Impact of goat milk powdered formulations on mineral absorption, peak bone mass and bone loss due to ovariectomy in rats

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Abstract

BACKGROUND: Goat milk is recognised as nutritious, with benefits to growth and skeletal development. The initial aim of this study was to investigate the effect of three different goat milk formulae – a whole milk, a skim milk and a goat milk growing-up formula fortified with pre- and probiotics (Formula 1) – on mineral absorption and retention in rats. The effect of long-term intake of the fortified formula diet on peak bone mass and post-ovariectomy bone loss in rats was then investigated in a follow-up study and was assessed by bone density dual-energy X-ray absorptiometry and biomechanical testing of bone *ex vivo*.

RESULTS: Goat whole milk and fortified milk formulations improved calcium and phosphorus absorption and retention. Body composition analysis showed that rats fed the fortified diet had higher body calcium and phosphorus content. The fortified diet was then tested in a long-term feeding trial. Rats fed the fortified diet from weaning had a higher peak bone mass than rats fed a soy protein control diet. Bone mineral content (BMC) and density (BMD) of the lumbar spine were higher in rats fed the fortified diet. After ovariectomy, all rats lost bone mass, but rats fed the fortified diet maintained significantly higher BMD and BMC values throughout the trial, though still lower than those of non-ovariectomised control rats. The fortified diet increased bone strength.

CONCLUSION: Goat milk specific nutrients supported by pre- and probiotics in Formula 1 may improve mineral status during growth and support attainment of peak bone mass.

Keywords: goat milk; mineral absorption; ovariectomised rat; bone density

INTRODUCTION

Osteoporosis is one of the most critical disorders occurring in women with advancing age. During menopause there is a change in bone turnover, with an imbalance between bone formation and bone resorption resulting in net bone loss. Optimising peak bone mass at adolescence or just after, up to the age of 30, can reduce the risk of developing osteoporosis later in life.[1–3]

Goat milk is recognised as being highly nutritious, benefiting growth and skeletal mineralisation in children.[4,5] Several animal-based studies have also indicated enhanced protein, mineral and fat utilisation from goat milk compared with cow milk.[6–10] Studies also demonstrate a higher calcium content of the femur, sternum and *Longissimus dorsi* muscle[7,10] and higher bone mineral density[6] following consumption of goat milk compared with cow milk. The constituents or synergistic relationships in goat milk contributing to these benefits are not known.

Based on the observations outlined above, it is possible that supplementation with goat milk could affect mineral retention during growth and may therefore also have a beneficial effect in later life in reducing bone mineral loss due to aging or menopause. Several studies show some benefit of goat milk over cow milk with regard to mineral absorption or availability,[6–10] but we are not aware of any studies investigating the impact of goat milk on functional outcomes such as bone loss or osteoporosis. In the present study we therefore endeavoured to investigate the effect of various forms of goat milk compared with each other on mineral absorption and balance; this was followed by a second study to investigate bone

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mineral acquisition when fortified goat milk is used to supplement calcium and other nutrients to a normal balanced diet.

In the present study we:

- investigated the effect of skim goat milk, whole goat milk and a fortified growing-up milk made from goat milk powder on mineral absorption and retention in the growing rat (study 1) and;
- tested whether long-term intake of the fortified goat milk supplementing a nutritionally adequate diet could increase peak bone mass and thereby reduce bone loss induced by ovariectomy in female rats (study 2).

We used a commercially available goat milk product (Karihome™ Goat Growing-up Milk) in both trials. In study 1, rats fed the fortified goat milk were compared with rats fed skim and whole goat milk powder. In study 2, rats fed the fortified goat milk product were compared with rats fed a diet devoid of dairy protein (soy protein) to avoid the presence of bioactive factors from milk that could have an impact on bone. The experimental diets (formula 1) in both trials provided goat milk as the source of protein and prebiotics, probiotics and medium-chain fatty acids, as these may have a potential role in mineral utilisation.[7,8,11]

MATERIALS AND METHODS

Study 1. Mineral absorption

The study was reviewed and approved by the Massey University Animal Ethics committee (03/103).

Animals

Three groups of male rats ($n = 10$ per group) aged 3 weeks were weaned onto balanced semi-synthetic diets formulated using goat milk powder as the protein source (diets were balanced for calcium and other minerals and vitamins according to the recommended daily intake for growing rats). The rats were maintained on the test diets for 3 weeks. They were housed individually in shoebox cages and accustomed to the diet for 2 weeks, then housed individually in metabolic cages for the third week.

The groups were as follows:

- goat whole milk powder (GWMP);
- goat skim milk powder (with added vegetable oils) (GSMP);
- goat milk growing-up formula with added pre- and probiotics, a commercially available formula (Karihome™ Goat Growing-up Milk) (Formula 1).

All milk powders were supplied by Dairy Goat Co-operative (N.Z.) Ltd (Hamilton, New Zealand).

Samples collected

During week 3, food and water intakes were measured daily while urinary and faecal excretions were collected. At the end of the week, faecal and urine samples were pooled for analysis. After week 3 the animals were euthanised by exsanguination and the livers were collected and weighed. The carcasses were processed for whole body macro- and micronutrient content chemical analyses. Samples of the three diets were also collected and analysed.

Chemical analysis

The following analyses were done on the various samples:

- total N (diet, faeces and urine);
- urea, ammonia and creatinine (urine);
- fat (diet and faeces);
- gross energy (diet, faeces and urine);
- P, Ca, Mg, Fe, Mn, Zn, Cu and Se (diet, faeces and urine).

Mineral contents were measured by inductively coupled plasma spectrophotometry. Protein was determined by Leco total combustion (AOAC method 968.06), fat by Soxtec extraction (AOAC method 991.36), moisture in a convection oven at 105 °C (AOAC methods 930.15 and 925.10) and ash in a furnace at 550 °C (AOAC method 942.05).

Study 2. Bone growth before and maintenance of bone after ovariectomy

The study was reviewed and approved by the Massey University Animal Ethics committee (04/166).

Animals

Forty-five weanling female Sprague-Dawley rats were obtained from the Small Animal Production Unit (SAPU) at Massey University. The rats were fed a semi-synthetic base diet with soy as the protein source (Table 1) for 4 weeks, after which they were randomly divided into three groups of 15 animals each. One group was fed the test goat milk diet while two groups (to be sham and control) were kept on the soy-based diet for 10 weeks. At week 11, rats fed the test diet and one group of control rats were ovariectomised (OVX). The sham operated animals (sham controls) underwent anaesthesia by isofluorane inhalation, and an abdominal incision was made but the ovaries were left intact. The ovaries were removed from the OVX rats. The animals were then fed the same diets as pre-OVX, after adjustment for the specific requirements of the older rat, for another 21 weeks.[12]

The animals were housed separately in shoebox cages and kept in a temperature (22 ± 2 °C)- and light (12 h day/night cycle)-controlled room at SAPU, Massey University with *ad libitum* access to deionised water. Daily food intake was measured and was adjusted weekly according to the sham group's body weight in order to reduce excess body weight gain due to the loss of oestrogen in the OVX groups. The mean food intake over the trial was 20 g day^{-1} per animal.

Table 1. Composition of various diets fed during growth and after ovariectomy

Component	Control (soy)	Formula 1	GWMP	GSMP
Standardised components				
Mean N (%)	2.7	2.7	3.2	3.1
Crude protein (%)	17.1	17.1	20.6	19.4
Gross energy (kJ g^{-1})	20.5	21.1	21.4	18.9
Fat (%)	22.2	22.6	21.8	20.3
Non-standardised components				
Goat milk fat (%)[a]	–	21.3	22.6	ND
SFA (%)[b]	11.2	12.2	ND	ND
MCFA (%)[c]	3.6	3.2	ND	ND
Dry matter (%)	93.5	96.7	93.8	93.9
Ash (%)	2.4	4.2	5.4	5.1
Prebiotics (g per 100 g)[d]	0	1.2	0	0
Probiotics (10^8 cfu per 100 g)[e]	0	2.4	0	0
Moisture (%)	4.8	3.3	6.1	6.1
Minerals				
Selenium (mg kg^{-1})	0.18	0.048	0.045	0.049
Potassium (g kg^{-1})	3.60	8.90	ND	ND
Magnesium (g kg^{-1})	0.70	0.70	0.92	0.74
Sodium (g kg^{-1})	2.3	1.7	ND	ND
Iron (mg kg^{-1})	291	336	270	278
Manganese (mg kg^{-1})	50	46	56	56
Zinc (mg kg^{-1})	32	70	54	57
Copper (mg kg^{-1})	9	11	7.7	7.9
Calcium (g kg^{-1})	3.50	6.30	6.60	5.90
Phosphorus (g kg^{-1})	1.70	4.80	6.50	5.70

ND, not determined.
[a] Percentage of fat content (Formula 1, 80%).
[b] Saturated fatty acids (calculated from composition of added ingredients).
[c] Medium-chain fatty acids (C6–C12; calculated from composition of added ingredients).
[d] Beneo Synergy 1, a specific, patented combination of chicory inulin and oligofructose from Orafti (Tienen, Belgium).
[e] Consisting of equal proportions of *Lactobacillus acidophilus* NCFM®, *Lactobacillus paracasei* and *Bifidobacterium lactis*.

Diets

The animals were fed a semi-synthetic diet including either soy or goat milk protein. The control and sham groups were fed a maintenance diet with soy protein as the protein source. The diet of the other group was based on a goat milk powder formulation, Formula 1, a commercially available formula (Karihome™ Goat Growing-up Milk) supplied by Dairy Goat Co-operative (N.Z.) Ltd. All diets were balanced for protein (17%), fat (22%) and energy (22 kJ g^{-1}). A mixture of vegetable oils, vitamins and minerals was added to adjust the total and saturated fat composition of the diets and the vitamin and mineral content according to AIN93M[12] (Table 1). The calcium content of the diets was not balanced, as the purpose of the study was to assess the effect of supplementing goat milk-based formulations on top of a diet containing a standard maintenance level of calcium. Thus the calcium level was 0.6% for the goat milk diet and 0.35% for the control diets; the calcium/phosphorus ratio was 1.28 for the goat milk diet and 2 for the soy diet. The goat milk powder contained added pre- and probiotics. The prebiotic included was Beneo Synergy 1 (Orafti, Tienen, Belgium), a specific, patented combination of chicory inulin and oligofructose which has been demonstrated to increase calcium absorption and enhance bone health with greater efficacy than either chicory inulin or oligofructose alone.[13,14] The probiotic consisted of equal proportions of *Lactobacillus acidophilus* NCFM®, *Lactobacillus paracasei* and *Bifidobacterium lactis*.

Dual-energy X-ray absorptiometry (DEXA) scans

Animals were scanned at baseline before starting the test diet and every 6 weeks thereafter until the end of the trial. For DEXA measurements, animals were weighed and anaesthetised with an appropriate dose of anaesthetic, i.e. 0.05 mL per 100 g body weight. The anaesthetic, a mixture of 0.2 mL of Acepromazine (ACP), 0.5 mL of Ketamine, 0.1 mL of Xylazine and 0.2 mL of sterile water in 1 mL total volume, was administered via intraperitoneal injection using a 25 g, 15 mm needle and a 1 mL syringe. The animals attained a suitable level of anaesthesia approximately 5–10 min after injection and remained under anaesthesia for 2 h.

Bone mineral measurements were taken using a Hologic QDR4000 bone densitometer utilising a pencil beam unit (Bedford, MA, USA). On the day preceding each set of scans, a quality control (QC) scan was taken to ensure that the unit's precision met the required coefficient of variation. The coefficient of variation (CV) for the QC data was 0.98–1.01%. Regional high-resolution scans were performed using

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a 1.5 mm diameter collimator with 0.31 mm point resolution and 0.5 mm line spacing. Rats were placed on an acrylic platform of uniform 37.5 mm thickness. Each rat underwent three regional high-resolution scans, one each of the spine and left and right femurs. Rats were positioned supine with right angles between the spine and femur and between the femur and tibia.

CVs for the femurs ranged between 0.92 and 0.85% with and without repositioning between scans respectively. Values for the spine ranged between 1 and 0.98%.

Euthanasia and tissue collection

After 40 weeks the animals were weighed and anaesthetised. Animals were euthanased by exsanguination under anaesthesia, blood samples were collected and the carcasses were dissected. Both the left and right femurs and spine were excised and frozen in phosphate-buffered saline (PBS) for further analysis. The uteri were removed and weighed to confirm OVX status. Blood was placed immediately in heparin-filled tubes, then centrifuged at $1000 \times g$ for 10 min. The plasma was removed, aliquoted, snap frozen and stored at $-80\,^{\circ}C$ until analysed.

Mechanical properties of bone

The right femurs were scraped clean of adhering flesh and stored in PBS at $-20\,^{\circ}C$. Before biomechanical testing, the bones were thawed. Femur length was measured using electronic callipers. The midpoint was marked and the width and thickness of the femurs were recorded. The femurs were held at room temperature ($23\,^{\circ}C$) before and during the test. Femurs were placed in a testing jig constructed for a three-point bending test. The distance between the supporting rods was fixed at 12 mm. Load was applied at a constant deformation rate of 50 mm min^{-1}. Maximum load (N), stiffness (N mm^{-2}) and energy (J) were measured using a Shimadzu Ezi-test texture analyser (Kyoto, Japan).

Bone biochemical markers

Plasma levels for telopeptides of type I collagen (CTx) and osteocalcin were measured using Ratlaps (CTx) and osteocalcin ELISA kits (Nordic Bioscience Diagnostics, A/S, Herlev, Denmark). Inter- and intra-assay CVs for both kits were 7–9 and 4–7% respectively. Plasma oestrogen was measured with a double-antibody radioimmunoassay for 17β-oestradiol (Diasorin, Stillwater, MN, USA).

Statistical analyses

Data were analysed using SAS® 9.1 (SAS Institute Inc., Cary, NC, USA).

Study 1

Differences between groups were determined using one-way analysis of variance (ANOVA) with Tukey *post hoc* comparisons between each group. All results are expressed as the mean ± the standard error of the mean. Statistical significance was set at $P < 0.05$.

Study 2

Bone density measurements were analysed with a repeated measures model fitted using SAS Proc Mixed. Two models were tested, both with body weight as a covariate. One model assumed no correlation between error terms for a particular individual at different times, while the other assumed a Toeplitz covariance structure between the error terms for an individual at different times. Body weight and bone marker measurements were analysed with a repeated measures model, with initial body weight or marker concentration as the covariate respectively. Pairwise comparisons between all groups at each week were made for bone density, body weight and bone markers. Differences between groups for bone mechanics and 17β-oestradiol results were determined using one-way ANOVA with Tukey *post hoc* comparisons between each group. All results are expressed as the mean ± the standard error of the mean.

RESULTS

Study 1

There were no significant differences between groups for weight and growth rate over the trial. Food intake and faecal output differed between groups over the 5 day balance study, with food intake being highest for the GSMP group (73.2 g) and lowest for the rats fed Formula 1 (63.2 g). Faecal excretion was significantly lower in the latter group (3 g) compared with the GSMP and GWMP groups (6–7 g), which may indicate higher digestibility of the fortified formula. Urinary output did not differ between groups (data not shown).

The % absorption and actual amount absorbed of various nutrients are given in Table 2. On a per 5 day basis, phosphorus absorption was lower in the Formula 1 group than in the GWMP and GSMP groups. Calcium absorption was higher in the GWMP group compared with the GSMP and Formula 1 groups. Magnesium absorption was significantly higher in the GWMP group on a 5 day basis. The % absorption of iron was supported by Formula 1 but not on a per 5 day basis. Absorption of iron from both GSMP and GWMP was similar. Zinc was better absorbed in the GWMP group on a per 5 day basis. Analysis for selenium in milk-based diets is variable, so the balance data for selenium are not presented. The data do suggest an effect by GSMP though.

Table 3 summarises the body composition of the animals after 3 weeks of being fed various diets. There were no significant differences between groups for body weight, water, dry matter, ash and fat. Body calcium and phosphorus contents were significantly higher in the Formula 1-fed rats compared with the

Table 2. Percentage and actual amount of dietary minerals absorbed during 5 day balance

Parameter	GSMP	GWMP	Formula 1
Total food intake per 5 days (g)	73.18 ± 5.02a	67.02 ± 5.45b	63.23 ± 4.51b
Phosphorus intake (g)	0.42 ± 0.03a	0.47 ± 0.04b	0.36 ± 0.03c
Phosphorus (%)	54.42 ± 6.11a	55.89 ± 5.87a	57.97 ± 6.42a
Phosphorus (g per 5 days)	0.23 ± 0.02ab	0.26 ± 0.03a	0.21 ± 0.03b
Calcium intake (g)	0.55 ± 0.04a	0.53 ± 0.04ab	0.50 ± 0.04b
Calcium (%)	62.40 ± 5.96a	72.70 ± 6.09b	66.54 ± 7.82ab
Calcium (g per 5 days)	0.34 ± 0.03ab	0.39 ± 0.03a	0.33 ± 0.05b
Magnesium intake (g)	0.062 ± 0.004a	0.075 ± 0.006b	0.054 ± 0.004c
Magnesium (%)	29.80 ± 3.98a	34.09 ± 3.51a	30.91 ± 5.43a
Magnesium (g per 5 days)	0.018 ± 0.002a	0.025 ± 0.003b	0.017 ± 0.004a
Iron intake (mg)	20.35 ± 1.40a	15.75 ± 1.28b	6.43 ± 0.46c
Iron (%)	21.89 ± 7.40ab	12.90 ± 13.15a	31.52 ± 12.64b
Iron (mg per 5 days)	4.44 ± 1.47a	2.01 ± 1.99a	2.05 ± 0.92a
Zinc intake (mg)	4.00 ± 0.27a	3.86 ± 0.31a	3.13 ± 0.22b
Zinc (%)	35.00 ± 5.30a	40.09 ± 6.54a	39.78 ± 7.84a
Zinc (mg per 5 days)	1.39 ± 0.19ab	1.54 ± 0.24a	1.25 ± 0.31b
Copper intake (mg)	0.58 ± 0.04a	0.52 ± 0.04b	0.31 ± 0.02c
Copper (%)	24.68 ± 6.11a	27.09 ± 7.06a	26.40 ± 9.68a
Copper (mg per 5 days)	0.14 ± 0.03a	0.14 ± 0.03a	0.08 ± 0.04b

Values are presented as mean ± standard error of mean. Different letters indicate statistically significant differences between means in a row ($P < 0.05$). Absorbed = [(diet − faeces)/diet] × 100%.

Table 3. Body composition of rats after 3 weeks of being fed various diets

Parameter	GSMP	GWMP	Formula 1
Body weight (g)	173.36 ± 12.39	170.03 ± 11.34	168.75 ± 6.92
Water (%)	69.12 ± 0.77	69.27 ± 1.96	69.23 ± 1.25
Dry matter (%)	30.87 ± 0.77	30.72 ± 1.96	30.76 ± 1.25
Ash (%)	10.06 ± 1.02	10.67 ± 1.62	10.61 ± 1.25
Crude protein (%)	61.69 ± 2.56	62.11 ± 4.66	63.36 ± 4.16
Fat (%)	26.03 ± 2.39	27.27 ± 5.36	26.59 ± 5.23
Zn (mg kg^{-1})	62.20 ± 2.04	64.70 ± 3.74	64.80 ± 3.32
Cu (mg kg^{-1})	7.30 ± 0.94a	8.21 ± 1.61b	7.40 ± 1.43a
Fe (mg kg^{-1})	128.0 ± 15.61a	153.80 ± 32.32b	128.10 ± 23.38a
Ca (%)	1.60 ± 0.41a	1.67 ± 0.13b	1.73 ± 0.25b
K (mg kg^{-1})	0.99 ± 0.04	0.97 ± 0.06	0.98 ± 0.05
P (%)	0.97 ± 0.04a	1.01 ± 0.06b	1.01 ± 0.10b
Mg (g kg^{-1})	0.54 ± 0.031	0.58 ± 0.097	0.55 ± 0.079
Na (g kg^{-1})	3.54 ± 0.02a	3.83 ± 0.02b	3.58 ± 0.04a

Values are presented as mean ± standard error of mean. Different letters indicate statistically significant differences between means in a row ($P < 0.05$).

GSMP group. Body iron content was highest for the GWMP group.

Study 2

Table 4 shows the final body weights, uterus weights and plasma oestradiol concentrations at week 40. The OVX control rats weighed significantly more at week 40 compared with the sham control and Formula 1-fed rats. Food intake was similar in all groups. There were significant differences in the uterus weights and oestradiol levels between sham and OVX groups at week 40, indicating that the OVX model was successfully employed.

At week 19, pre-OVX bone mineral density (BMD) and content (BMC) values for the lumbar spine were significantly higher in the Formula 1-fed rats compared with both control groups (Fig. 1). All OVX rats lost bone mass after OVX, but Formula 1-fed rats maintained significantly higher BMD and BMC values than the OVX control group. Although bone mass for the Formula 1-fed group was not maintained at sham levels, there was a tendency for slower bone loss after OVX. Up to week 33 the BMD values for the Formula 1-fed group were similar to those of the sham control group and higher than those of the OVX control group. At week 40 the group of rats fed Formula 1 had BMD values that were significantly higher than those of the OVX group but significantly lower than those of the sham group.

The observed bone loss pattern was confirmed by the BMD and BMC values for the femurs (Fig. 2). At week 19, femur BMC and BMD were significantly

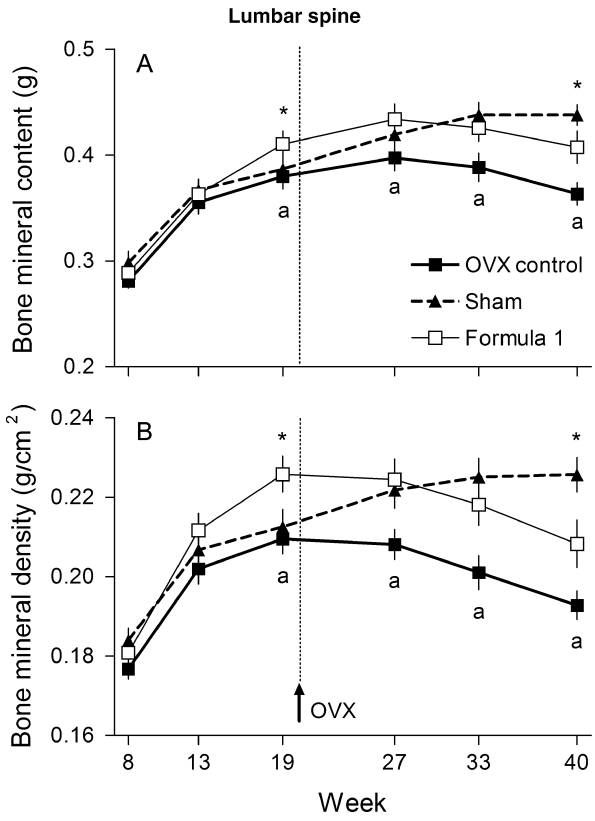

Figure 1. Lumbar spine bone mineral content (A) and density (B) of female Sprague-Dawley rats fed either a soy protein based diet (Sham and OVX control) or with a goat milk powdered formulae (Formula 1) from 8 to 40 weeks of age. An 'a' represents $P < 0.05$ for Formulae 1 *vs* OVX control. An 'a' represents $P < 0.05$ for Formulae 1 *vs* OVX control. An asterisk (*) represents $P < 0.05$ for Formula 1 *vs* sham.

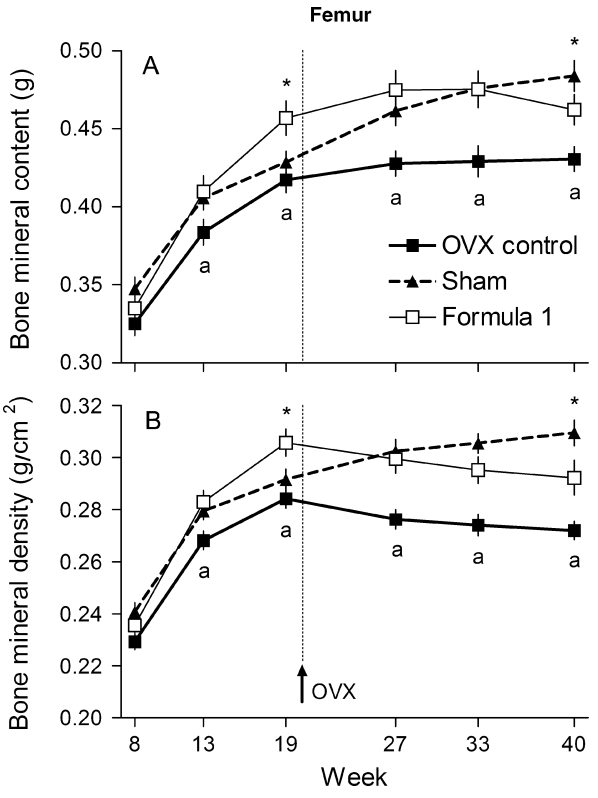

Figure 2. Femur mineral content (A) and density (B) of female Sprague-Dawley rats fed either a soy protein based diet (sham and OVX control) or with a goat milk powdered formulae (Formula 1) from 8 to 40 weeks of age. An 'a' represents $P < 0.05$ for Formulae 1 *vs* OVX control. An asterisk (*) represents $P < 0.05$ for Formulae 1 *vs* sham.

higher in the Formula 1-fed group compared with the sham and OVX controls. Feeding the goat milk powder-based diet therefore impacted on peak bone mass gain in the growing rats. After OVX, all rats lost bone in a similar manner. At week 33, Formula 1-fed rats had BMC and BMD values similar to those of the sham group. At week 40, BMC and BMD for the Formula 1-fed rats were significantly higher than those

Table 4. Body and uterus weights and serum oestradiol concentration of various groups of animals at termination of trial

Parameter	Sham	OVX control	Formula 1
Body weight (g)	415.9 ± 14.4a	480.3 ± 15.0b	417.2 ± 14.4a
Uterus weight (g)	0.71 ± 0.25a	0.17 ± 0.02b	0.15 ± 0.03b
17β-Oestradiol (pg mL^{-1})	27.49 ± 5.08a	16.90 ± 4.51b	15.10 ± 3.79b

Values are presented as mean ± standard error of mean. Different letters indicate statistically significant differences between means in a row ($P < 0.05$).

Table 5. Plasma osteocalcin and CTx of various groups of animals before OVX (week 19), at weeks 27 and 33 and at termination of trial (week 40)

Parameter	Week	Sham	OVX control	Formula 1
Osteocalcin (ng mL^{-1})	19	93.1 ± 8.1a	111.6 ± 14.8a	75.0 ± 7.7a
	27	90.0 ± 10.2a	160.4 ± 11.1b	148.2 ± 21.2b
	33	77.9 ± 10.3a	91.5 ± 7.2a	82.1 ± 10.2a
	40	54.8 ± 3.6a	81.8 ± 9.6a	70.9 ± 10.5a
CTx (ng mL^{-1})	19	11.7 ± 1.2a	11.6 ± 1.1a	11.1 ± 0.9a
	27	10.2 ± 1.2a	14.4 ± 0.7b	16.4 ± 1.3b
	33	8.5 ± 0.5a	11.8 ± 1.1b	10.9 ± 1.1ab
	40	7.9 ± 1.1a	10.7 ± 1.1a	10.6 ± 1.4a

Values are presented as mean ± standard error of mean. Different letters indicate statistically significant differences between means in a row ($P < 0.05$).

Table 6. Biomechanics of right femur of various groups of animals at termination of trial

Parameter	Sham	OVX control	Formula 1
Maximum load (N)	179.4 ± 5.4ab	166.8 ± 4.0a	191.7 ± 5.8b
Break load (N)	179.1 ± 5.3ab	166.0 ± 4.0a	190.0 ± 5.5b
Energy (J)	0.155 ± 0.007a	0.148 ± 0.005a	0.172 ± 0.009a
Elasticity (N mm^{-2})	817.0 ± 25.5a	751.3 ± 30.8a	699.4 ± 39.8a

Values are presented as mean ± standard error of mean. Different letters indicate statistically significant differences between values in a row ($P < 0.05$).

of the OVX control but significantly lower than those of the sham control.

Bone turnover increases significantly with ovariectomy owing to the loss of oestrogen. Plasma osteocalcin, a marker for bone turnover, increased significantly in all groups at week 27, except for the sham group (Table 5). At week 27, values for the Formula 1-fed rats were significantly higher than those of the sham group. All groups returned to pre-OVX levels by week 40 of the trial. There were no significant differences between groups at week 40.

Plasma levels of Ratlaps (CTx), a marker of bone resorption, increased following OVX in both OVX groups relative to the sham group at week 27 (Table 5). At week 27, CTx levels for OVX control and Formula 1-fed rats were still significantly higher than those of the sham group. At weeks 33 and 40 there were no significant differences between groups.

Table 6 summarises the biomechanical properties of the rat bones after 40 weeks of feeding the various test diets. There were no significant differences except for maximum load and break load. For these, Formula 1 appeared to increase the load needed to fracture the femurs compared with the OVX control. There was no significant effect by Formula 1 on the energy absorbed before fracture or elasticity in comparison with the two controls, which is an indication that bone stiffness was not affected.

DISCUSSION

Study 1 showed that GWMP and fortified goat milk affect mineral absorption as well as whole body mineral content (as determined using chemical analyses) compared with GSMP. This observation could be due to goat milk fat, as values for whole goat milk were in some cases significantly higher than those for skim goat milk to which vegetable oils were added. Goat milk contains a high amount of medium-chain fatty acids, which affect digestibility and can also affect mineral absorption.[4] Iron bioavailability of goat milk has been shown to be superior to that of cow milk.[15] Similarly Alferez *et al.*[9] showed that goat milk improves zinc and selenium bioavailability by about 60 and 15% respectively. The latter study, however, used a different method of determining retention, so its results cannot be directly compared with those of the present study. The results from study 1 suggest that the body's retention of calcium and phosphorus is higher when using whole goat milk and fortified goat milk compared with skim goat milk. Fortification of goat milk with pre- and probiotics further supported mineral absorption. Whether the mineral was deposited in bone could not be ascertained in study 1. These observations therefore prompted us to investigate whether fortified whole goat milk, providing extra nutrients and possible bioactive factors, supplemented to an adequate diet could increase peak bone mass gain and reduce bone loss after ovariectomy.

Animals in trial 2 all consumed a similar amount of diet, between 107 and 114 g per rat, during the last week of the trial, but the final body weights were significantly different. The Formula 1-fed animal weights were similar to those for the sham rats, while the OVX control group was the heaviest. The possibility that goat milk can affect satiety and/or body weight gain was a surprising observation not reported before and is being investigated in further trials. For statistical analyses for trial 2, body weight was used as a covariate.

Skeletal growth is dependent on the availability of dietary calcium, and it is important that sufficient calcium is available to support growth and attainment of peak bone mass. The results of study 2 indicate that supplementation of calcium using a goat milk formula was able to raise peak bone mass above that of control rats fed a soy protein-based diet alone. Furthermore, rats fed goat milk maintained a significantly higher bone mineral density in the lumbar spine and significantly higher femur bone mineral content compared with the OVX control rats throughout the post-OVX stage. However, except for a transient effect on osteocalcin, the goat milk formula was not able to alter bone turnover following OVX. Our results corroborate the suggestion that increasing peak bone mass in growing children and young adults provides the advantage of higher bone mass in later life and therefore protection against the development of osteoporosis.[16]

Goat milk supplementation also affected the biomechanical properties of the bones by increasing the maximum load that the bones could withstand as well as the load needed to break them (Table 6). Bone-breaking strength (N) is the maximum power that is required to break bone by the three-point bending method. Breaking energy (J) is an integration value of the force required to make the break. Measured stiffness (N mm^{-2}) reflects the amount a bone can bend under an applied load without

permanent deformation (plasticity). Breaking strength reflects the mineral content of bone as well as the protein component, while breaking energy is thought to reflect bone collagen content.[17,18] Differences in breaking strength are linearly related to femur calcium content.[17,18] The only significant effects observed in the present trial were on maximum load and break load, and it is therefore feasible that bone calcium content was affected by supplementation with Formula 1. This is supported by the increased thickness and width of the femurs from the rats fed Formula 1 compared with the control rats (data not shown). There was no observed effect on bone stiffness, and it is possible that there was no specific effect on bone collagen by the goat milk supplement.

Aliaga *et al*.[19] showed that goat milk improves mineral deposition in the femur, sternum and *Longissimus dorsi* muscle in rats fed a goat milk diet as opposed to a control (non-milk) or cow milk diet. Similarly, Campos *et al*.[10] showed that goat milk improves mineral retention in rats with intestinal resection. In that study, goat milk was compared with a non-milk diet as well as cow milk. Goat milk may contain factors that assist with mineral absorption, which in turn may affect the attainment of peak bone mass and OVX-induced bone loss. Lipids have also been shown to affect calcium and mineral absorption to a certain degree depending on the level of unsaturation.[20] Barrionuevo *et al*.[8,21] suggested that minerals were better absorbed from goat milk than from cow milk owing to the naturally higher levels of medium-chain fatty acids, which would affect intestinal absorption. Similarly, Lopez-Aliaga *et al*.[7] hypothesised that the greater bioavailability of calcium from goat milk could be due to the higher medium-chain fatty acid content and higher vitamin D (250 ng per 100 mL) and vitamin C contents when compared with cow milk. In the present trial the protein and total fat levels were balanced across all treatment groups, but the fat composition was different. The soy diets contained a mixture of saturated and unsaturated vegetable oils, while Formula 1 contained natural goat milk fat and possibly had a different profile of medium-chain fatty acids.

The goat milk formulation (Formula 1) used in both trials contained added prebiotic Beneo Synergy 1 (a specific, patented combination of chicory inulin and oligofructose) as well as probiotics (Table 1). Several studies in rats have shown that inulin-type fructans increase intestinal calcium absorption.[22–25] Scholz-Ahrens *et al*.[11,26] also showed that various doses of dietary inulin increased the amount of calcium absorbed and bone mineral density in ovariectomised rats. The addition of pre- and probiotics to the goat milk formula could have contributed to the mineral absorption and retention observed in both studies 1 and 2.

CONCLUSION

These trials demonstrate that goat milk, when used as a source of proteins and minerals and supplemented with the prebiotic Beneo Synergy 1, improves mineral absorption and retention in growing rats. This improved peak bone mass attained after adolescence and was maintained post-OVX in the presence of sufficient dietary calcium. These results support and extend observations from earlier studies, showing that goat milk formulations are able to promote growth and skeletal mineralisation in children.[4,5]

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