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.
Effect of Goat milk on bone mass, morphology and biomechanics

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy
in
Anatomy and Physiology

at Massey University, Palmerston North
New Zealand.

Hilary McKinnon

2012
Abstract

Milk is a major source of dietary calcium which is essential for bone growth and maintenance, and is seen as a beneficial resource in the prevention and alleviation of osteoporotic bone loss. The objectives of this thesis were to investigate the effects of a bioactive component of goat milk, Casein phosphopeptide (CPP), and its ability to increase calcium solubility for improved calcium absorption and retention. To investigate the effect of a formulated goat milk diet as a nutritional supplement on bone growth and mineral accretion; and to investigate the effect of the long term consumption of goat milk as a nutritional supplement with or without a drug therapy (Sodium Alendronate) to determine any complementary effects on ovariectomy induced osteoporosis in the female rat. The effect of CPP on calcium bioavailability was investigated in growing rats during a period of rapid bone growth. The diets that contained 80% and 57% of goat milk protein as casein delivered increased calcium absorption compared to the diet containing 17% casein, suggesting a minimum level of casein is needed to optimise calcium absorption from goat milk. However, increased calcium absorption did not result in increased mineral retention in the femur or lumbar spine.

The next trial had two animal experiments with a total of 200 rats involved (Chapter 4 and 5); in the first experiment all 200 rats were fed either a non-milk diet, a formulated cow’s milk diet, or a formulated goat milk diet from 3 weeks of age until 5 months of age. At its conclusion 60 rats were euthanized and ex vivo samples taken for analysis. The second experiment saw the remaining mature rats either ovariectomized or sham operated then grown until 10 months. The consumption of the goat milk diet increased mineral accretion during the phase of rapid bone growth beyond ‘Peak bone mass’ at approximately 12 weeks of age until maturity at 5 months of age. Mineral retention in the femoral shaft showed that the rats fed the goats milk diet had significantly greater quantities of mineral (p<0.001) compared to the not-milk group. Investigation of the marrow cavity showed that bone formation at the two cross sections examined at the femoral mid-shaft were more significant for the rats fed the goat milk diet compared to the rats fed the non-milk diet (p<0.034 and p<0.007) respectively. Ovariectomy surgery at 5½ months caused osteoporotic like conditions in bone to develop resulting in the rapid loss of bone mass in the ovariectomized rats. This saw both periosteal and
endosteal expansion resulting in larger overall marrow cavities (p<0.0001) in the femoral shaft and larger overall cross sectional area (p<0.002). Ovariectomy was also found to have an uneven effect on bone loss within the femoral shaft of ovariectomized rats (OVX), where bone at the endosteal surface had a tendency to be lost at a greater rate than the distal region compared to sham operated rats (SHAM) (p<0.061). This regional change showed that the SHAM rats had relatively larger bone areas in the proximal region, whereas, OVX rats had relatively larger bone areas in the distal region (p<0.0005).

Dual energy x-ray absorptiometry (DEXA) measurements of the lumbar spine and femur did not show any significant differences between OVX and ovariectomized alendronate groups (OVX ALD) fed either of the milk diets (Chapter 5). However, there was a potentially differing, almost opposite effect within each of the two milk diets in the bone area of the femoral shaft. The GOAT OVX rats showed a trend for larger overall mean bone areas than the GOAT OVX ALD rats (p<0.063), yet in contrast to this the COW OVX rats showed a trend for smaller overall mean bone areas than the COW OVX ALD rats in the femoral shaft although not significant.

The rats fed a long term diet of formulated goat milk and dosed with alendronate had a tendency to have tougher bone material per unit of bone (J/mm²) than rats fed cow’s milk and dosed with alendronate (p<0.073) in the femoral mid-shaft. Whereas, in the proximal femoral shaft the rats fed either of the milk diets and dosed with alendronate had tougher bone material per unit of bone (J/mm²) than the rats fed either of the milk diets and dosed with the placebo (p<0.05).

Analysis of the trabecular structure of the proximal tibia showed that the rats fed goats milk and dosed with alendronate increased the prevalence of rod shaped trabeculae (p<0.048), increased surface volume to bone ratio (p<0.001), reduced the connectivity between trabeculae struts within the structure (p<0.004), decreased the fractal dimensions of the trabecular structure (p<0.018), and had thinner trabeculae (p<0.006) compared to the rats fed the Goat milk diet and dosed with the placebo.
In conclusion, this thesis has found that the long-term consumption of goat milk may provide some protection against ovariectomy bone loss in rats. This may be in part due to increased mineral accretion during the phase of rapid bone growth. The co-administration of goat milk and alendronate had a significant effect on the toughness of the bone material per unit area of bone in the proximal and mid-shaft of the femur, however, potentially weakened the trabecular structure of the proximal tibia.
Acknowledgements

I would like to thank my supervisors for their time and effort in getting me through my PhD. To Professor Marlena Kruger I sincerely thank her for her help and support throughout my PhD. Her guidance and experience got me through some rough times and she often gave me the strength to carry on. I wish to offer special thanks to Dr Patrick Janssen for his guidance and advice on the biomechanics and help developing the toughness method in Chapter 7.

My sincere thanks to the Dairy Goat Co-operative who provided funding for the work conducted in Chapter 2 and Chapter 4. I would also like to thank them for contributing to my scholarship in partnership with the Tertiary Education Commission New Zealand in the form of an Enterprise Scholarship.

To the team at IFNHH I owe a debt of gratitude for their support and long hours of hard work that they gave without hesitation. Especially to Mrs Anne Broomfield and Mrs Kim Wylie for the seemly endless days of helping feed rats and clean cages, ovariectomy surgeries and DEXA scans. To Chris Booth for keeping me sane and being there to help out when the chips were down. For Mr Ben Schon and Mrs Gabrielle Plimmer for assisting with DEXA scans and blood work. To Wei Hung Chua for his guidance with stats and helping out with lab work. To Dr Fran Wolber I would like to offer special thanks for her support in helping me track down the course of the diet deficiency and providing unwavering support through tough times.

I would like to thank Ms Felicity Jackson and the rest of the team from the Nutrition Lab, IFNHH, Massey University for allowing me into their lab, offering me support and guidance with equipment. I would also like to thank them for analysing my samples for my calcium balance work in Chapter 2.

Particularly I would like to thank Mr Alasdair Noble, Mr A. Johnathan R. Godfrey and Ms Penelope Bilton from the Massey Post Graduate Stats clinic for coming to my rescue and assisting me with understanding my statistical analysis.
I would like to offer my deepest thanks to the Massey Post mortem room staff, the IFNHH Nutrition lab staff, Dr Kathleen Parton, Dr Juliet Cayzer for their support during the very difficult months while my rats suffered from the Vitamin A deficiency.

I would like to thank Dr Jillian Cornish of Auckland University for making their μCT scanner available and especially Ms Maureen Watson for running the scans and preparing the data investigated in Chapter 7.

I would like to thank Associate Prof Roger Lentle for his contributions to this thesis.
# Table of Contents

Abstract .............................................................................................................................. iii
Acknowledgements ........................................................................................................... vi
Table of Contents ............................................................................................................. viii
List of Tables .................................................................................................................. xiii
List of Figures ................................................................................................................ xv
Abbreviations .................................................................................................................. xvii
Introduction .................................................................................................................... 1-1

## 1. LITERATURE REVIEW .............................................................. 1-3

### 1.1. Biomechanical Definitions ..................................................... 1-4
  1.1.1. Stress and Strain ................................................................. 1-4
  1.1.2. Extrinsic and Intrinsic properties .................................... 1-5
  1.1.3. Strength and toughness .................................................. 1-6

### 1.2. Overview of Bone ................................................................. 1-9

### 1.3. Bone composite ................................................................. 1-11
  1.3.1. Mineral ........................................................................... 1-11
  1.3.2. Collagen .......................................................................... 1-11
  1.3.3. Mineralised Collagen Fibrils ........................................... 1-13
  1.3.4. Collagen Fibril Array ..................................................... 1-14
  1.3.5. Non-collagenous proteins .............................................. 1-15
  1.3.6. Water ............................................................................ 1-16
  1.3.7. Haversian Bone (secondary Osteons) ......................... 1-16

### 1.4. Bone cells ............................................................................ 1-17
  1.4.1. Osteoblasts .................................................................. 1-17
  1.4.2. Osteocytes .................................................................... 1-18
  1.4.3. Osteoclasts .................................................................. 1-18
  1.4.4. Immune cells ................................................................ 1-19

### 1.5. Remodelling ................................................................. 1-19
  1.5.1. Initiation of bone remodelling ...................................... 1-20
  1.5.2. Regulation of bone remodelling ................................. 1-22

### 1.6. Structural design of bone .................................................. 1-23

### 1.7. Osteoporosis ................................................................. 1-26
  1.7.1. Misregulation of remodelling ...................................... 1-28
  1.7.2. Effects of Bisphosphonates on Osteoporotic bone .... 1-29

### 1.8. Goat milk and bone health .................................................. 1-32
  1.8.1. Mechanisms affecting calcium absorption ................ 1-33
      Casein phosphopeptides .................................................... 1-33
      Goat milk fat ................................................................. 1-34
  1.8.2. Human studies .............................................................. 1-35
  1.8.3. Animal studies .............................................................. 1-35

### 1.9. Motivation and objectives of the thesis ............................. 1-36

### 1.10. References ...................................................................... 1-38
2. THE EFFECTS OF FORMULATED GOATS MILK ON CALCIUM BIOAVAILABILITY IN MALE GROWING RATS ........................................ 2-1

2.1. Introduction .................................................................................................................. 2-2

2.2. Methods & materials .................................................................................................... 2-2
   2.2.1. Animals .................................................................................................................. 2-2
   2.2.2. Diets ...................................................................................................................... 2-2
   2.2.3. Metabolism experiment ........................................................................................ 2-3
   2.2.4. Duel energy X-ray Absorptiometry (DEXA) scans .............................................. 2-3
   2.2.5. Euthanasia and tissue collection ............................................................................ 2-4
   2.2.6. Bone marker ......................................................................................................... 2-4
   2.2.7. Ashing and calcium content of femur ................................................................. 2-4
   2.2.8. Biomechanical properties of the femur ............................................................... 2-4
   2.2.9. Statistical analysis ............................................................................................... 2-5

2.3. Results .......................................................................................................................... 2-6
   2.3.1. Mineral balance .................................................................................................... 2-6
   2.3.2. DEXA .................................................................................................................... 2-7
   2.3.3. Ex vivo femur ....................................................................................................... 2-8
   2.3.4. CTx ...................................................................................................................... 2-8

2.4. Discussion .................................................................................................................... 2-10
   2.4.1. Conclusion ........................................................................................................... 2-11

2.5. References ................................................................................................................... 2-13

3. THE EFFECTS OF OVARIECTOMY ON THE ARCHITECTURE OF DIFFERENT REGIONS WITHIN THE FEMORAL SHAFT OF FEMALE RATS. 3-1

3.1. Introduction .................................................................................................................. 3-2

3.2. Methods & materials .................................................................................................... 3-3
   3.2.1. Animals .................................................................................................................. 3-3
   3.2.2. Diets ...................................................................................................................... 3-3
   3.2.3. Bone densitometry by Duel energy X-ray Absorptiometry (DEXA) ..................... 3-4
   3.2.4. Euthanasia and tissue collection ............................................................................ 3-4
   3.2.5. Right femurs ......................................................................................................... 3-4
   3.2.6. Ash content of femoral slices .............................................................................. 3-4
   3.2.7. Left Femurs .......................................................................................................... 3-6
   3.2.8. Biomechanical properties of the Left femoral segments ..................................... 3-7
   3.2.9. Statistical Analysis ............................................................................................... 3-8
      Principal component analysis ...................................................................................... 3-8

3.3. Results .......................................................................................................................... 3-11
   3.3.1. Morphology of the femoral shaft ....................................................................... 3-11
      Bone area (mm²) ........................................................................................................ 3-11
      Marrow cavity area (mm²) ....................................................................................... 3-13
      Total cross sectional area (mm²) ............................................................................ 3-14
   3.3.2. Mineral content of femoral shaft ....................................................................... 3-16
      Duel energy X-ray Absorptiometry (DEXA) .......................................................... 3-16
      Mineral composition ............................................................................................... 3-16
   3.3.3. Biomechanical strength of the femoral shaft ..................................................... 3-17
      Cross sectional moment of inertia (mm²) (CSMI) .................................................. 3-17
      Compression strength ............................................................................................ 3-18

3.4. Discussion .................................................................................................................... 3-20
5.2. Morphological changes in the femoral shaft following ovariectomy or sham ovariectomy..3-20
3.4.2. Changes in mineralization of the femoral shaft following ovariectomy ........................................3-21
3.4.3. Changes in bone strength following ovariectomy ......................................................................3-22
3.4.4. Conclusion .................................................................................................................................3-23

3.5. References .....................................................................................................................................3-24

4. THE EFFECT OF FORMULATED GOAT MILK ON BONE GROWTH AND MINERAL ACCRETION AND MORPHOLOGY IN GROWING FEMALE RATS. 4-1

4.1. Introduction ......................................................................................................................................4-5

4.2. Methods and materials ........................................................................................................................4-6
  4.2.1. Animals .......................................................................................................................................4-6
  4.2.2. Diets .........................................................................................................................................4-6
  4.2.3. Bone densitometry by Duel energy X-ray Absorptiometry (DEXA) .........................................4-7
  4.2.4. Euthanasia and sample collections ............................................................................................4-8
  4.2.5. Femurs ......................................................................................................................................4-8
  4.2.6. Imaging .....................................................................................................................................4-9
  4.2.7. Ash content ...............................................................................................................................4-9
  4.2.8. Statistical analysis .....................................................................................................................4-9

4.3. Results ..............................................................................................................................................4-10
  4.3.1. Duel energy X-ray Absorptiometry (DEXA) .............................................................................4-10
    Bodyweight and Whole body composition ......................................................................................4-10
    Lumbar spine .................................................................................................................................4-12
    Femur .............................................................................................................................................4-13
  4.3.2. Mineral composition ....................................................................................................................4-13
  4.3.3. Morphology of the femoral shaft ..............................................................................................4-16
    Bone area (mm²) ..............................................................................................................................4-16
    Total cross sectional area (mm²) ......................................................................................................4-17
    Marrow cavity area (mm²) ................................................................................................................4-18

4.4. Discussion .........................................................................................................................................4-20
  4.4.1. Effect of diet deficiency .............................................................................................................4-20
  4.4.2. Bodyweight and Whole body composition ..............................................................................4-20
  4.4.3. Morphological changes ............................................................................................................4-21
  4.4.4. Conclusion ...............................................................................................................................4-23

4.5. References .........................................................................................................................................4-24

5. EFFECT OF FORMULATED GOAT MILK AND COW’S MILK DIETS WITH AND WITHOUT SODIUM ALENDRONATE ON BONE MASS AND REGIONAL BONE MORPHOLOGICAL CHANGES IN THE Ovariectomized RAT. ...........................................................................5-1

5.1. Introduction .......................................................................................................................................5-2

5.2. Methods & materials ..............................................................................................................................5-3
  5.2.1. Animals ..................................................................................................................................5-3
  5.2.2. Diets .......................................................................................................................................5-4
  5.2.3. Drug treatment ..........................................................................................................................5-4
  5.2.4. Bone densitometry by Duel energy X-ray Absorptiometry (DEXA) ....................................5-5
  5.2.5. Euthanasia and sample collections .........................................................................................5-5
  5.2.6. Femurs .....................................................................................................................................5-6
  5.2.7. Statistical analysis .....................................................................................................................5-6

X
5.3. **Results** ................................................................................................................. 5-7

5.3.1. **Duel energy X-ray Absorptiometry (DEXA)** ....................................................... 5-7
   Bodyweight and whole body composition ................................................................. 5-7
   Lumbar spine ............................................................................................................. 5-11
   Femur ......................................................................................................................... 5-13

5.3.2. **Morphology of the femoral shaft** ........................................................................ 5-15
   Bone area (mm$^2$) ..................................................................................................... 5-15
   Marrow cavity area (mm$^2$) ..................................................................................... 5-18
   Total cross sectional area (mm$^2$) .......................................................................... 5-20

5.4. **Discussion** .......................................................................................................... 5-22

5.4.1. **Diet deficiency impact on the femur** ................................................................. 5-22
5.4.2. **Diet and Alendronate effect on the ovariectomized rat** .................................... 5-23
5.4.3. **Conclusion** ...................................................................................................... 5-25

5.5. **References** ....................................................................................................... 5-27

6. **THE EFFECTS OF FORMULATED GOAT MILK AND COW’S MILK DIETS WITH AND WITHOUT SODIUM ALENDRONATE ON TOUGHNESS OF DIFFERENT REGIONS OF THE FEMORAL SHAFT OF Ovariectomized Rats** ........................................................................................................... 6-1

6.1. **Introduction** ....................................................................................................... 6-2

6.2. **Method & materials** .......................................................................................... 6-3

6.2.1. **Summary of groups** ......................................................................................... 6-3
6.2.2. **Femur sections** ............................................................................................... 6-4
6.2.3. **Izod test** .......................................................................................................... 6-4
6.2.4. **Statistical analysis** .......................................................................................... 6-7
   Groups fed the Goat or Cow’s milk diets ................................................................. 6-8
   Groups fed the Non-milk diet .................................................................................. 6-8

6.3. **Results** ................................................................................................................. 6-9

6.3.1. **Milk diets – Segment A** .................................................................................. 6-9
   Specific energy .......................................................................................................... 6-9
   Morphology – Bone area and Total cross sectional area .......................................... 6-10
   Relationship between impact energy and bone area, and total cross sectional area .... 6-11

6.3.2. **Milk diets – Segment B** .................................................................................. 6-11
   Specific energy .......................................................................................................... 6-11
   Morphology – Bone area and Total cross sectional area .......................................... 6-12
   Relationship between fracture energy and bone area, and total cross sectional area .... 6-13

6.3.3. **Non-milk diet – Segment A** ........................................................................... 6-14
   Morphology and treatment – Bone area and Total cross sectional area .................... 6-15
   Relationship between fracture energy and bone area, and total cross sectional area .... 6-15

6.3.4. **Non-milk diet – Segment B** ........................................................................... 6-17
   Morphology and treatment - Bone area and Total cross sectional area ..................... 6-17
   Relationship between fracture energy, total cross sectional area and bone area ......... 6-18

6.4. **Discussion** ....................................................................................................... 6-19

6.4.1. **Testing method** ............................................................................................... 6-19
6.4.2. **The effect of diet and drug on impact energy** .................................................... 6-20
6.4.3. **Regional variation of impact energy** ................................................................. 6-21
6.4.4. **Effect of morphology on the energy needed to break segments** ....................... 6-21
6.4.5. **Conclusion** ..................................................................................................... 6-22

6.5. **References** ....................................................................................................... 6-23
7. EFFECT OF A FORMULATED GOAT MILK DIET ON TRABECULAR BONE IN THE TIBIA USING MICRO-CT IN OVARIECTOMIZED RATS...... 7-1

7.1. Introduction .................................................................................................................. 7-2

7.2. Methods & materials ........................................................................................................ 7-3
   7.2.1. Bone samples ........................................................................................................... 7-3
   7.2.2. Treatment groups ................................................................................................... 7-3
   7.2.3. Micro-computed tomography .................................................................................. 7-3
   7.2.4. Biomechanical testing ......................................................................................... 7-5
   7.2.5. Statistical analysis .................................................................................................. 7-5

7.3. Results ....................................................................................................................... 7-5

7.4. Discussion .................................................................................................................... 7-8

7.5. Conclusion .................................................................................................................. 7-11

7.6. References .................................................................................................................. 7-12

8. DISCUSSION AND CONCLUSION................................................................. 8-1

8.1. Conclusion .................................................................................................................. 8-6

8.2. Recommendations for Future Research ................................................................. 8-6

8.3. References .................................................................................................................. 8-8
List of Tables

Chapter 1
Table 1-1. Table of stress and strain definitions. ........................................................... 1-4

Chapter 2
Table 2-1. Analysis of diet composition of the six formulated goat milk diets. ...........2-6
Table 2-2. Size measurements, dry weight, ash content, calcium content and biomechanical testing for femurs, as well as concentration levels (ng/mL) of serum C-terminal cross-linking telopeptide of type I collagen (CTx), for the six diets. ..2-9

Chapter 3
Table 3-1. Means, SD’s and repeated measures ANOVAs of overall bone area (mm$^2$), marrow cavity area (mm$^2$), and total cross sectional area (mm$^2$) of slices of the femoral shaft from ovariectomized and sham operated rats. 3-12
Table 3-2. Means and SD’s of the bone areas of consecutive slices from the femoral shafts of ovariectomized and sham operated rats and results of principal component analysis. 3-13
Table 3-3. Means and SD’s of the area of the marrow cavities of consecutive slices from the femoral shafts of ovariectomized and sham operated rats and results of principal component analysis. 3-14
Table 3-4. Means and SD’s of the total cross sectional areas of consecutive slices from the femoral shafts of ovariectomized and sham operated rats and results of principal component analysis. 3-15
Table 3-5. 2-way ANOVA, means, SD’s of the mineral composition (relative to the amount of organic and inorganic material: g/g air dry bone mass) of the three segments of the femoral shaft from ovariectomized and sham operated rats. ....3-17
Table 3-6. Means and SD’s of the total cross sectional areas of consecutive slices from the femoral shafts of ovariectomized and sham operated rats and results of principal component analysis. 3-18
Table 3-7. 2-way ANOVA, means and SD’s of the extrinsic stiffness and maximum load of the three segments of the femoral shaft from ovariectomized and sham operated rats. .............................................................. 3-19

Chapter 4
Table 4-1. Diet composition (%) for non-milk and experimental diet formulations. ...4-7
Table 4-2. Repeated measures ANOVAs of Whole body area (cm$^2$), bone mineral content (BMC)(g), and bone mineral density (BMD) (g/cm$^2$), fat (g), lean (g), bone mass (g) and percentage fat (pfat). .............................................................. 4-11
Table 4-3. Repeated measures ANOVAs of Lumbar spine bone area (cm$^2$), bone mineral content (BMC) (g), and bone mineral density (BMD) (g/cm$^2$). ........4-12
Table 4-4. Repeated measures ANOVAs of Femur bone area (cm$^2$), bone mineral content (BMC)(g), and bone mineral density (BMD) (g/cm$^2$) ..................4-13
Table 4-5. Means, SDs, and repeated measures ANOVAs of overall bone area (mm$^2$), marrow cavity area (mm$^2$), total cross sectional area (mm$^2$), and mineral composition (g/g per dry weight bone mass) of segments of the femoral shaft from the three diets groups. .............................................................. 4-15
Table 4-6. Table of mineral composition (g/g per dry weight bone mass).................4-16
Table 4-7. Table of for bone area (mm$^2$) .................................................................. 4-17
Table 4-8. Table of for Total cross sectional area (mm$^2$) ...........................................4-18
Chapter 5
Table 5-1. Seven animal groups in the trial ................................................................. 5-3
Table 5-2. Diet powder composition (%) for non-milk and experimental diet formulations. .............................................................................................................. 5-4
Table 5-3. Repeated measures ANOVAs of Whole body area (cm²), bone mineral content (BMC)(g), and bone mineral density (BMD) (g/cm²), fat (g), lean (g), bone mass (g) and percentage fat (pfat). ........................................................................ 5-9
a) The three rat groups fed the non-milk diet (SHAM, OVX and OVX ALD) .... 5-9
b) The four rat groups fed the milk diets (GOAT OVX, GOAT OVX ALD, COW OVX, COW OVX ALD)...................................................................................... 5-10
Table 5-4. Repeated measures ANOVAs of Lumbar spine bone area (cm²), bone mineral content (BMC) (g), and bone mineral density (BMD) (g/cm²). ............. 5-11
a) The three rat groups fed the non-milk diet (SHAM, OVX and OVX ALD). ...... 5-11
b) The four rat groups fed the milk diets (GOAT OVX, GOAT OVX ALD, COW OVX, COW OVX ALD)...................................................................................... 5-12
Table 5-5. Repeated measures ANOVAs of Femur bone area (cm²), bone mineral content (BMC)(g), and bone mineral density (BMD) (g/cm²). ............................ 5-13
a) The three rat groups fed the non-milk diet (SHAM, OVX and OVX ALD). ...... 5-13
b) The four rat groups fed the milk diets (GOAT OVX, GOAT OVX ALD, COW OVX, COW OVX ALD)...................................................................................... 5-14
Table 5-6. Repeated measures ANOVAs of overall bone area (mm²), marrow cavity area (mm²), and total cross sectional area (mm²) of slices of the femoral shaft from the seven groups .................................................................................................. 5-15
Table 5-7. Table of bone area (mm²) and component loadings from a principal component analysis of the 12 slices of the femoral shaft........................................ 5-16
Table 5-8. Table of for marrow cavity area (mm²) and component loadings from a principal component analysis of the 12 slices of the femoral shaft. .................... 5-18
Table 5-9. Table of for total cross sectional area (mm²) and component loadings from a principal component analysis of the 12 slices of the femoral shaft. .......... 5-20
Table 5-10. Means and Standard deviations (SD’s) for the Bone areas of the 16 slices of the femoral shaft. ...................................................................................... 5-32
Table 5-11. Means and Standard deviations (SD’s) for the Marrow cavity areas of the 16 slices of the femoral shaft. ................................................................. 5-33
Table 5-12. Means and Standard deviations (SD’s) for the Cross sectional areas of the 16 slices of the femoral shaft. ................................................................. 5-34

Chapter 6
Table 6-1. The table shows the seven treatment groups in the mature OVX rat trial... 6-4

Chapter 7
Table 7-1. μCT operation settings ............................................................................... 7-4
Table 7-2. Means, SD’s and significant differences for μCT measurements for the cortical bone of the tibia. ................................................................. 7-6
Table 7-3. Means, SD’s and significant differences for μCT measurements for the trabecular bone of the tibia ........................................................................................... 7-7
Table 7-4. Means, SD’s and significant differences for biomechanical testing extrinsic measurements of the proximal tibia. ................................................................. 7-8
List of Figures

Chapter 1
Figure 1-1. Stress-strain curve divided into elastic strain region and plastic strain region.................................................................1-5
Figure 1-2. Notched milled sample under tension, as the strain increases a new crack forms as the site of the pre-existing crack ..................................................1-8
Figure 1-3. Diagram of the longitudinal cross section of the human femur, showing the epiphyseal and diaphyseal regions .................................................................1-10
Figure 1-4. Diagram showing arrangement of the topocollagen molecule and the formation of cross linked collagen fibrils .................................................................1-13
Figure 1-5. Bone mass lifecycle for Women, highlighting ‘Peak bone mass’ and the decline of bone with aging .................................................................1-27
Figure 1-6. Structure of Bisphosphonate, alendronate and the chemical name (alendronate, sodium salt) .................................................................1-30

Chapter 2
Figure 2-1. Fractional calcium absorption (%) and SD from the metabolism trial at seven weeks of age, for the six diets, (n = 12 for each diet) ........................................2-7
Figure 2-2. Means and SD for the change in Lumbar spine BMC between the two time points (8 and 12 weeks of age) for the six diets (n = 12 for each diet) ........2-8

Chapter 3
Figure 3-1. Picture describing the cross sectional profile of the femur slice ..........3-6
Figure 3-2. Diagram of rat femur showing the segments A, B and C in relation to slice numbers ...........................................................................................................3-7
Figure 3-3. Scree plot of the cumulative eigenvalue sum versus the number of principal components .................................................................................................3-10
Figure 3-4. Mean mineral composition ± standard deviation (relative to the amount of organic and inorganic material: g/g air dry bone mass) of the two treatments sham-operated rats (SHAM) and ovariectomized rats (OVX), for the three segments of the femoral shaft .................................................................3-16

Chapter 4
Figure 4-1. Timeline of diet changes in an attempt to correct suspected diet deficiencies ..................................................................................................................4-3
Figure 4-2. Picture describing the five sections cut from the entire femur, including the three segments that make up the femoral shaft used in this study. They are identified as Segment A (proximal shaft), Segment B (mid-shaft), and Segment C (distal shaft) ...........................................................................................................4-8
Figure 4-3. Picture describing the transverse cross sectional profile of the femur slice ..................................................................................................................4-9
Figure 4-4. Graphs showing means and SDs for rat bodyweights, and the seven DEXA whole body composition measurements taken at 7, 13 and 19 weeks of age of the three diet groups. Non-milk (n=19), Goat (n=18), Cow (n=20) ...........................................4-26
Figure 4-5. Graphs showing means and SDs for Lumbar Spine area, BMC, and BMD measurements from DEXA scan taken at 7,13, and 19 weeks of age. Non-milk (n=19), Goat (n=18), Cow (n=20) .................................................................4-27
Figure 4-6. Graphs showing means and SDs for Femur area, BMC, and BMD measurements from DEXA scans taken at 7, 13, and 19 weeks of age. Non-milk (n=19), Goat (n=18), Cow (n=20)...

Chapter 5
Figure 5-1. Picture describing the cross sectional profile of the femur slice.......
Figure 5-2. Graphs showing means and SD for Bodyweights and the seven DEXA whole body composition measurements taken at 20, 30, and 38 weeks of age for the seven treatment groups...
Figure 5-3. Graphs showing Lumbar Spine BMC, and BMD at 20, 30, and 38 weeks of age for the seven treatment groups...
Figure 5-4. Graphs showing Femur BMC, and BMD at 20, 30, and 38 weeks of age for the seven treatment groups...

Chapter 6
Figure 6-1. Diagram of Izod test...
Figure 6-2. Diagrams showing orientation of bone samples in Izod tester...
Figure 6-3. Means and SD’s of specific energy (J/mm²) required to fracture segment A for rats fed milk diets...
Figure 6-4. Means and SD’s of a) bone areas (mm²) and b) cross sectional areas (mm²) of femoral segments A for rats that received milk diets...
Figure 6-5. Means and SD’s of specific energy (J/mm²) required to fracture segment B for rats that received milk diets...
Figure 6-6. Means and SD’s of a) bone areas (mm²) and b) cross sectional areas (mm²) in femoral segment B for rats that received milk diets...
Figure 6-7. Means and SD’s of specific energy (J/mm²) required to fracture segment A for rats that received the non-milk diet...
Figure 6-8. Means and SD’s of a) bone areas (mm²) and b) cross sectional areas (mm²) in femoral segment B for rats that received the non-milk diet...
Figure 6-9. Means and SD’s of specific energy (J/mm²) required to fracture segment B for rats that received the non-milk diet...
Figure 6-10. Means and SD’s of a) bone areas (mm²) and b) cross sectional areas (mm²) in femoral segment B for rats that received the non-milk diet...

Chapter 7
Figure 7-1. Sample μCT scans of proximal tibia cortical bone from a) a GOAT OVX rat, and b) a GOAT OVX ALD rat...
Figure 7-2. Sample μCT scans of proximal tibia trabecular bone from a) a GOAT OVX rat, and b) a GOAT OVX ALD rat...
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACP</td>
<td>Acepromazine</td>
</tr>
<tr>
<td>AGE’s</td>
<td>Advanced glycation end-products or non-enzymic cross-links</td>
</tr>
<tr>
<td>αs2-casein</td>
<td>Alpha s2 casein</td>
</tr>
<tr>
<td>α-lactalbumin</td>
<td>Alpha lactalbumin</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of co-variance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ASTM</td>
<td>American society for testing and materials</td>
</tr>
<tr>
<td>BA</td>
<td>Bone area</td>
</tr>
<tr>
<td>β-casein</td>
<td>Beta casein</td>
</tr>
<tr>
<td>β-lactoglobulin</td>
<td>Beta lactoglobulin</td>
</tr>
<tr>
<td>BMC</td>
<td>Bone mineral content</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone mineral density</td>
</tr>
<tr>
<td>BMU</td>
<td>Basic multicellular unit</td>
</tr>
<tr>
<td>BMPs</td>
<td>Bone morphogenetic proteins</td>
</tr>
<tr>
<td>BPM</td>
<td>Bone perimeter</td>
</tr>
<tr>
<td>BS/BV</td>
<td>Surface to volume ratio</td>
</tr>
<tr>
<td>BS/TV</td>
<td>Bone surface density</td>
</tr>
<tr>
<td>BS</td>
<td>Bone surface</td>
</tr>
<tr>
<td>BS/BV</td>
<td>Surface to volume ratio</td>
</tr>
<tr>
<td>BV</td>
<td>Bone volume</td>
</tr>
<tr>
<td>BV/BT</td>
<td>Percentage of bone volume</td>
</tr>
<tr>
<td>Ca^{2+}</td>
<td>Calcium ions</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>CPP</td>
<td>Casein phosphopeptides</td>
</tr>
<tr>
<td>CSTH</td>
<td>Cortical thickness</td>
</tr>
<tr>
<td>CSMI</td>
<td>Cross section moment of inertia</td>
</tr>
<tr>
<td>CTx</td>
<td>C-terminal telopeptides of type 1 collagen</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DA</td>
<td>Degree of anisotropy</td>
</tr>
<tr>
<td>DEXA</td>
<td>Dual energy x-ray absorptiometry</td>
</tr>
<tr>
<td>Dpi</td>
<td>Dots per square inch (resolution)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix proteins</td>
</tr>
<tr>
<td>EFA</td>
<td>Essential fatty acid</td>
</tr>
<tr>
<td>EPFM</td>
<td>Elastic-plastic fracture mechanics (J-integral measurement)</td>
</tr>
<tr>
<td>FD</td>
<td>Fractal dimension</td>
</tr>
<tr>
<td>F</td>
<td>Femur</td>
</tr>
<tr>
<td>$G_c$</td>
<td>Critical strain energy release rate</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GLM</td>
<td>General linear model</td>
</tr>
<tr>
<td>H1-ATPase</td>
<td>Electrogenic proton pump H1–adenosine triphosphatase</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>ICPOES</td>
<td>Inductively coupled plasma optical emission spectroscopy</td>
</tr>
<tr>
<td>J</td>
<td>Joules</td>
</tr>
<tr>
<td>$J/mm^2$</td>
<td>Modulus of toughness or Specific energy</td>
</tr>
<tr>
<td>$K_c$</td>
<td>Critical stress intensity factor</td>
</tr>
<tr>
<td>$k$-casein</td>
<td>Kappa casein</td>
</tr>
<tr>
<td>kN</td>
<td>Kilonewton</td>
</tr>
<tr>
<td>LEFM</td>
<td>Linear-elastic fracture mechanics</td>
</tr>
<tr>
<td>LS</td>
<td>Lumbar spine</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Monocyte chemoattractant protein-1</td>
</tr>
<tr>
<td>M-CSF</td>
<td>Macrophage-colony stimulating factor or CSF-1</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>mL</td>
<td>Millilitre</td>
</tr>
<tr>
<td>mm</td>
<td>Millimetre</td>
</tr>
<tr>
<td>MPa</td>
<td>Megapascal</td>
</tr>
<tr>
<td>N</td>
<td>Newton</td>
</tr>
<tr>
<td>N/mm</td>
<td>Extrinsic stiffness</td>
</tr>
<tr>
<td>N/mm$^2$</td>
<td>Ultimate stress</td>
</tr>
<tr>
<td>NCP</td>
<td>Noncollagenous proteins</td>
</tr>
<tr>
<td>ng</td>
<td>Nanogram</td>
</tr>
<tr>
<td>OVX</td>
<td>Ovariectomized rat</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal component analysis</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>QC</td>
<td>Quality control</td>
</tr>
<tr>
<td>RANK</td>
<td>Receptor activator of nuclear factor kappa B</td>
</tr>
<tr>
<td>RANKL</td>
<td>Receptor activator of nuclear factor kappa B ligand</td>
</tr>
<tr>
<td>R curves</td>
<td>Crack resistant curves</td>
</tr>
<tr>
<td>RUNX2</td>
<td>Runt-related transcription factor 2 or Cbf-alpha-1(Cbfa1)</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SHAM</td>
<td>Sham-operated rat</td>
</tr>
<tr>
<td>SMI</td>
<td>Structure model index</td>
</tr>
</tbody>
</table>