrolysis: A process by which complex molecules (those in proteins) are split into simpler units by a chemical reaction with water molecules. The reaction may be produced by an enzyme, catalyst, or acid, or by heat and pressure.

Iodine Value: A measure of the degree of unsaturation in a lipid. It is obtained by finding the percentage of iodine by weight absorbed by the sample in a given time under standard conditions (Daintith, 1985).

Magnetron: The vacuum (electron) tube that converts electricity to microwaves (Pitt, 1977).

Maillard Reactions (browning): Non-enzymatic browning reaction between proteins and carbonyl compounds, particularly reducing sugars.

Pelagic: Pertaining to the organism(s) or habitat within the mass bodies of water, in contrast to the sea or the lake bottom (Abercrombie *et al.*, 1980).

Sublimation Front: A sharp and discrete dividing surface between a region which is fully hydrated and frozen and a region which is nearly completely dry.

Triple Point of Water: The intersection of three phase boundaries, liquid, solid, and vapour. At 32°F and at a pressure of 4.7 mm of mercury, water is in such a condition (Daintith, 1985).

Vacuum: A space in which there is a low pressure of gas (few atoms or molecules). Pressure below atmospheric pressure but above the triple point of water (Daintith, 1985).