

# The effect of formulated goats' milk on calcium bioavailability in male growing rats

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## Abstract

**BACKGROUND:** There are two main proteins in milk; whey and casein. Casein contains casein phosphopeptides (CPP), which are released on digestion of the milk. These may increase calcium solubility by binding calcium in the small intestine. Thus increasing casein in the diet may help to stimulate bioavailability of calcium and increase bone density. The present study tested this hypothesis in growing male rats fed diets containing three different concentrations of casein from goat milk.

**RESULTS:** Rats fed the diet containing no casein had significantly lower calcium absorption when compared to rats fed the diets that contained 80% and 57% of goat milk protein as casein; however, no significant difference was observed between rats fed diets with 80% and 57% casein. The varying amounts of casein had no effect on mineral uptake or retention in the femur. Biomechanical testing and mineral analysis of the femurs showed no differences between diet groups. The mechanism to explain this lack of retention remains unclear.

**CONCLUSION:** The diets containing 80% and 57% of goat milk protein as casein delivered increased calcium absorption compared to the diet containing no casein, suggesting a minimum level of casein is needed to optimize calcium absorption from goat milk.

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**Keywords:** goat milk; casein phosphopeptides; calcium absorption; DEXA

## INTRODUCTION

Milk is the major source of dietary calcium in the western world, and is generally seen as a positive factor in improving bone health. The composition of milk is believed to have optimal ratios of calcium and phosphorus (2:1) for the enhanced absorption of minerals, and the bioactive peptides found in milk protein are believed to play an active role in the bioavailability of calcium for absorption. Casein, one of the main proteins found in goat milk, contains the bioactive protein casein phosphopeptides (CPP). It has been suggested that CPP increase calcium solubility<sup>1,2</sup> by binding to calcium in the small intestine, where passive calcium absorption takes place.<sup>2,3</sup>

Goat milk fat may also contribute to increased calcium absorption. Previous work has indicated greater absorption of calcium with diets containing goat milk fat compared to goat skim milk with added vegetable oils.<sup>4</sup> It has been suggested that this is in part due to a higher percentage of the more readily absorbed medium chain-fatty acids found in whole goat milk.<sup>4-6</sup>

The present study investigated the effect of different ratios of whey and casein and milk fat in goat milk formulations on calcium bioavailability in growing male rats.

## METHODS

### Animals

Seventy-two male Sprague Dawley rats, aged 3 weeks, were randomly allocated into six treatment groups. The rats were housed in individual shoebox cages and weighed weekly. Rats

from all treatment groups were kept at a constant 22 °C (±2 °C) in a light-controlled 12/12 h light/dark lighting regime.

### Diets

All rats were maintained *ad libitum* on a semi-synthetic diet, with 50% of protein derived from egg albumin and the rest from goat milk. The goat milk protein consisted of three different ratios of 20:80 (diets 1 and 4), 43:57 (diets 2 and 5) or 83:17 (diets 3 and 6) whey:casein. Diets 1-3 had milk fat, whereas diets 4-6 contained a mixture of palm, coconut and soybean oil in proportions used in previous work at this institute calculated to provide a similar fatty acid profile to milk fat.<sup>4</sup> All diets had 10 g kg<sup>-1</sup> corn oil to prevent essential fatty acid (EFA) deficiency. Vitamin and minerals known to affect bone growth and mineral accretion were balanced where possible, including calcium (0.5%), phosphorus (0.3%) and fat (~9%). All minimum dietary needs for growing rats were met as according to AIN93G (National Research Council, 1995). Goat whole milk, goat skim milk, goat whey protein concentrate and goat fat powder were supplied by the Dairy Goat Co-operative,

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Hamilton, New Zealand. All rats were given *ad libitum* access to deionized water.

### Metabolism experiment

The rats were housed in metabolism cages for 5 days at 7 weeks of age (week 4 of dietary treatment). Daily food intakes were measured, and urine and faeces collected for calcium and magnesium content. The quantity of calcium and magnesium absorbed and retained was determined by measuring total intake from diet and lost minerals via faeces and urine.

### Dual energy X-ray absorptiometry (DEXA) scans

*In vivo* DEXA scans were performed on anaesthetized rats at 8 and 12 weeks of age (week 5 and 9 of dietary treatment). DEXA was used to determine total body composition for comparisons between diets. Body composition is defined by whole body bone mineral density ( $\text{g cm}^{-2}$ ) (BMD), bone mineral content (g) (BMC), fat mass (g), lean mass (g), mass of total body (g) and percentage of fat. Sub-regional scans of mineral deposited in the L1–L4 lumbar spine and femur were measured using BMD and BMC.

Bone mineral measurements were taken using a Hologic Discovery A bone densitometer (Bedford, MA, USA). On each day that scans were undertaken, a quality control (QC) scan was taken to ensure that its precision met the required DEXA manufacturer's coefficient of variation (CV). The CV for the QC data was 0.98–1.01%. Each rat underwent three regional high-resolution scans of the spine and left and right femurs. Rats were positioned supine with right angles between the spine and femur, and between femur and tibia.

Coefficient of variance for the femurs ranged between 0.60% and 1.20% without and with repositioning between scans. These values ranged between 0.61% and 1.38% for the lumbar spine.

### Euthanasia and tissue collection

The rats were euthanized by exsanguination under anesthesia. Fasting blood samples were collected and the serum stored at  $-80^{\circ}\text{C}$ . The lumbar spine and both femurs were removed by simple dissection and stored at  $-20^{\circ}\text{C}$  pending further analysis.

### Bone marker

Blood serum was tested for determination of C-terminal telopeptides of type 1 collagen (CTX). This test was run using Ratlaps Elisa kits, supplied by Nordic Bioscience Diagnostic A/S, Herlev, Denmark.

### Ashing and calcium content of femur

All adherent soft tissue was removed from left femurs and wet weights and lengths were recorded. Femurs were dried overnight at  $105^{\circ}\text{C}$ , then ashed at  $660^{\circ}\text{C}$  overnight, and both dry and ash weights were recorded. Ashed bones were dissolved in 2 mL of  $6\text{ mol L}^{-1}$  hydrochloric acid (HCl) and then analyzed using a Vista model inductively coupled plasma optical emission spectroscopy (ICPOES) machine (Varian, Palo Alto, CA, USA) for calcium analysis.

### Biomechanical properties of the femur

Right femurs were thawed and any adherent soft tissue removed. The midpoint of the femur was marked and the width and thickness recorded using Mitutoyo vernier calipers ( $\pm 0.02\text{ mm}$ ).

The bones were warmed to  $23^{\circ}\text{C}$  for 30 min in a water bath prior to mechanical testing. This temperature remained constant throughout the testing procedure. The biomechanical testing was done using a texture analyzer (Ezi test series, Shimadzu, Kyoto, Japan) and results interpreted using Shimadzu WinAGSLite 2000 software. The femurs were placed on the three point-bending jig with a fixed 12 mm span, and subjected to a constant deformation rate of  $50\text{ mm min}^{-1}$  with a 500 N load cell.

### Statistical analysis

Body weights and food intake were analyzed with a one-way ANOVA repeated-measures model using SYSTAT 11.0. DEXA results were analyzed by one-way ANOVA for each time point (8 and 12 weeks of age). DEXA results were then analyzed for the change in lumbar spine BMC and BMD between the two time points (8 and 12 weeks of age), before running a general linear model (GLM) comparing the diets. As the lumbar spine BMC and BMD were nonlinear the raw data were log transformed. Any significant differences identified were examined using a Tukey HSD post hoc pairwise comparison test. Biomechanics, femur size measurements, bone ash and calcium content results were analyzed using a one-way ANOVA. Results from CTx and some parameters measured from the metabolism experiment were nonlinear and therefore log transformed and a one-way ANOVA was used to investigate significance between groups. The metabolism data were pooled over the 3 days of the experiment. All results were expressed as the mean (standard error of the mean) and statistical significance was set at  $P < 0.05$ .

## RESULTS

Diet analysis shows balanced minerals, fats and proteins for the six formulated diets in the trial (Table 1). Examination of diet intake and rat bodyweights showed no significant differences between treatment groups throughout the trial.

### Mineral balance

The diets containing lowest casein had significantly lower absolute calcium absorption ( $P < 0.0005$ ) compared to those diets with 57% and 80% of the goat milk protein as casein, this is also reflected in fractional calcium absorption (Fig. 1). Intake of calcium did not differ between treatments; however, excretion levels in the non-casein diets were significantly greater. Diet 3 had significantly higher calcium excretion from the urine ( $P < 0.0005$ ) against all other diets, whereas diet 6 with lowest casein and no goat milk fat had significantly higher calcium excretion from the faeces ( $P < 0.0005$ ) against all other diets. Magnesium intake differed significantly between diets and no obvious pattern of effect was found. Diet 3 had significantly lower magnesium excretion from the faeces ( $P < 0.0005$ ) against all other diets except diet 6 (data not shown).

### DEXA

Investigation of changes between the two time points (8 and 12 weeks of age) showed a significant difference in BMC (d.f. 5,  $F 2.528$ ,  $P < 0.038$ ) and BMD (d.f. 5,  $F 2.383$ ,  $P < 0.048$ ) in the lumbar spine region (Fig. 2). Post hoc testing showed diet 5 had a significantly higher BMC ( $P < 0.036$ ) and BMD ( $P < 0.046$ ) than diet 4 in the lumbar spine region; no other diet pairs differed significantly. No significant differences were noted in the other parameters and body sites tested with bone densitometry, nor

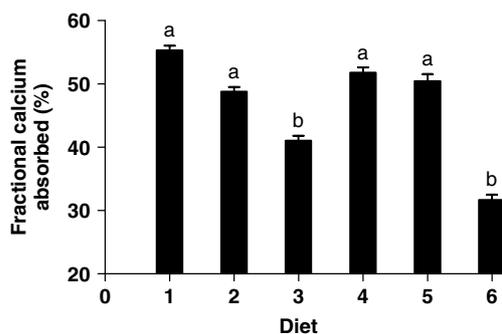
**Table 1.** Analysis of diet composition of the six formulated goat milk diets

Diet composition	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
N (%)	3.25	3.24	3.21	3.17	3.31	3.36
Crude protein (%)	20.33	20.26	20.09	19.78	20.67	20.97
Protein from casein (%) <sup>a</sup>	39	28	8	40	28	8
Dry matter (%)	94.04	94.12	94.31	93.90	92.47	94.43
Ash (%)	5.44	5.44	5.49	5.17	5.37	4.61
Fat (%)	10.04	10.13	9.48	8.81	8.59	9.08
Fat from milk fat (%) <sup>b</sup>	86	85	92	4	7	11
Carbohydrates (%)	58.23	58.29	59.25	60.13	57.84	57.32
Minerals						
Calcium (g kg <sup>-1</sup> )	5.1	5.3	5.8	5.4	5.5	6.3
Magnesium (g kg <sup>-1</sup> )	1.5	1.5	1.4	1.5	1.5	1.4
Phosphorus (g kg <sup>-1</sup> )	3.4	3.3	3.3	3.5	3.4	3.5

Diet 1, protein ratio 20:80 whey:casein with milk fat; diet 2, protein ratio 43:57 whey:casein with milk fat; diet 3, protein ratio 83:17 whey:casein with milk fat; diet 4, protein ratio 20:80 whey:casein with vegetable oils; diet 5, protein ratio 43:57 whey:casein with vegetable oils; diet 6, protein ratio 83:17 whey:casein with vegetable oils.

<sup>a</sup> Calculated from the amount of goat casein divided by the total protein from the dietary analysis.

<sup>b</sup> Calculated from the amount of goat milk fat divided by the total fat content from the dietary analysis.

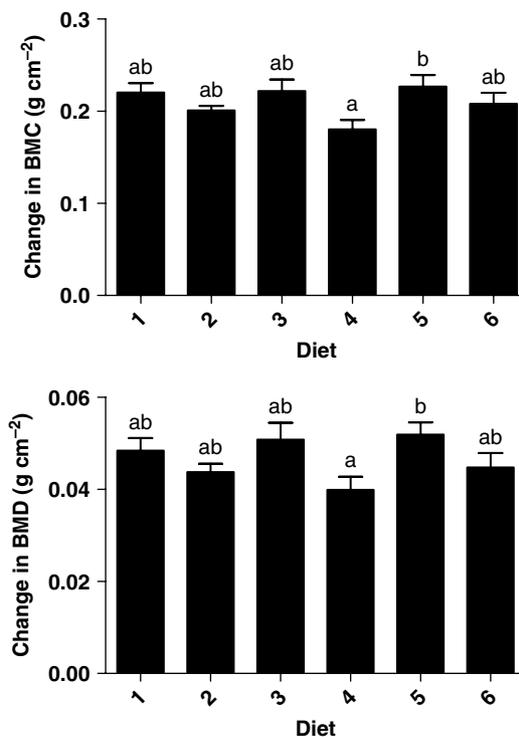


**Figure 1.** Fractional calcium absorption (%) from the metabolism trial at 7 weeks of age, for the six diets ( $n = 12$  for each diet). Diet 1, protein ratio 20:80 whey:casein with milk fat; diet 2, protein ratio 43:57 whey:casein with milk fat; diet 3, protein ratio 83:17 whey:casein with milk fat; diet 4, protein ratio 20:80 whey:casein with vegetable oils; Diet 5, protein ratio 43:57 whey:casein with vegetable oils; diet 6, protein ratio 83:17 whey:casein with vegetable oils. Values with different letters are significantly different ( $P < 0.05$ ) between diets.

were any differences found when the two time points were individually examined (data not shown).

**Ex vivo femur**

Examination of the femur *ex vivo* showed that the variations in whey and casein ratios had no significant effect on the size of the bone or on biomechanical strength, ash content or calcium content (Table 2).



**Figure 2.** Means and errors of means for the change in lumbar spine BMC and BMD between the two time points (8 and 12 weeks of age) for the six diets ( $n = 12$  for each diet). Diet 1, protein ratio 20:80 whey:casein with milk fat; diet 2, protein ratio 43:57 whey:casein with milk fat; diet 3, protein ratio 83:17 whey:casein with milk fat; diet 4, protein ratio 20:80 whey:casein with vegetable oils; diet 5, protein ratio 43:57 whey:casein with vegetable oils; diet 6, protein ratio 83:17 whey:casein with vegetable oils. Values with different letters are significantly different ( $P < 0.05$ ) between diets.

**CTx**

Investigations of bone markers for resorption revealed there were no significant differences found between serum concentration levels (ng mL<sup>-1</sup>) of CTx in the rats fed the six formulated diets (Table 2).

**DISCUSSION**

This study investigated various ratios of whey and casein in goat milk formulations and their subsequent effect on calcium bioavailability in growing male rats. Over the 9-week period of the trial the diets containing 80% and 57% of goat milk protein as casein delivered increased calcium and magnesium absorption compared to the diet containing only 17% casein. These results suggest that a minimum level of casein is needed to optimize calcium absorption from goat milk. The difference found between levels of casein and apparent calcium absorption seen in this trial could be explained by the theory examined by Erba *et al.*<sup>7</sup> In their study, Erba *et al.*<sup>7</sup> investigated different ratios of casein phosphopeptides and calcium on passive calcium transport in the small intestine, and suggested that there was an optimum ratio for increased absorption. However, the increased calcium absorption did not result in significant differences in bone mineral content or density throughout the trial. Nor was it reflected in *ex vivo* ash (mineral) content or calcium content of the femurs, suggesting that the varying ratios of protein tested did not affect mineral

**Table 2.** Size measurements, dry weight, ash content, calcium content and biomechanical testing for femurs, as well as concentration levels (ng mL<sup>-1</sup>) of serum C-terminal cross-linking telopeptide of type I collagen (CTx), for the six diets

	Diet 1 N = (12)	Diet 2 N = (12)	Diet 3 N = (12)	Diet 4 N = (11)	Diet 5 N = (12)	Diet 6 N = (11)	F-stat	P-value
Femur length (mm)	35.47 ± 0.2	35.54 ± 0.2	35.29 ± 0.3	35.61 ± 0.2	35.26 ± 0.2	35.55 ± 0.3	0.378	NS
Femur thickness (mm)	3.51 ± 0.1	3.61 ± 0.1	3.49 ± 0.1	3.48 ± 0.1	3.58 ± 0.1	3.54 ± 0.1	0.793	NS
Femur width (mm)	4.67 ± 0.1	4.77 ± 0.1	4.75 ± 0.1	4.71 ± 0.1	4.79 ± 0.1	4.67 ± 0.1	0.357	NS
Ash (%)	0.48 ± 0.01	0.48 ± 0.01	0.48 ± 0.01	0.47 ± 0.01	0.47 ± 0.01	0.47 ± 0.01	0.286	NS
Calcium (mg g <sup>-1</sup> )	188.80 ± 4.7	196.99 ± 4.1	196.83 ± 4.0	192.84 ± 5.7	189.16 ± 3.9	187.65 ± 2.5	0.957	NS
CTx (ng mL <sup>-1</sup> )	14.882 ± 0.66	17.356 ± 1.20	16.521 ± 0.69	15.658 ± 1.02	15.447 ± 0.08	15.219 ± 0.71	1.086	NS
Max Load (N)	183.06 ± 4.3	180.59 ± 4.5	176.01 ± 4.8	172.89 ± 3.5	175.59 ± 5.4	176.81 ± 6.2	0.573	NS
Break load (N)	182.03 ± 4.5	170.40 ± 6.9	171.57 ± 5.0	171.95 ± 3.7	167.92 ± 9.9	175.79 ± 5.9	0.628	NS
Elasticity (N mm <sup>-2</sup> )	545.26 ± 39.9	472.03 ± 26.5	526.12 ± 33.9	528.35 ± 41.3	483.85 ± 30.9	540.38 ± 52.3	0.662	NS
Energy (J)	0.186 ± 0.01	0.187 ± 0.01	0.173 ± 0.01	0.174 ± 0.01	0.177 ± 0.01	0.166 ± 0.01	0.825	NS

Values are mean ± error of means, with statistical significance set at  $P < 0.05$ . NS, no significant difference. Diet 1, protein ratio 20:80 whey:casein with milk fat; diet 2, protein ratio 43:57 whey:casein with milk fat; diet 3, protein ratio 83:17 whey:casein with milk fat; diet 4, protein ratio 20:80 whey:casein with vegetable oils; diet 5, protein ratio 43:57 whey:casein with vegetable oils; diet 6, protein ratio 83:17 whey:casein with vegetable oils.

uptake or retention into the femur during the period of this trial. This was also the case with the biomechanical results, indicating that there was no effect on bone strength.

This appears to be in line with some reported findings, where studies investigating casein levels in cows' milk noted that changes found in increased calcium absorption were temporary. Bennett *et al.*<sup>8</sup> found that a high-casein diet enhanced calcium absorption, although the efficiency of the calcium absorption had reduced within 2 weeks, whereas other studies found no effect at all. Howe and Beecher<sup>9</sup> found that, on a diet of 9% calcium and 3.5% phosphorus, an increase in protein as casein from 25% to 45% did not have an effect on calcium absorption in young growing rats. Yuan and Kitts<sup>10</sup> found a decrease in feed efficiency, femur calcification and physical measurements from the group fed a low-casein diet (60 g kg<sup>-1</sup>) compared to the control group on 200 g kg<sup>-1</sup> of casein. They did not find any difference, however, between levels of casein and calcium absorption. Allen and Hall<sup>11</sup> investigated the effects of two different levels of protein (18% and 36%) on calcium absorption, with casein as the protein source. They found that there was no difference in absorption, bone turnover or bone calcium.

One study found referred to the effects of CPP on calcium absorption from goat milk in rats. This study compared the effects of adding CPP from bovine and caprine sources to calcium fortified milk. That study concluded that both sources of CPP increased calcium absorption when added to calcium fortified milks.<sup>12</sup>

The vegetable oils used as a fat source in the diets may have also reduced mineral absorption compared to the diets with whole goat milk. Diet 6, containing the highest levels of vegetable oils, had the lowest levels of calcium absorption in the study. Kruger *et al.*<sup>4</sup> using the same mix of vegetable oils found that the diet containing whole goat milk had significantly greater absorption of calcium than the diet containing goat skim milk powder and vegetable oils. They theorized, as did López Aliaga *et al.*,<sup>13</sup> that this is in part due to a higher percentage of the more readily absorbed medium-chain fatty acids found in goat milk. This theory was also shared by Nestares *et al.*<sup>14</sup> on the bioavailability of magnesium, where they found that anaemic rats fed goat milk had greater mineral absorption. Oddly, in terms of bone mineral retention the only significant difference found was between two diets using

vegetable oil as their fat source. Although neither diet was significantly different from any of the other diets, it would suggest that during the latter stages of growing male rats a casein level of 57% was more advantageous than 80% in the lumbar spine. However, any differences in BMC and BMD were lost by peak bone growth.

However, this still leaves the question concerning the lack of calcium retention in bone in the rats with greater calcium absorption. It could be suggested that the mechanisms that allow for greater calcium absorption became less efficient over time, allowing for the less bioavailable diets to catch up, thereby introducing balanced results between the diets tested.<sup>8</sup>

## CONCLUSION

The diets containing 80% and 57% goat milk protein as casein delivered increased calcium absorption compared to the diet containing no casein, suggesting a minimum level of casein is needed to optimize calcium absorption from goat milk. However, increased calcium absorption did not appear to impact mineral uptake or retention in the femur within growing male rats. The mechanism to explain this lack of retention remains unclear.

## REFERENCES

- 1 Lee Y, Noguchi T and Naito H, Phosphopeptides and soluble calcium in the small intestine of rats given a casein diet. *Br J Nutr* **43**:457–467 (1980).
- 2 Sato R, Noguchi T and Naito H, Casein phosphopeptide (CPP) enhances calcium absorption from the ligated segment of rat small intestine. *J Nutr Sci Vitaminol* **32**:67–76 (1986).
- 3 Tsuchita H, Toshiyuki G, Shimizu T, Yonehara Y and Kuwata T, Dietary casein phosphopeptides prevent bone loss in aged ovariectomized rats. *J Nutr* **126**:86–93 (1996).
- 4 Kruger M, Chua W, Darragh A, Booth C, Prosser C and Lowry D, Impact of goat milk powdered formulations on mineral absorption, peak bone mass and bone loss due to ovariectomy in rats. *J Sci Food Agr* **88**:1080–1090 (2008).
- 5 Haenlein G, Goat milk in human nutrition. *Small Rumin Res* **51**:155–163 (2004).
- 6 Campos M, Lopez-Aliaga I, Alferrez M, Nestares T and Barrionuevo M, Effects of goats' or cows' milk on nutritive utilization of calcium and phosphorus in rats with intestinal resection. *Br J Nutr* **90**:61–67 (2003).

- 7 Erba D, Ciappellano S and Testolin G, Effect of the ratio of casein phosphopeptides to calcium (w/w) on passive calcium transport in the distal small intestine of rats. *Nutrition* **18**:743–746 (2002).
- 8 Bennett T, Desmond A, Harrington M, McDonagh D, Fitzgerald R and Flynn A, The effect of high intakes of casein and casein phosphopeptide on calcium absorption in the rat. *Br J Nutr* **83**:673–680 (2000).
- 9 Howe J and Beecher G, Effect of dietary protein and phosphorus levels on calcium and phosphorus metabolism of the young fast growing rat. *J Nutr* **111**:708–720 (1981).
- 10 Yuan Y and Kitts D, Calcium absorption and bone utilization in spontaneously hypertensive rats fed on native and heat-damaged casein and soya-bean protein. *Brit J Nutr* **71**:583–603 (1994).
- 11 Allen L and Hall T, Calcium metabolism, intestinal calcium-binding protein, and bone growth of rats fed high protein diets. *J Nutr* **108**:967–972 (1978).
- 12 Mora-Gutierrez A, Farrell H, Attaie R, McWhinney V and Wang C, Influence of bovine and caprine casein phosphopeptides differing in  $\alpha_{s1}$ -casein content in determining the absorption of calcium from bovine and caprine calcium-fortified milks in rats. *J Dairy Res* **74**:356–366 (2007).
- 13 López Aliaga I, Alférez M, Barrionuevo M, Lisbona F and Campos M, Influence of goat and cow milk on the digestive and metabolic utilization of calcium and iron. *J Physiol Biochem* **56**:201–208 (2000).
- 14 Nestares T, Diaz-Castro J, Alférez M, López Aliaga I, Barrionuevo M and Campos M, Calcium-enriched goat milk, in comparison with similarly enriched cow milk, favours magnesium bioavailability in rats with nutritional ferropenic anaemia. *J Sci Food Agr* **88**:319–327 (2008).