MILK COMPOSITION OF THE NEW ZEALAND SEA LION AND FACTORS THAT INFLUENCE IT

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ABSTRACT

The objectives of the present study were to: 1) describe the gross chemical milk composition of the New Zealand sea lion (NZSLs), *Phocarctos hookeri*, in early lactation; 2) validate an analytical method for sea lion milk composition; 3) investigate a series of temporal, individual and dietary factors that influence the milk composition of the NZSL and; 4) investigate the temporal and spatial differences in the fatty acids signatures of sea lion milk.

A comprehensive literature review revealed that data on milk composition in otariid species is either missing or limited, that to be able to fully describe their milk composition extensive sampling was required and that the temporal, maternal and offspring factors that influence milk composition in pinnipeds are poorly understood. The review identified that considerable work has been conducted to infer diet via the application of fatty acids signature analysis of milk and blubber. There are many factors (i.e. metabolism, *de novo* synthesis and endogenous sources) that contribute to the differences in fatty acid composition between the diet and milk or blubber.

Milk samples from NZSL were used to test whether a new method would give similar results as the standard methods of milk analysis. Agreement between analytical methods for milk components was assessed using different measures of statistical fitness and the results indicated that the new method was comparable to the standard methods and applicable to the milk of sea lions, pinnipeds and to ecological studies of lactation. Milk from NZSLs was collected over a period of seven years (1997, 1999 to 2003, and 2005) in early lactation to describe the composition of milk of NZSL and to test for differences between years. The results indicated that: i) the milk protein concentration was comparable to other species of pinnipeds; ii) the milk fat concentration and the milk energy content of NZSL is the lowest reported for otariids in early lactation; however iii) the milk fat concentration was significantly different between years. These results suggested that the milk composition of NZSLs was influenced by annual changes in the environment; however, there may be other unidentified factors. Month, maternal body

condition, age, body weight and length, offspring sex and age, and attendance pattern were compared with milk components. The results identified that month, maternal body condition and age significantly affected milk fat concentration. These results and the fact that maternal body condition varied significantly between years and mothers nursing male pups had lower body condition and produced milk lower in energy content suggested that local food resources along with other unidentified factors have an effect on the reproductive success of NZSLs. To test whether the fatty acid signature analysis (FASA) of lipid rich tissues (milk, blubber and serum) of otariids could be used to infer diet a mixture of vegetable oil (with distinctive fatty acid signature) was fed to 24 lactating NZSL and tissue samples were collected at different time intervals. Significant increases in the concentration of specific fatty acids in serum and milk were observed with peaks within 12hrs and 24hrs respectively of ingestion. Concentrations in milk remained elevated for up to 72hrs and there were differential rates of incorporation into milk. These findings confirm the potential of FASA to infer the composition of the diet. The variation in milk fatty acid signatures from lactating NZSL from four years (1997, 2003, 2004 and 2005) of sampling were measured in order to test whether differences occurred between years. Fatty acids signatures from five potential prey species including the commercially important arrow squid were incorporated into the analysis to associate the changes in milk fatty acids with a shift in prey choice. The results indicated that milk fatty acid signatures were different in 1997 and 2003; however, it was not possible to relate these differences to the five prey species. The variability in the annual arrow squid catch data suggested that local food resources around the Auckland Islands may also be variable.

In conclusion, the milk produced by the NZSL has the lowest concentration of fat and energy in early lactation reported for any otariid species. The main factors that contributed to changes in milk quality were stage of lactation, year and maternal body condition. The yearly variation in the quality of milk appears to be a result of their lactation strategy or to variable local food conditions that also affect maternal body condition. Therefore monitoring the annual milk quality may be a means to monitor the health of a pinniped

population and potential management tool for pinniped species. This thesis has shown that annual changes in the diet of NZSL can be assessed with milk fatty acid signatures.

PREFACE

A lack of understanding of the lactation strategies adopted by New Zealand sea lions (NZSL) was the keystone that initiated the work presented in this thesis. *A priori* there were some questions that were proposed such as what's the gross chemical milk composition of the milk?; Does it vary in relation to environmental conditions?; What are the factors that are determining the milk quality produced by NZSLs?; Do these factors have detrimental effects on the quality of the milk of NZSLs and thus on their reproductive success as a measured by pup survival?

The interaction with the commercial squid fisheries is evident and thus the question that comes to mind is whether both fisheries and NZSLs are targeting the same food source, and if so would the competition adversely impact on the lactating NZSL? Would this interaction reduce the quality and quantity of milk produced by the NZSL?

I started this project with the idea of analysing the gross chemical composition of milk of NZSL and relating its composition to a number of maternal and offspring characteristics and temporal factors. Although there has been some work in this area but not necessarily on sea lions, I realize that there were many bias incorporated in these studies, for instance, methodologies were not standardized for the analysis of milk composition.

The first step in this project was to validate for the milk of NZSL an analytical method based on infra-red technology that is usually applied in the analysis of milk of dairy animals. Next it was evident that the factors scuh as maternal characteristics that influence the milk composition needed to be investigated and eventually the long and short term effects of diet. It soon became obvious that the complex mechanisms of milk fat synthesis/secretion related to the physiology of sea lions and the factors governing this mechanism would make it difficult to fully understand the relation between milk and diet and to draw objective conclusions. I found that there was little known about the mechanism of transfer of dietary lipids to milk lipids, in particular in pinnipeds.

Finally, my study focused on the milk composition and the factors that affect it because of its importance in the dynamics and recovery of the population of the species. Information on the quality of the milk can be used as an indirect index of the reproductive success and as a measure of the health of the population.

DEDICATION

"I dedicate this thesis to both my grandmother, Pochola (Jorgelina Bustamante de Riet), and my grandfather Hector (Hector Maria Sapriza) that passed away while doing this thesis, and of course to my parents. They more than anyone, have been the best support and encouragement I could have hoped for. Any accomplishment of mine is due in no small part to their support."

"Quiero dedicar esta tesis de doctorado a mi abuela Pochola (Jorgelina Bustamante de Riet) y a mi abuelo Héctor (Héctor Maria Sapriza), que fallecieron en el periodo de esta tesis, y por supuesto a mis padres. Ellos, más que nadie, han sido el mejor apoyo y ánimo que yo haya podido esperar. Cualquiera de mis logros, son en parte, debido a su apoyo."

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LIST OF ABBREVATIONS

- AMF — Anhydrous milk fat
- AOAC — Association of Official Analytical Chemists
- BCF — Bias Correction Factor
- BCI — Body condition index
- CART — Classification and regression tree
- CCC — Concordance correlation coefficient
- CDA — Canonical Discriminant analysis
- CLO — Cod liver oil
- CoNVO — Cocktail of Natural Vegetable Oils
- DFA — Discriminant Function Analysis
- ENSO — El Niño Southern Oscillation
- FA — Fatty acid
- FAME — Fatty acid methyl esters
- FASA — Fatty Acid Signature Analysis
- ICC — Intraclass correlation coefficient
- MUFA — Monounsaturated fatty acids
- NEFA — Non-esterified fatty acids
- NPN — Non-protein nitrogen
- NZSL — New Zealand sea lion
- PUFA — Polyunsaturated fatty acids
- QFASA — Quantitative Fatty Acid Signature Analysis
- r — Pearson correlation coefficient
- r^2 — Coefficient of determination
- RPE — Relative prediction error
- R-G — Roese Gottlieb method for fat determination
- SAFA — Saturated fatty acids
- SDA — Stepwise Discriminant analysis
- TAG — Triacylglycerol