

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**EVALUATION OF THE ANTI-NUTRITIVE AND
HYPOCHOLESTEROLEMIC EFFECTS OF A β -GLUCAN
PREPARATION EXTRACTED FROM NEW ZEALAND BARLEY**

A thesis presented in partial fulfilment of the requirements for the Degree
of Master of Science in Nutritional Science at Massey University

MARIA DE LOURDES MAQUEDA DE GUEVARA

1999

ABSTRACT

The present study evaluated the anti-nutritive as well as the hypocholesterolemic effects of barley β -glucan through the use of a commercially prepared barley β -glucan extract. Two experiments with broiler chickens were performed. In the first experiment (Chapter 3), a commercially prepared barley β -glucan extract was added (15 g/kg diet) to a synthetic diet based on cornstarch and casein. In addition, the diet was heated to evaluate the effects of heat treatment on the anti-nutritive effects of β -glucan. The diet was fed to twenty 15-day-old male broilers for 7 days. Diets containing β -glucan reduced ($P<0.01$) nitrogen (N) and carbon (C) digestibility, increased in the full weight of the whole gut ($P=0.08$) and the caeca ($P<0.05$), and increased ($P<0.01$) the insoluble solids volume (ISV) and water holding capacity (WHC) of digesta of birds. Heating the diets decreased ($P<0.01$) N digestibility and increased the ISV of the digesta.

In the second experiment (Chapter 4), different amounts of the β -glucan extract were added to a barley-based diet to provide three different levels of dietary β -glucan (low (19.8 g β -glucan /kg diet), medium (50.7 g β -glucan/kg diet), and high (68.3 g β -glucan/kg diet)). In addition, a β -glucanase was added to each of the diets to assess its influence on digestibility, performance, and digesta physico-chemical properties. The diets were fed to 36, 15-day-old male broilers for seven days. Results showed variable responses. The values obtained for gross energy (GE) digestibility, and the weights of the whole gut empty and the second part of the small intestine full and empty were similar between diets of low and medium β -glucan content, but lower ($P<0.05$) than the values obtained with diet high. In the case of WHC and ISV, the values obtained with diets medium and high were similar, but higher ($P<0.01$ for WHC; $P<0.05$ for ISV) than the values obtained with diet low. For viscosity, the higher ($P<0.01$) values were obtained with diet low, while the values obtained with diets medium and low were similar. The variable responses were attributed to the soft gelatinisation of β -glucan when dispersed in water, which could have encapsulated the native β -glucan from barley, hindering any anti-nutritive effect it could have. Another possibility suggested was in terms of the threshold level of β -glucan needed to elicit its anti-nutritive effects, which was not reached in some of the diets. The β -glucanase

inclusion improved N, C ($P<0.05$), and GE ($P<0.01$) digestibility, decreased the viscosity of the digesta ($P<0.01$) and the weights of the whole gut and the second part of the small intestine ($P<0.01$), and improved the weight gain and feed conversion ratio (FCR) of the birds ($P<0.05$). The experiment also suggested that viscosity is not the main mechanism needed for the anti-nutritive effects of β -glucan because although the viscosity of most of the diets was low, the β -glucan still elicited some anti-nutritive effects. It is possible that the gelling capacity of the β -glucan extract was one of the main factors influencing the anti-nutritive effects of the extract.

In addition to these two studies, an *in vitro* digestibility method simulating the chicken's gut was developed to evaluate the anti-nutritive effects of β -glucan on N and C digestibility as well as on the physico-chemical properties of the digesta (Chapter 5). The method showed acceptable accuracy ($r=0.93$, $P<0.01$) in the prediction of N digestibility *in vivo* in a wide range of diets. In the case of C digestibility, although a significant correlation ($r=-0.64$, $P<0.05$) between the *in vitro* and *in vivo* values was found, the *in vitro* digestion did not reflect what happened during the *in vivo* digestion. This was possibly due to the fact that the *in vitro* assay was not designed to analyse C digestibility, and the results obtained were in fact an artifact of the data set. The physico-chemical properties of the digesta were not predicted accurately by the *in vitro* method because conditions such as the churning effect of the intestine and the action of the microorganisms present in the intestine, which greatly affect digesta, could not be replicated in the method.

The hypocholesterolemic effects of the β -glucan extract were evaluated in growing male rats through the inclusion of β -glucan in synthetic diets based on cornstarch and casein (Chapter 6). In addition, the effects of coconut oil (rich in saturated fatty acids (SFA)), and flax oil (rich in polyunsaturated fatty acids (PUFA)) in blood lipids were also evaluated. The diets were fed to 36 28-day-old male rats for 28 days. The results of this experiment showed a reduction ($P=0.07$) in total cholesterol (TC) levels by the inclusion of β -glucan. The kind of oil used in the diets did not affect TC levels ($P>0.05$). Serum TG levels were decreased ($P<0.01$) by inclusion of β -glucan. When coconut oil was used, β -glucan inclusion decreased ($P<0.05$) serum TG levels. However, when flax oil was used, β -glucan inclusion did not have any effect on TG ($P>0.05$). In addition, it is suggested that the hypocholesterolemic effects of the β -

glucan extract used were due to a decrease in lipid absorption in the small intestine, which was caused by the β -glucan inducing a gel formation, which delayed nutrient absorption. This gel formation induced by the β -glucan extract is considered to be one of the main factors responsible for the hypocholesterolemic effects of the β -glucan extract.

It is concluded that the anti-nutritive and hypocholesterolemic effects of barley β -glucans were demonstrated through the use of a commercially prepared barley β -glucan extract, and that the gel formation induced by the β -glucan extract could be one of the main mechanisms responsible for the anti-nutritive and hypocholesterolemic effects of this NSP, which could be equally important to increases in viscosity induced by intact β -glucan.

ACKNOWLEDGEMENTS

First of all I would like to express my deepest gratitude to my supervisors Dr. Patrick C.H. Morel and Dr. John R. Pluske from the Monogastric Research Centre, Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand, for their enthusiastic, valuable, and friendly guidance, advice, support, encouragement, help, and patience throughout my research. Special thanks to Dr. Patrick Morel for his help and guidance regarding the statistical part of my thesis.

I am extremely grateful to Ms Heather McClean for the support she offered since I arrived to New Zealand, for helping me to get unconditional admission to Massey University and to get a NZODA: PGS Scholarship, and most of all for the warm friendship she and her family offered me and my husband.

I am also grateful to Dr. Kathy Kitson for the special help she gave me when I first came to New Zealand and for her help and encouragement at the end of my degree.

I want to thank the New Zealand Ministry of Foreign Affairs and Trade for awarding me a NZODA: PGS Scholarship to obtain the degree of Master of Science. Without their help it would have been very difficult for me to study in New Zealand. Also, many thanks to the Foundation for Research and Science Technology and to the Crop and Food Research Institute for providing research funding.

Special thanks to Dr. Graeme Coles from the Crop & Food Research Institute, Christchurch New Zealand, for providing the β -glucan extract and the barley used in the experiments, and for his valuable advice.

I want to thank very much Dr. John Monro from the Crop and Food Research Institute, Palmerston North, New Zealand for his valuable help and advice in dealing with the physico-chemical features of the β -glucan extract, as well as for the digesta analyses he made and for developing the *in vitro* digestibility method, and allowing me to work with him and share his knowledge and experience.

My special thanks to Mr. Don Thomas for his enthusiastic help, advice, and support regarding the chickens' experiments and the preparation of the diets for all my experiments, and for his warm friendship and encouragement throughout my research.

Many thanks to Mr. Blake Camden for his enthusiastic help with the animals and diets throughout all my experiments.

To all the people that helped me with the rats' experiment in one or another way, specially Ms. Debbie Chesterfield, Ms. Margaret Scott, Ms. Anne Broomfield, Ms. Yvette Cottam, Dr. Isam Kadim, Mr. Graham Pearson, Dr. Peter Kunz, and Mr. Mike King, thank you very, very much. Also, special thanks to Mr. Shane Rutherford, Ms. Suzanne Hodgkinson, and Dr. Dean Revell for all their advise.

I am extremely grateful to the team of the Nutrition Laboratory, Institute of Food, Nutrition and Human Health, Massey University, especially to Ms. Fliss Jackson, Ms. Maggie Zou, Ms. Wibha Desai, Ms. Marie Russell, Ms. Florence Chung, Mr. Hian Voon, Ms. Zirsha Sadler and Dr. John McIntosh for their expert technical laboratory assistance, their friendly help, and for allowing me to work with them and share their knowledge and experience.

I am very grateful to Dr. Phil Pearce and Ms. Yvette Cottam from the Physiology Laboratory, Institute of Food, Nutrition and Human Health, Massey University, for their expert technical assistance.

Also, many thanks to Ms. Rebecca Abbot from the Crop and Food Research Institute for helping me to get paramount information regarding the β -glucan extract.

I would like to thank in a special way all the animals that had to be sacrificed in order for me to complete this work, and for the sake of science.

My deepest gratitude goes to my husband Aurelio for his love, support, and encouragement throughout the time we have been in New Zealand, as well as for his invaluable help and advice during my master degree.

Special thanks to Ms Ruth Hodgson, Mr. Arthur and Ms. Joyce Worboys, and to Ms. Jo Donovan for all their love and support throughout the years my husband and I have been in New Zealand, for making us feel part of their families, and for helping me improve my English skills.

I wish to thank all the Latin American and International friends I have had in New Zealand for their friendship and for sharing their cultures with me, as well as for teaching me another way to see the world.

Last but not least, special thanks to my families (mine and my husband's) back in Mexico for their love, support, encouragement, and letters, and for waiting patiently back home for us to return.

TABLE OF CONTENTS

		Page
ABSTRACT		ii
ACKNOWLEDGEMENTS		v
TABLE OF CONTENTS		viii
LIST OF TABLES		x
LIST OF FIGURES		xiv
LIST OF ABBREVIATIONS		xv
CHAPTER ONE	General Introduction	1
CHAPTER TWO	Literature Review	6
CHAPTER THREE	β -glucan and heat treatment decrease nutrient digestibility in young broilers	50
CHAPTER FOUR	The use of β -glucanase in barley-based diets containing three different levels of β -glucan and fed to broiler chickens	70
CHAPTER FIVE	Development of an <i>in vitro</i> digestibility assay to simulate <i>in vivo</i> digesta properties and nitrogen and carbon digestibility in chickens	90

CHAPTER SIX	Effects of a β -glucan extract in combination with flax or coconut oil on serum cholesterol and triglyceride levels in rats	102
CHAPTER SEVEN	General discussion and conclusions	122
APPENDICES		129

LIST OF TABLES

	Page
Table 2.1. Factors affecting intestinal viscosity	18
Table 2.2. Risk factors, which will augment the negative effects of high viscosity	19
Table 2.3 Primary enzyme activity required for hydrolysis of cereal grain cell wall β -glucan	24
Table 2.4 Possible mechanisms of dietary fibre influences on lipid absorption	32
Table 2.5. Major dietary FA	35
Table 3.1 Composition of experimental diets (as is basis)	54
Table 3.2. Height (cm) of known volumes added to tubes	56
Table 3.3 Interaction least-square mean values for N, C, and GE digestibility (%) of the experimental diets	58
Table 3.4 Level of significance of β -glucan, heat, and their interaction on N, C, and GE digestibility	58

Table 3.5. Interaction least-square mean weight (g) of digestive organs of birds fed the experimental diets	59
Table 3.6. Level of significance of β -glucan, heat, and their interaction on the weights of digestive organs	60
Table 3.7. Interaction least-square mean WHC and ISV values of digesta of birds fed the experimental diets	60
Table 3.8. Level of significance of β -glucan, heat, and their interaction on the physical characteristics of the digesta	60
Table 4.1 Composition of experimental diets (g/kg air-dry basis)	74
Table 4.2 Levels of significance of β -glucan, enzyme, and their interaction on N, C, and GE digestibility	77
Table 4.3 Interaction least-square means for N, C, and GE digestibility (%).	77
Table 4.4 Levels of significance of β -glucan, enzyme, and their interaction, on weights of digestive organs (using bird weight as a covariate)	79
Table 4.5 Interaction least-square mean weights of digestive organs (g)	79
Table 4.6 Level of significance of β -glucan, enzyme, and their interaction on the digesta physico-chemical properties	80

Table 4.7 Interaction least-square mean values for digesta physico-chemical properties	80
Table 4.8 Interaction least-square mean values for total gain (TG) and feed conversion ratio (FCR)	81
Table 4.9 Level of significance of β -glucan, enzyme, and their interaction on total gain (TG) and feed conversion ratio (FCR)	81
Table 5.1 <i>In vitro</i> simulation of chicken digestion process for testing the effects of β -glucan on digesta properties and nutrient digestibility	92
Table 5.2 Mean values for <i>in vitro</i> and <i>in vivo</i> N and C digestibility, ISV, WHC and Visc of the digesta	94
Table 5.3 Correlation (r) values coefficient of determination (R^2) values, the standard error of the estimate (SEE), and their significance (P-value) for <i>in vitro</i> and <i>in vivo</i> N and C digestibility, ISV, WHC, and Visc.	95
Table 5.4 Correlation (r) and coefficient of determination (R^2) values, the standard error of the estimate (SEE), and their significance (P-value) for <i>in vitro</i> and <i>in vivo</i> N and C digestibility, ISV, and WHC, of pooled data	95

Table 6.1 Ingredient composition of experimental diets (g/kg as-is basis)	106
Table 6.2 Nutrient composition of experimental diets	106
Table 6.3 Interaction least-square means of TC, HDL (observed and calculated) and LDL-cholesterol, and TG (mmol/l) of rats fed the experimental diets	111
Table 6.4 Level of significance of β -glucan, oil, and their interaction on TC, HDL and LDL-cholesterol, and TG	111
Table 6.5 Interaction least-square mean digestibility for Nitrogen (%).	112
Table 6.6 Level of significance of β -glucan, oil, and their interaction on N digestibility	112
Table 6.7 Interaction least-square mean daily feed intake (DFI), average daily gain (ADG), and feed conversion ratio (FCR)	113
Table 6.8 Level of significance of β -glucan, oils and their interaction on DFI, ADG, and FCR	113

LIST OF FIGURES

	Page
Figure 2.1. NSP Classification	10
Figure 2.2. β -glucan structure	12
Fig 5.1 Comparison of <i>in vitro</i> and <i>in vivo</i> N digestibility (%)	96
Fig 5.2 Comparison of <i>in vitro</i> and <i>in vivo</i> C digestibility (%)	97

LIST OF ABBREVIATIONS

ADG	AVERAGE DAILY GAIN
C	CARBON
DFI	DAILY FEED INTAKE
DHA	DECOSAHEXAENOIC
EMP	EMPTY
EPA	EICOSAPENTAENOIC
FA(S)	FATTY ACID(S)
FCR	FEED CONVERSION RATIO
GE	GROSS ENERGY
HDL	HIGH DENSITY LIPOPROTEIN
HMG CoA	β -HYDROXY- β -METHYLGLUTARYL COENZYME A
INT	INTESTINE
ISV	INSOLUBLE SOLIDS VOLUME
LDL	LOW DENSITY LIPOPROTEIN
Lp (a)	LIPOPROTEIN (a)
MUFA(S)	MONOUNSATURATED FATTY ACID(S)
N	NITROGEN
NSP	NON-STARCH POLYSACCHARIDES
PUFA(S)	POLYUNSATURATED FATTY ACID(S)

RO	REVERSE OSMOSIS
SFA(S)	SATURATED FATTY ACID(S)
TC	TOTAL CHOLESTEROL
TG	TRIGLYCERIDES
UFA(S)	UNSATURATED FATTY ACID(S)
UWL	UNSTIRRED WATER LAYER
VISC	VISCOSITY
VLDL	VERY LOW DENSITY LIPOPROTEIN
WHC	WATER HOLDING CAPACITY
β-GLUC	β-GLUCAN