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# **Effect of Goat milk on bone mass, morphology and biomechanics**

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## 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 ( $J/mm^2$ ) 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 ( $J/mm^2$ ) 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.

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## Abbreviations

ACP	Acepromazine
AGE's	Advanced glycation end-products or non-enzymic cross-links
$\alpha_{s2}$ -casein	Alpha s2 casein
$\alpha$ -lactalbumin	Alpha lactalbumin
ANCOVA	Analysis of co-variance
ANOVA	Analysis of variance
ASTM	American society for testing and materials
BA	Bone area
$\beta$ -casein	Beta casein
$\beta$ -lactoglobulin	Beta lactoglobulin
BMC	Bone mineral content
BMD	Bone mineral density
BMU	Basic multicellular unit
BMPs	Bone morphogenetic proteins
BPM	Bone perimeter
BS/BV	Surface to volume ratio
BS/TV	Bone surface density
BS	Bone surface
BS/BV	Surface to volume ratio
BV	Bone volume
BV/BT	Percentage of bone volume
Ca <sup>2+</sup>	Calcium ions
CO <sub>2</sub>	Carbon dioxide
CPP	Casein phosphopeptides
CSTH	Cortical thickness
CSMI	Cross section moment of inertia
CTx	C-terminal telopeptides of type 1 collagen
CV	Coefficient of variation
DA	Degree of anisotropy
DEXA	Dual energy x-ray absorptiometry
Dpi	Dots per square inch (resolution)

ECM	Extracellular matrix proteins
EFA	Essential fatty acid
EPFM	Elastic-plastic fracture mechanics (J-integral measurement)
FD	Fractal dimension
F	Femur
$G_c$	Critical strain energy release rate
g	Gram
GLM	General linear model
H1-ATPase	Electrogenic proton pump H1–adenosine triphosphatase
HCl	Hydrochloric acid
ICPOES	Inductively coupled plasma optical emission spectroscopy
J	Joules
$J/mm^2$	Modulus of toughness or Specific energy
$K_c$	Critical stress intensity factor
<i>k</i> -casein	Kappa casein
kN	Kilonewton
LEFM	Linear-elastic fracture mechanics
LS	Lumbar spine
MCP-1	Monocyte chemoattractant protein-1
M-CSF	Macrophage-colony stimulating factor or CSF-1
mg	Milligram
mL	Millilitre
mm	Millimetre
MPa	Megapascal
N	Newton
N/mm	Extrinsic stiffness
$N/mm^2$	Ultimate stress
NCP	Noncollagenous proteins
ng	Nanogram
OVX	Ovariectomized rat
PBS	Phosphate buffered saline
PCA	Principal component analysis
PTH	Parathyroid hormone

QC	Quality control
RANK	Receptor activator of nuclear factor kappa B
RANKL	Receptor activator of nuclear factor kappa B ligand
R curves	Crack resistant curves
RUNX2	Runt-related transcription factor 2 or Cbf-alpha-1(Cbfa1)
SD	Standard deviation
SHAM	Sham-operated rat
SMI	Structure model index