Table 3-1. Composition of the experimental diets (g/100 g).

Ingredients		CE	CH	CHVC	CHCa
Cornstarch		41.3192	41.3192	39.8192	39.0692
Casein		14	14	14	14
D-cornstarch		15.5	15.5	15.5	15.5
Sucrose		10	10	10	10
Soybean oil		4	4	4	4
Chitosan		—	10	10	10
Cellulose		10	—	—	—
Mineral mixture(Ca Free)[*1]		3.5	3.5	3.5	3.5
Vitamin mixure[*2]		1	1	1	1
L-Cystine		0.18	0.18	0.18	0.18
Choline bitartrate		0.25	0.25	0.25	0.25
T-butyhydroquinone		0.0008	0.0008	0.0008	0.0008
$CaCO_3$		0.25	0.25	0.25	2.5
Vitamin C[*3]		—	—	1.5	—
Total		100	100	100	100
Measured value of Ca and P in each diet (mg/ 100g diet)	Ca[*4]	95.8	99.1	99.1	1071.6
	P[*5]	537	374	383	383

Composition of diets are prepared according to the AIN-93M prescription. (Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr. 1993;123(11):1939-51.)

[*1]: AIN-93M mineral mixture without Ca. [*2]: AIN-93M vitamin mixture. [*3]: L(+)-Ascorbic Acid Sodium Salt (Wako, Japan) were used. [*4]: by atomic absorption spectrometry. [*5]: by molybdenum blue method. CE: cellulose, CH: chitosan, CHVC: chitosan with vitamin C, CHCa: chitosan with 1%Ca.

Table 3-2. Body weight and food intake of OVX rats fed the experimental diets for 6wk.

	CE	CH	CHVC	CHCa
Initial body weight (g)	188±3	193±4	192±4	192±4
Final body weight (g)	230±4	232±5	230±3	239±3
Body weight gain (g)	42±3	40±3	38±3	47±2+
Food intake (g/ 6wk)	570±6	576±11	574±12	644±11***###+++

Values are means ± SE (CE: n=6, CH, CHVC, CHCa: n=7). ***: P<0.001 vs CE. ###: P<0.001 vs CH. +: P<0.05, +++: P<0.001 vs CHVC. CE: cellulose, CH: chitosan, CHVC: chitosan with vitamin C, CHCa: chitosan with 1%Ca.

Table 3-3. Organ weights (g) of OVX rats fed the experimental diets for 6 wk.

	CE	CH	CHVC	CHCa
Liver	5.66±0.17	5.23±0.20	5.45±0.07	5.20±0.12
Abdominal fat	7.99±0.48	7.53±1.01	6.53±0.48	8.02±0.55
Uterus	0.12±0.02	0.10±0.01	0.11±0.02	0.10±0.01

Values are means ± SE (CE: n=6, CH, CHVC, CHCa: n=7). CE: cellulose, CH: chitosan, CHVC: chitosan with vitamin C, CHCa: chitosan with 1%Ca.

Table 3-4. Calcium and phosphorus balances of OVX rats fed the experimental diets for 6wk.

	CE	CH	CHVC	CHCa
Ca (mg/ 4d)				
Intake	46.4±1.3	52.4±2.1	51.5±1.0	612.1±14.8
Fecal excretion	28.7±1.6	29.7±1.9	27.7±1.4	502.7±13.9
Urinary excretion	5.2±0.3	12.6±0.6***	8.8±0.7**##	27.2±1.1
Total excretion	33.9±1.6	42.4±2.0	36.5±1.5	529.9±14.4
Absorption	17.8±0.9	22.7±1.8	23.8±1.6	109.4±4.7
Absorption rate(%)	38.4±2.2	43.3±2.8	46.2±2.8*	17.9±0.8
Retention	12.6±0.8	10.1±1.8	15.0±1.6	82.2±4.9
Retention rate(%)	27.3±2.0	19.0±3.1*	29.1±3.0##	13.4±0.8
P (mg/ 4d)				
Intake	260.5±7.1	197.9±8.1***	199.3±3.7***	218.7±5.3***#+
Fecal excretion	32.4±2.5	42.3±2.1*	44.3±2.6*	130.2±4.2***###+++
Urinary excretion	82.8±2.4	83.8±3.3	82.7±2.8	12.6±1.2***###+++
Total excretion	115.2±2.9	126.1±4.4	127.0±4.5	142.8±3.9 ***###+++
Absorption	228.2±6.1	155.6±7.5***	155.0±2.8***	88.6±4.9 ***###+++
Absorption rate(%)	87.6±0.8	78.5±1.1***	77.8±1.1***	40.4±1.8 ***###+++
Retention	145.4±5.2	71.8±4.8***	72.3±3.0***	75.9±4.4***
Retention rate(%)	55.8±0.8	36.1±1.4***	36.4±1.6***	34.7±1.5***

Values are means ± SE (CE: n=6, CH, CHVC, CHCa: n=7). ***: P<0.001, **: P<0.01, *: P<0.05 vs CE. ###: P<0.001, ##: P<0.01 vs CH. +++: P<0.001, +: P<0.05 vs CHVC. Feces and urine were collected for 4 days. Absorption: Intake-fecal excretion. Absorption rate(%): {(Intake-fecal excretion)/intake} ×100. Retention: Intake-total excretion. Retention rate(%): {(Intake-total excretion)/intake} ×100. CE: cellulose, CH: chitosan, CHVC: chitosan with vitamin C, CHCa: chitosan with 1%Ca.

Table 3-5. Effects of the experimental diets on serum mineral levels in OVX rats

	CE	CH	CHVC	CHCa
Calcium (mg/dL)	10.4±0.1	9.6±0.3*	9.4±0.2*	9.7±0.3
Phosphate (mg/dL)	4.0±0.8	6.7±1.1*	8.0±0.1**	7.5±0.6*
Magnesium (mg/dL)	2.3±0.1	2.0±0.1	2.1±0.1	2.0±0.1

Values are means ± SE (CE: n=6, CH, CHVC, CHCa: n=7). *: $P<0.05$, **: $P<0.01$ vs CE. CE: cellulose, CH: chitosan, CHVC: chitosan with vitamin C, CHCa: chitosan with 1%Ca.

Table 3-6. Bone-related metabolic parameters in serum and urine of OVX rats fed the experimental diets for 6wk.

	CE	CH	CHVC	CHCa
Serum				
Alkaline phosphatase (IU/L)	142.7±20.1	67.8±2.9***	68.2±2.0***	70.2±2.4***
Urine				
DPD/creatinine (nmol/mmol)	418.5±19.0	447.0±26.8	474.7±13.3	312.9±29.6**###+++

Values are means ± SE (CE: n=6, CH, CHVC, CHCa: n=7). **: $P<0.01$, ***: $P<0.001$ vs CE. ###: $P<0.001$ vs CH. +++: $P<0.001$ vs CHVC. DPD: deoxypyridinoline. CE: cellulose, CH: chitosan, CHVC: chitosan with vitamin C, CHCa: chitosan with 1%Ca.

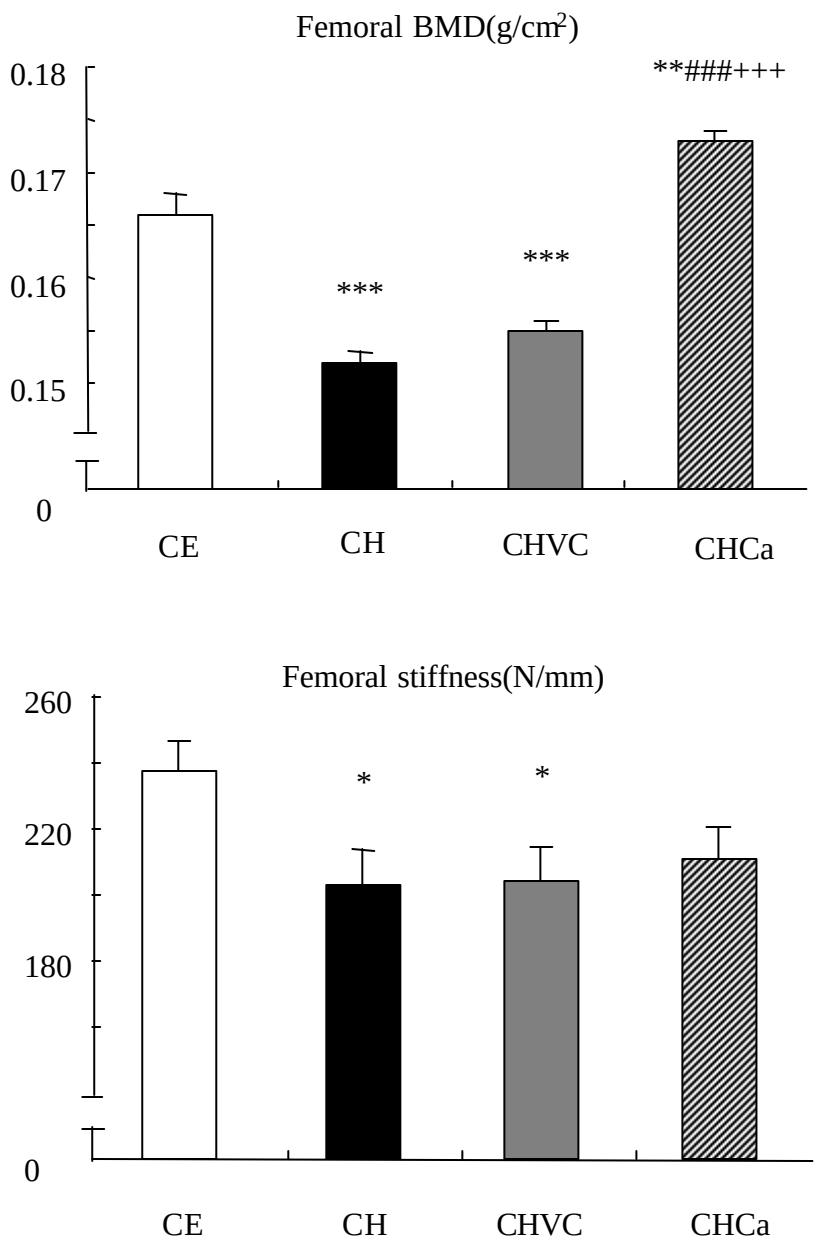

Fig. 3-1. Effects of the experimental diets on femoral BMD and stiffness in OVX rats. Values are means ± SE (CE: n=6, CH, CHVC, CHCa: n=7). *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$ vs CE. ###: $P<0.001$ vs CH. +++: $P<0.001$ vs CHVC. CE: cellulose, CH: chitosan, CHVC: chitosan with vitamin C, CHCa: chitosan with 1%Ca.

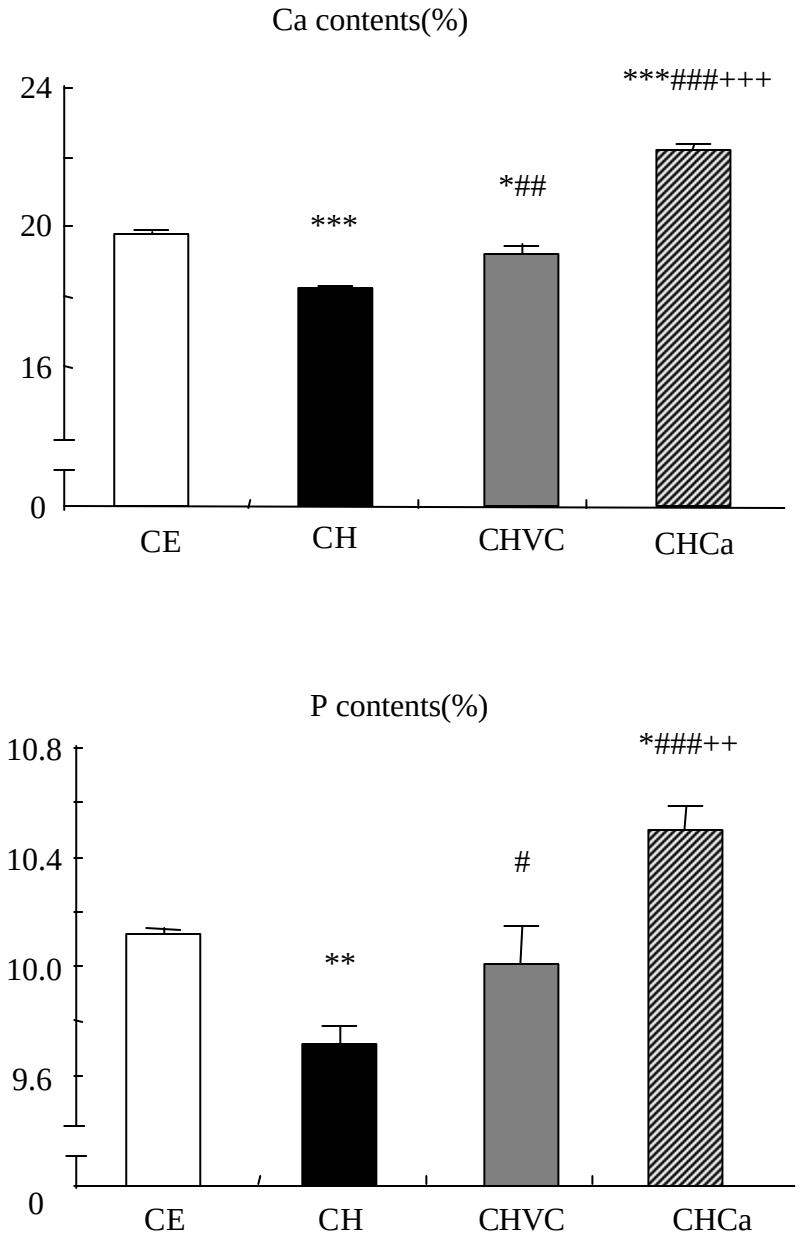

Fig. 3-2. Effects of the experimental diets on femoral Ca and P contents in OVX rats. Values are means ± SE (CE: n=6, CH, CHVC, CHCa: n=7). *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$ vs CE. #: $P<0.05$, ##: $P<0.01$, ###: $P<0.001$ vs CH. ++: $P<0.01$, +++: $P<0.001$ vs CHVC. Femoral Ca contents (%): {Ca (mg)/dry femur weight (mg)} × 100. Femoral P contents (%): {P (mg)/dry femur weight (mg)} × 100. CE: cellulose, CH: chitosan, CHVC: chitosan with vitamin C, CHCa: chitosan with 1%Ca.

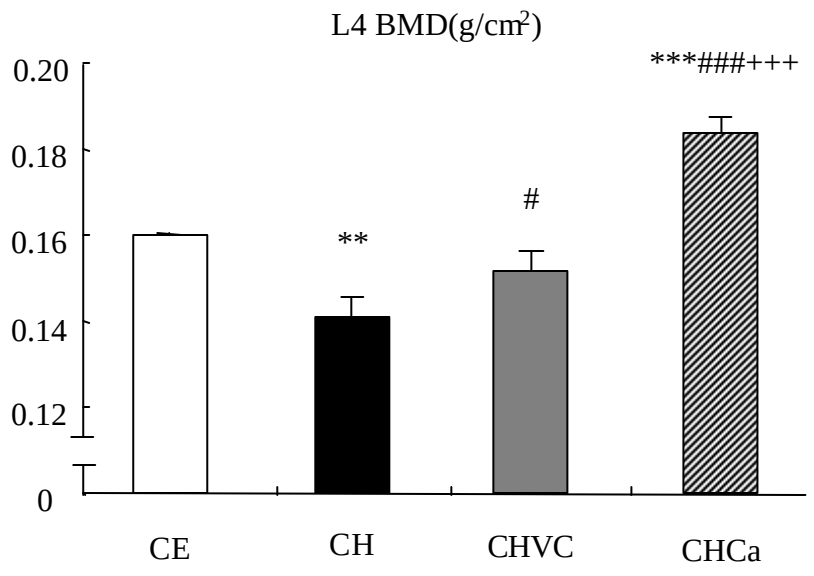

Fig. 3-3. Effects of the experimental diets on L4 BMD and stiffness in OVX rats. Values are means ± SE (CE: n=6, CH, CHVC, CHCa: n=7). **: $P<0.01$, ***: $P<0.001$ vs CE. #: $P<0.05$, ###: $P<0.001$ vs CH. +++: $P<0.001$ vs CHVC. L4: the fourth lumbar vertebrae. BMD: bone mineral density. CE: cellulose, CH: chitosan, CHVC: chitosan with vitamin C, CHCa: chitosan with 1%Ca.

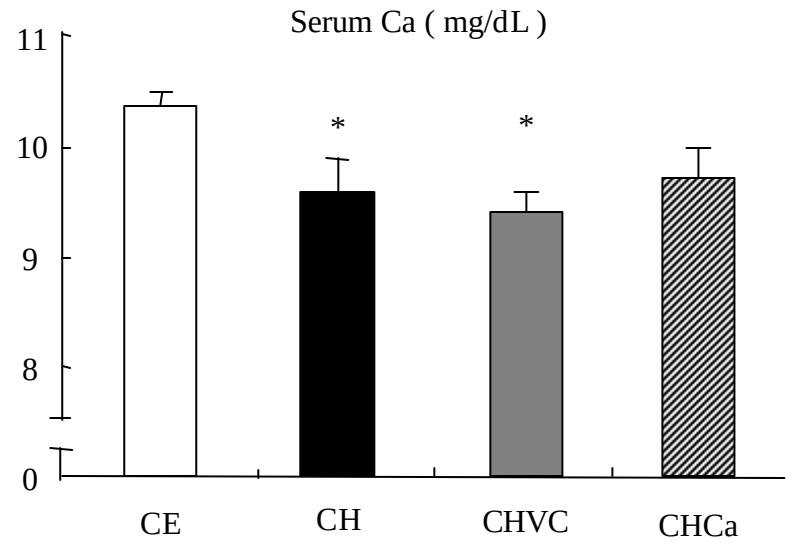

Fig. 3-4. Effects of the experimental diets on serum Ca and P levels in OVX rats. Values are means ± SE (CE: n=6, CH, CHVC, CHCa: n=7). *: $P<0.05$, **: $P<0.01$ vs CE. CE: cellulose, CH: chitosan, CHVC: chitosan with vitamin C, CHCa: chitosan with 1%Ca.

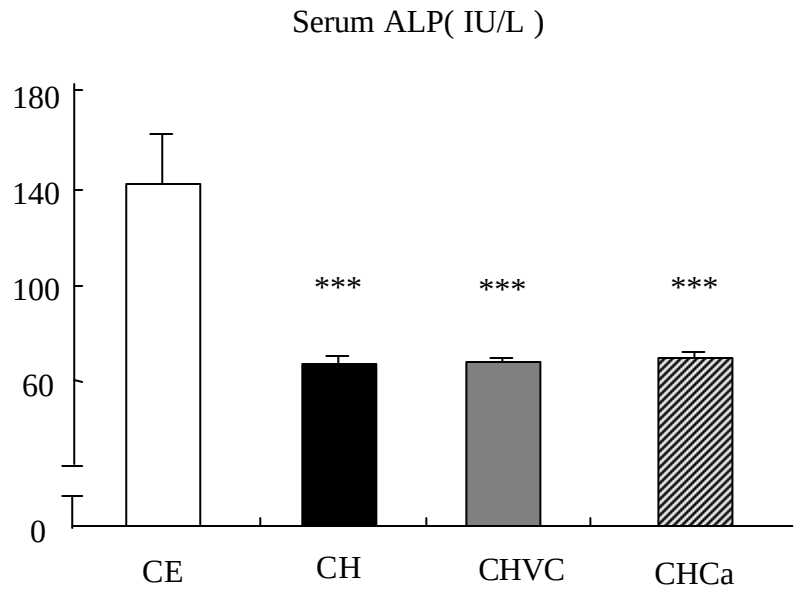

Fig. 3-5. Effects of the experimental diets on serum Alkaline phosphatase and urinary DPD levels in OVX rats. Values are means ± SE ((CE: n=6, CH, CHVC, CHCa: n=7). **: $P<0.01$, ***: $P<0.001$ vs CE. ###: $P<0.001$ vs CH. +++: $P<0.001$ vs CHVC. DPD: deoxypyridinoline. CE: cellulose, CH: chitosan, CHVC: chitosan with vitamin C, CHCa: chitosan with 1%Ca.

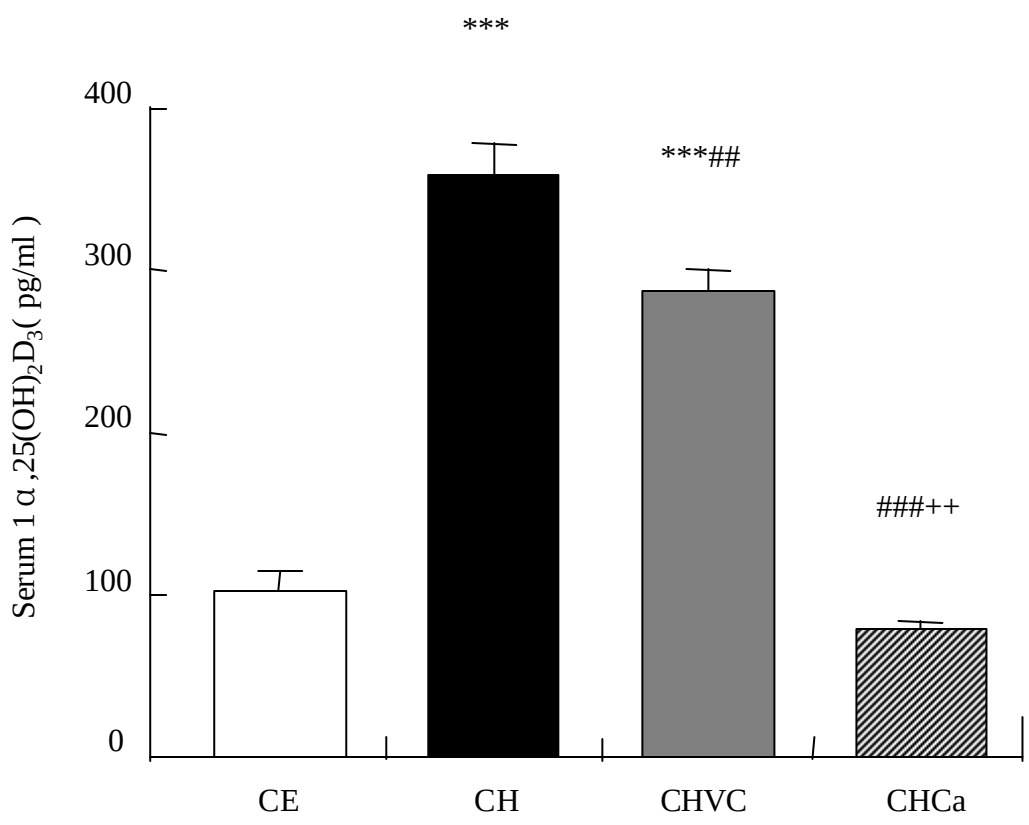

Fig. 2-6. Effects of several diets on serum Serum 1 α ,25(OH)$_2$D$_3$(pg/ml) levels in OVX rats. Values are means ± SE ((CE: n=6, CH, CHVC, CHCa: n=7). ***: P<0.001 vs CE. ##: P<0.01, ###: *P*<0.001 vs CH. ++: P<0.01 vs CHVC. CE: cellulose, CH: chitosan, CHVC: chitosan with vitamin C, CHCa: chitosan with 1%Ca.

Table 2-7. Biochemical parameters in serum and urine of OVX rats fed the experimental diets for 6wk.

	CE	CH	CHVC	CHCa
Serum				
Alkaline phosphatase (IU/L)	142.7±20.1	67.8±2.9***	68.2±2.0***	70.2±2.4***
PTH (pg/mL)	26.2±9.9	46.2±6.3	—	—
1,25-Dihydroxyvitamin D(pg/mL)	102.4±12.7	359.7±18.6***	286.8±13.6***##	78.7±5.2###++
Urine				
Dpd/creatinine (nmol/mmol)	418.5±19.0	447.0±26.8	474.7±13.3	312.9±29.6**###+++

Values are means ± SE (CE: n=6, CH, CHVC, CHCa: n=7). **: $P<0.01$, ***: $P<0.001$ vs CE. ##: $P<0.01$, ###: $P<0.001$ vs CH. ++: $P<0.01$, +++: $P<0.001$ vs CHVC. PTH: parathyroid hormone, DPD: deoxypyridinoline. CE: cellulose, CH: chitosan, CHVC: chitosan with vitamin C, CHCa: chitosan with 1%Ca.

Table 2-4. Effect of the experimental diets on bone properties of femurs and the fourth lumbar vertebrae (L4) in OVX rats.

	CE	CH	CHVC	CHCa
Femoral BMD	0.166±0.002	0.152±0.001***	0.155±0.001***	0.173±0.001**###+++
Femoral Stiffness	237.3±9.3	203.1±10.3*	204.2±10.2*	211.1±9.2
Femoral Ca content(%)	19.8±0.1	18.3±0.1***	19.2±0.3*##	22.2±0.2***###+++
Femoral P content (%)	10.1±0.0	9.7±0.1**	10.0±0.1#	10.5±0.1*###++
L4 BMD	0.160±0.000	0.141±0.005**	0.152±0.004#	0.184±0.004***###+++
L4 Stiffness	325.6±35.3	241.7±25.5	310.8±47.2	305.2±19.7

Values are means ± SE (CE: n=6, CH, CHVC, CHCa: n=7). *: P<0.05, **: P<0.01, ***: P<0.001 vs CE. #: P<0.05, ##: P<0.01, ###: P<0.001 vs CH. ++: P<0.01, +++: P<0.001 vs CHVC. Femoral Ca contents: {Ca (mg)/dry femur weight (mg)} ×100. Femoral P contents: {P (mg)/dry femur weight (mg)} ×100. BMD(g/cm^2): Bone mineral density. L4: the fourth lumbar vertebrae.