

Table 2-1-1. Composition of the experimental diets (g/100 g).

Ingredients		CE	CH
Cornstarch		41.3192	41.3192
Casein		14	14
D-cornstarch		15.5	15.5
Sucrose		10	10
Soybean oil		4	4
Chitosan			10
Cellulose		10	
Mineral mixture(Ca Free) <sup>*1</sup>		3.5	3.5
Vitamin mixture <sup>*2</sup>		1	1
L-Cystine		0.18	0.18
Choline bitartrate		0.25	0.25
T-butylhydroquinone		0.0008	0.0008
CaCO <sub>3</sub>		0.25	0.25
Total		100	100
Measured value of Ca and P in each diet ( mg/ 100g diet)	Ca <sup>*3</sup>	95.8	99.1
	P <sup>*4</sup>	537	374

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.)

<sup>\*1</sup>: AIN-93M mineral mixture without Ca. <sup>\*2</sup>: AIN-93M vitamin mixture.

<sup>\*3</sup>: by atomic absorption spectrometry. <sup>\*4</sup>: by molybdenum blue method.

CE: cellulose, CH: chitosan.

Table 2-1-2. Conditions for semi-quantitative RT-PCR.

		Sequence	Product size (bp)	Cycles	Tm ( )
CaBP-D9K	(+)	5'-ATGAGCGCTAAGAAATCTCCC-3'	237	24	51
	(-)	5'-TTGTGATAACTTTTTGAAGAAA-3'			
VDR	(+)	5'-GTGACTTTGACCGGAACGTG-3'	280	24	53
	(-)	5'-ATCATCTCCCTCTTACGCTG-3'			
GAPDH	(+)	5'-CCATGGAGAAGGCTGGGG-3'	194	26	53
	(-)	5'-CAAAGTTGTCATGGATGACC-3'			

Table 2-1-3. Body weight, food intake and organ weights of OVX rats fed the experimental diets for 6wk.

	CE	CH
Initial body weight (g)	188 ± 3	193 ± 4
Final body weight (g)	230 ± 4	232 ± 5
Body weight gain (g)	42 ± 3	40 ± 3
Food intake (g/ 6wk)	570 ± 6	576 ± 11
Liver (g)	5.66 ± 0.17	5.23 ± 0.20
Abdominal fat (g)	7.99 ± 0.48	7.53 ± 1.01
Uterus (g)	0.12 ± 0.02	0.10 ± 0.01

Values are means ± SE (CE: n=6, CH: n=7). CE: cellulose, CH: chitosan

Table 2-1-4. Calcium and phosphorus balances of OVX rats fed the experimental diets for 6wk.

	CE	CH
<b>Ca (mg/ 4d)</b>		
Intake	46.4 ± 1.3	52.4 ± 2.1
Fecal excretion	28.7 ± 1.6	29.7 ± 1.9
Urinary excretion	5.2 ± 0.3	12.6 ± 0.6***
Total excretion	33.9 ± 1.6	42.4 ± 2.0
Absorption	17.8 ± 0.9	22.7 ± 1.8
Absorption rate(%)	38.4 ± 2.2	43.3 ± 2.8
Retention	12.6 ± 0.8	10.1 ± 1.8
Retention rate(%)	27.3 ± 2.0	19.0 ± 3.1*
<b>P (mg/ 4d)</b>		
Intake	260.5 ± 7.1	197.9 ± 8.1***
Fecal excretion	32.4 ± 2.5	42.3 ± 2.1*
Urinary excretion	82.8 ± 2.4	83.8 ± 3.3
Total excretion	115.2 ± 2.9	126.1 ± 4.4
Absorption	228.2 ± 6.1	155.6 ± 7.5***
Absorption rate(%)	87.6 ± 0.8	78.5 ± 1.1***
Retention	145.4 ± 5.2	71.8 ± 4.8***
Retention rate(%)	55.8 ± 0.8	36.1 ± 1.4***

Values are means ± SE (CE: n=6, CH: n=7). \*:  $P < 0.05$ , \*\*\*:  $P < 0.001$  vs CE. Feces and urine were collected for 4 days. Absorption: Intake-fecal excretion. Absorption rate(%):  $\{(Intake - fecal\ excretion) / intake\} \times 100$ . Retention: Intake-total excretion. Retention rate(%):  $\{(Intake - total\ excretion) / intake\} \times 100$ . CE: cellulose, CH: chitosan.

Table 2-1-5. Effects of the experimental diets on serum mineral levels in OVX rats

	CE	CH
Calcium (mg/dL)	10.4 ± 0.1	9.6 ± 0.3*
Phosphate (mg/dL)	4.0 ± 0.8	6.7 ± 1.1*
Magnesium (mg/dL)	2.3 ± 0.1	2.0 ± 0.1

Values are means ± SE (CE: n=6, CH: n=7). \*:  $P < 0.05$  vs CE. CE: cellulose, CH: chitosan.

Table 2-1-6. Biochemical parameters in serum and urine of OVX rats fed the experimental diets for 6wk.

	CE	CH
<b>Serum</b>		
Alkaline phosphatase (IU/L)	142.7 ± 20.1	67.8 ± 2.9***
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***
<b>Urine</b>		
DPD/creatinine (nmol/mmol)	418.5 ± 19.0	447.0 ± 26.8

Values are means ± SE (CE: n=6, CH: n=7). \*\*\*:  $P < 0.001$  vs CE. PTH: parathyroid hormone, DPD: deoxypyridinoline. CE: cellulose, CH: chitosan.

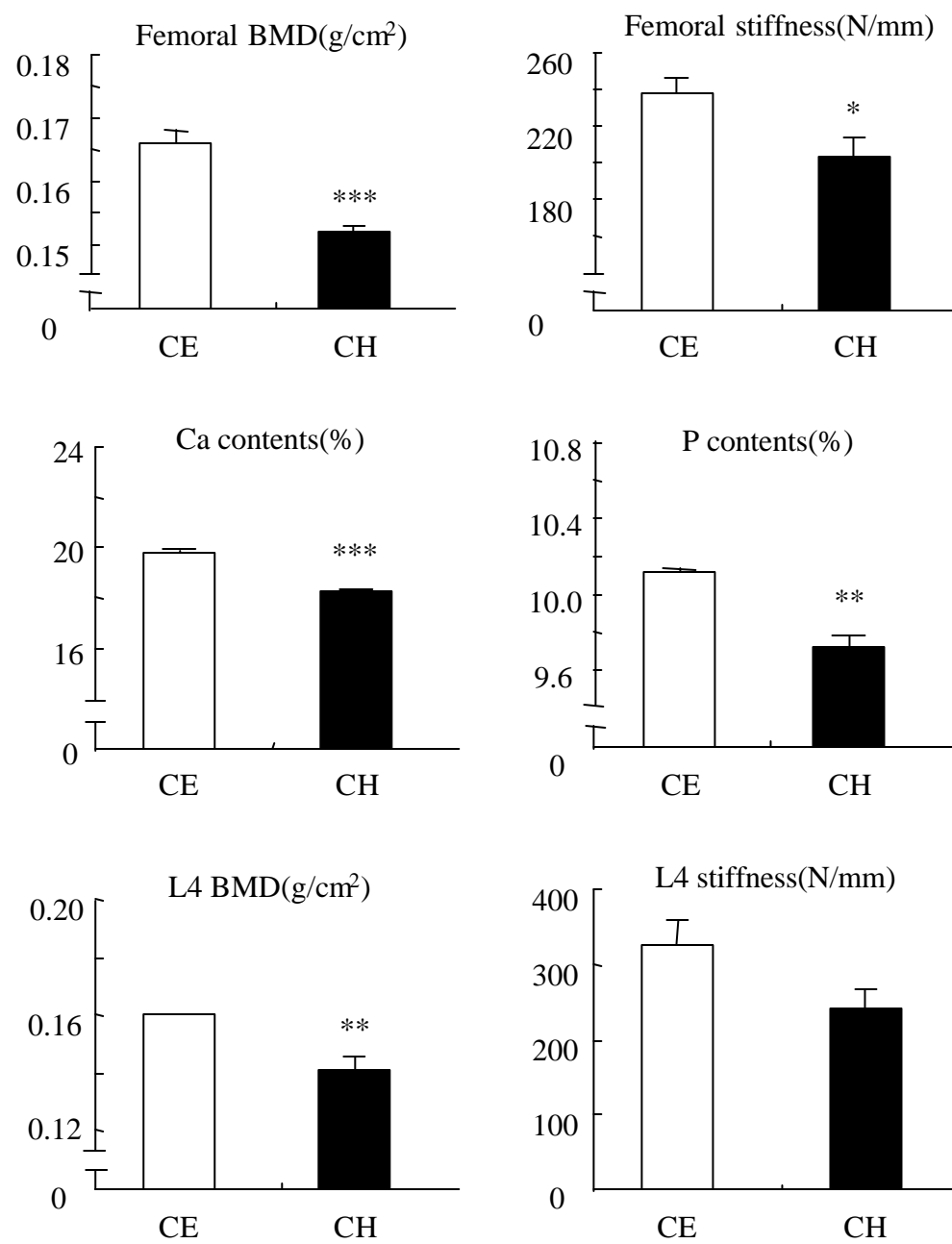


Fig. 2-1-1. Effects of the experimental diets on bone properties of femurs and the fourth lumbar vertebrae (L4) in OVX rats. Values are means  $\pm$  SE (CE: n=6, CH: n=7). \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$  vs CE. CE: cellulose, CH: chitosan. Femoral Ca contents: {Ca (mg)/dry femur weight (mg)}  $\times$  100. Femoral P contents: {P (mg)/dry femur weight (mg)}  $\times$  100. BMD(g/cm<sup>2</sup>): Bone mineral density.

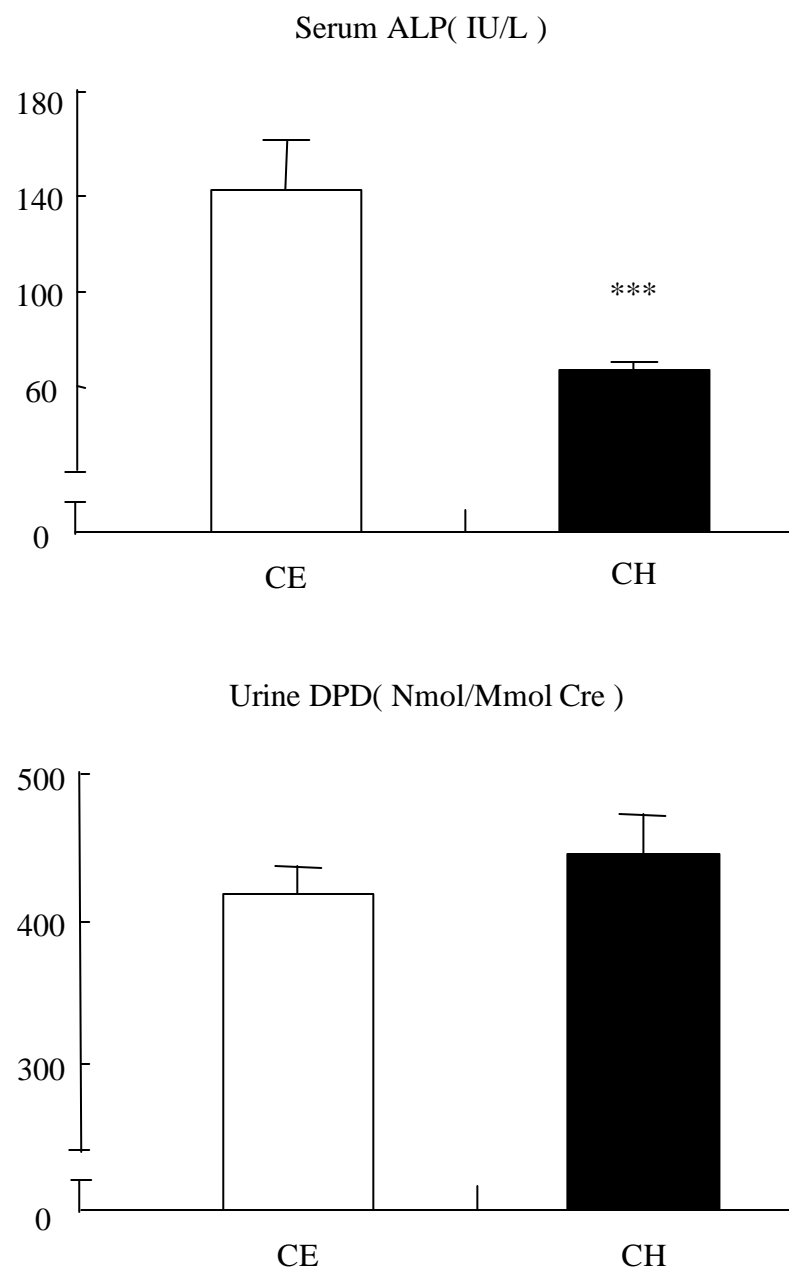


Fig. 2-1-2. Effects of the experimental diets on serum Alkaline phosphatase and urinary DPD levels in OVX rats. Values are means  $\pm$  SE (CE: n=6, CH: n=7). \*\*\*:  $P < 0.001$  vs CE. CE: cellulose, CH: chitosan. DPD: deoxypyridinoline



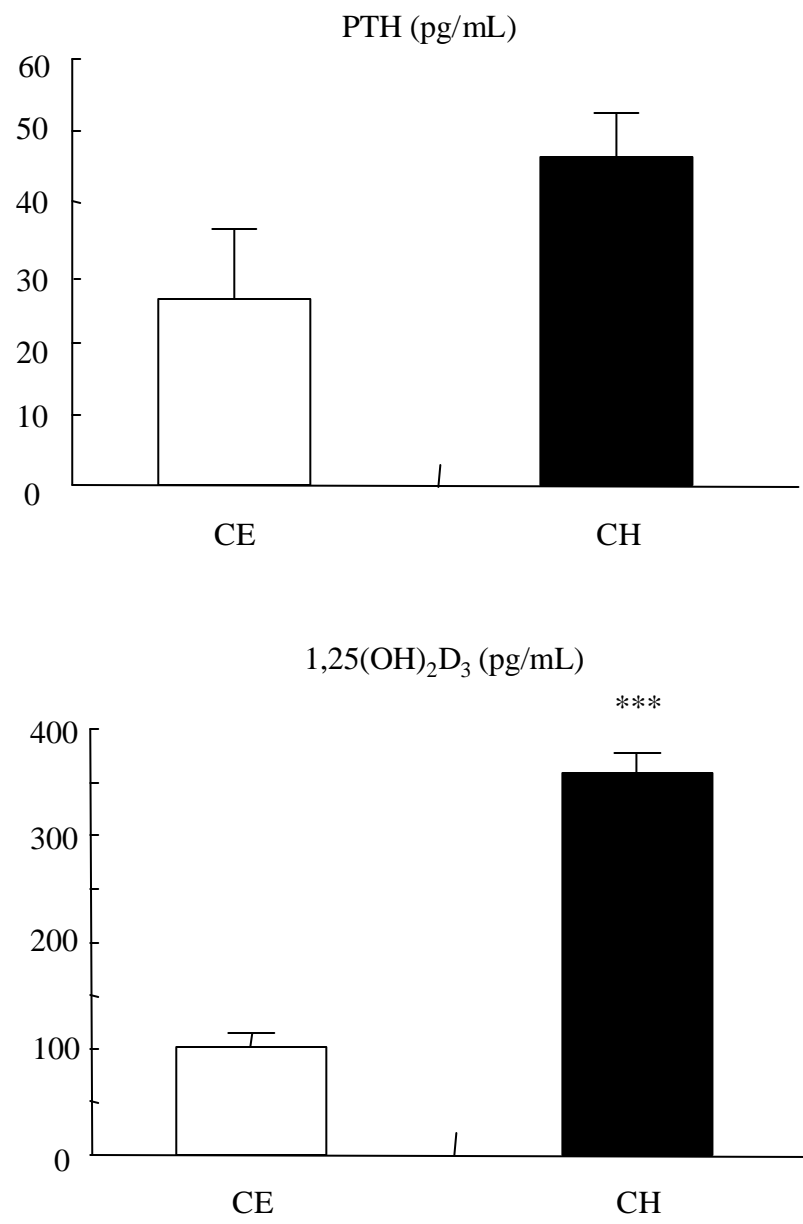


Fig. 2-1-3. Effects of the experimental diets on serum PTH and 1,25(OH)<sub>2</sub>D<sub>3</sub> levels in OVX rats. Values are means  $\pm$  SE (CE: n=6, CH: n=7). \*\*\*:  $P < 0.001$  vs CE. CE: cellulose, CH: chitosan. PTH: parathyroid hormone.

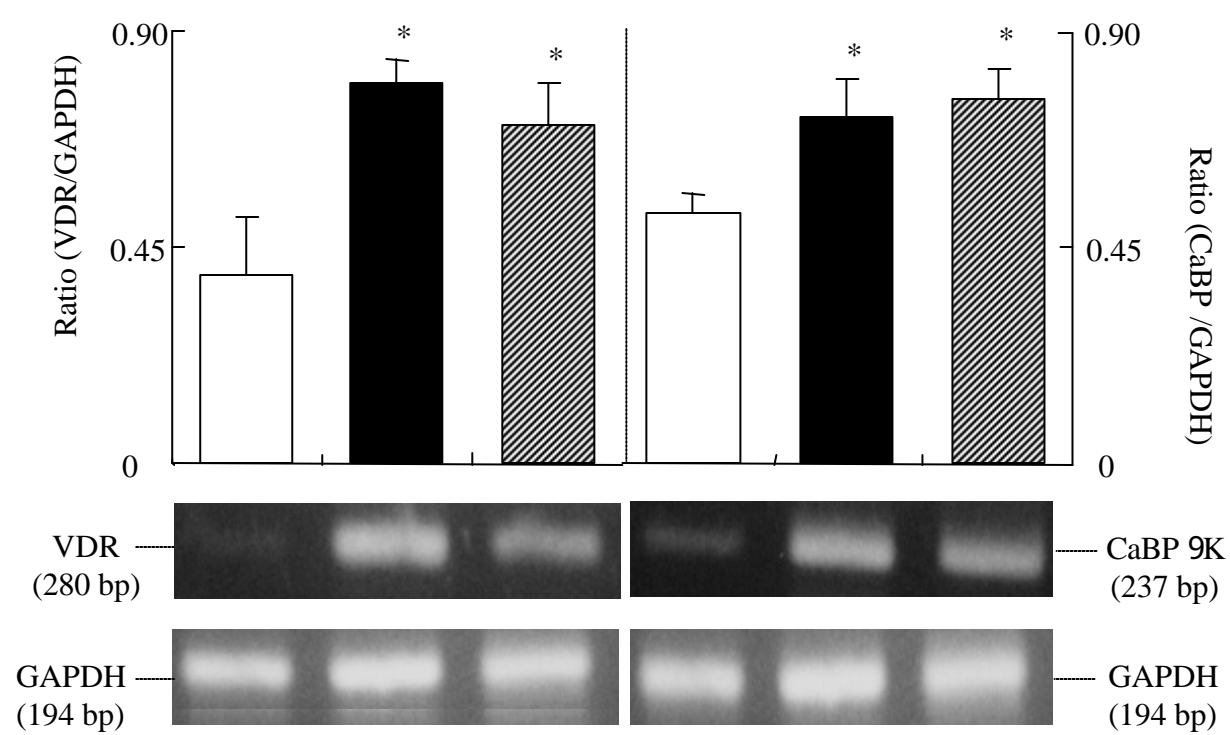


Fig. 2-1-4. Expression of VDR mRNA and CaBP D9K mRNA in duodenum of rats fed the experimental diets for 6 wk. Values are means  $\pm$  SE, \* :  $P < 0.05$  vs CE group. □ : CE, ■ : CH, ▨ : CHVC group. RT-PCR was performed to detect VDR and CaBP D9K transcripts. The results were evaluated as the relative ratio in mRNA of VDR and CaBP D9K to that of GAPDH.

Table 2-2-1. Composition of the experimental diets (g/100 g).

Ingredients	LP	P
Cornstarch	50.8792	49.9992
Casein	14	14
D-cornstarch	15.5	15.5
Sucrose	10	10
Soybean oil	4	4
Fiber		
Mineral mixture(Ca, P Free)* <sup>1</sup>	3.5	3.5
Vitamin mixture* <sup>2</sup>	1	1
L-Cystine	0.18	0.18
Choline bitartrate	0.25	0.25
T-butylhydroquinone	0.0008	0.0008
CaCO <sub>3</sub>	0.25	0.25
KH <sub>2</sub> PO <sub>4</sub>	0.44	1.32
Total	100	100

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.)

\*<sup>1</sup>: AIN-93M mineral mixture without Ca and P. \*<sup>2</sup>: AIN-93M vitamin mixture. LP: 0.1% P diet, P: 0.3% P diet.

Table 2-2-2. Body weight, food intake and organ weights of OVX rats fed the experimental diets for 4wk.

	P	LP
Initial body weight (g)	195 ± 5	184 ± 3
Final body weight (g)	226 ± 6	225 ± 3
Body weight gain (g)	31 ± 2	41 ± 3*
Food intake (g/ 4wk)	260 ± 5	278 ± 6
Liver (g)	5.35 ± 0.12	5.30 ± 0.12
Abdominal fat (g)	4.54 ± 0.39	4.88 ± 0.40
Uterus (g)	0.12 ± 0.02	0.12 ± 0.01

Values are means ± SE (P: n=6, LP: n=6). \*:  $P < 0.05$  vs P. P: 0.3% P diet, LP: 0.1% P diet.

Table 2-2-3. Effects of the experimental diets on serum mineral levels in OVX rats

	P	LP
Calcium (mg/dL)	8.8 ± 0.6	9.7 ± 0.4
Phosphate (mg/dL)	4.7 ± 0.8	4.5 ± 0.4

Values are means ± SE (P: n=6, LP: n=6). P: 0.3% P diet, LP: 0.1% P diet.

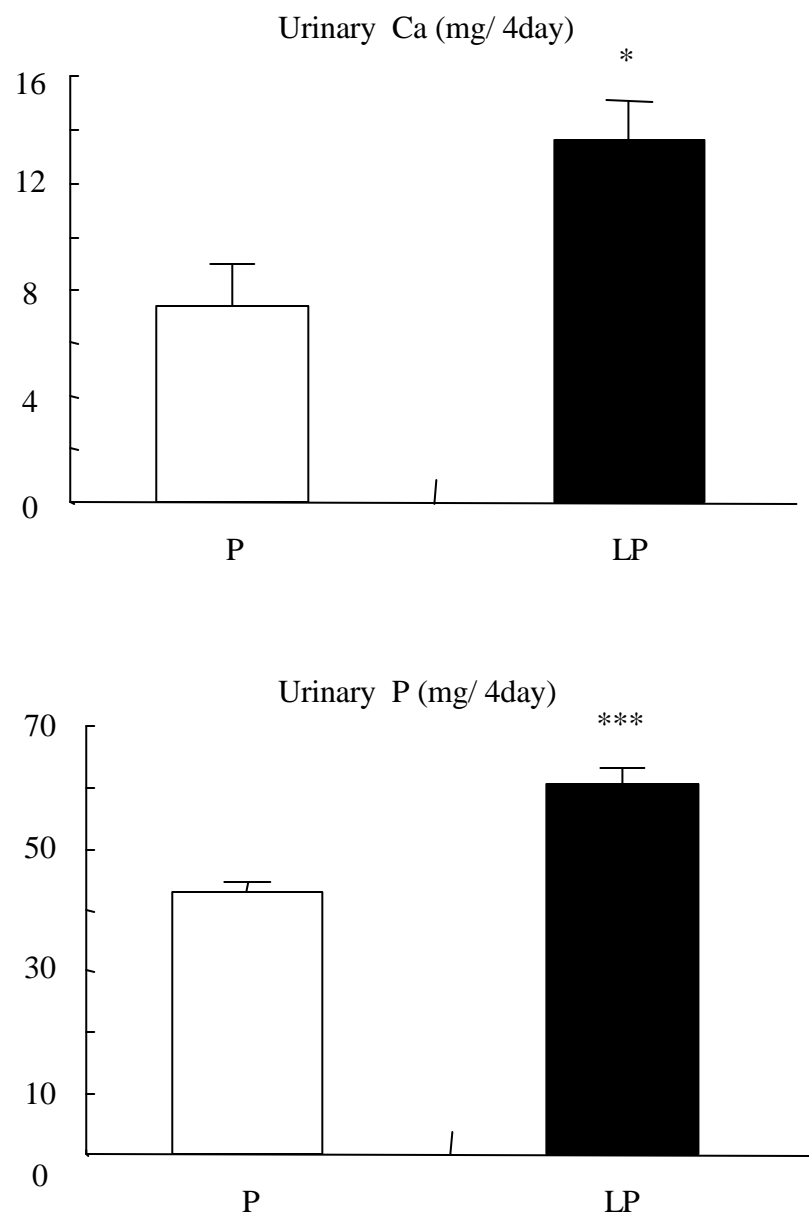


Fig. 2-2-1. Effects of the experimental diets on urinary Ca and P levels in OVX rats. Values are means  $\pm$  SE (P: n=6, LP: n=6). \*:  $P < 0.05$ , \*\*\*:  $P < 0.001$  vs P. P: 0.3% P diet, LP: 0.1% P diet.

Table 2-3-1. Composition of the experimental diets (g/100 g).

Ingredients	FF	CE	CH
Cornstarch	51.3192	41.3192	41.3192
Casein	14	14	14
D-cornstarch	15.5	15.5	15.5
Sucrose	10	10	10
Soybean oil	4	4	4
Cellulose		10	
Chitosan			10
Mineral mixture(Ca Free)* <sup>1</sup>	3.5	3.5	3.5
Vitamin mixture* <sup>2</sup>	1	1	1
L-Cystine	0.18	0.18	0.18
Choline bitartrate	0.25	0.25	0.25
T-butylhydroquinone	0.0008	0.0008	0.0008
CaCO <sub>3</sub>	0.25	0.25	0.25
Total	100	100	100

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.)

\*<sup>1</sup>: AIN-93M mineral mixture without Ca. \*<sup>2</sup>: AIN-93M vitamin mixture. FF: fiber-free. CE: cellulose, CH: chitosan.

Table 2-3-2. Body weight, organ weights and food intake of OVX rats fed the experimental diets for 6w.

	FF	CE	CH
Initial body weight (g)	168 ± 7	168 ± 4	168 ± 5
Final body weight (g)	212 ± 13	223 ± 4	216 ± 6
Body weight gain (g)	43 ± 10	55 ± 4	48 ± 3
Food intake (g/ 45days)	489 ± 31	563 ± 9*	534 ± 13
Liver (g)	7.08 ± 0.52	6.14 ± 0.23	6.09 ± 0.15
Abdominal fat (g)	3.60 ± 0.88	3.49 ± 0.51	3.16 ± 0.38
Uterus (g)	0.14 ± 0.04	0.12 ± 0.01	0.12 ± 0.01

Values are means ± SE (FF: n=5, CE: n=6, CH: n=7). \*: P<0.05 vs FF. FF: fiber-free. CE: cellulose, CH: chitosan.



Table 2-3-3. Effects of the experimental diets on cecal pH value and the volatile fatty acid pool

( $\mu$ mol/ cecum)	FF	CE	CH
Succinic acid	0.62 $\pm$ 0.24	1.07 $\pm$ 0.40	0.87 $\pm$ 0.11
Lactic acid	0.05 $\pm$ 0.05	0.07 $\pm$ 0.05	0.03 $\pm$ 0.03
Acetic acid	4.71 $\pm$ 1.25	8.29 $\pm$ 1.56	13.25 $\pm$ 1.50***#
Propionic acid	1.70 $\pm$ 0.44	2.31 $\pm$ 0.47	4.94 $\pm$ 0.48***##
Butyric acid	0.56 $\pm$ 0.22	1.78 $\pm$ 0.54	1.70 $\pm$ 0.47
Total	7.49 $\pm$ 2.67	14.48 $\pm$ 2.88	21.35 $\pm$ 2.57**
Water	82.43 $\pm$ 1.03	73.54 $\pm$ 0.51***	84.88 $\pm$ 0.20####
Cecal pH	6.98 $\pm$ 0.18	6.86 $\pm$ 0.12	7.40 $\pm$ 0.08*##

Values are means  $\pm$  SE (FF:  $n=5$ , CE:  $n=6$ , CH:  $n=7$ ). \*:  $P<0.05$ , \*\*:  $P<0.01$ , \*\*\*:  $P<0.001$  vs FF, #:  $P<0.05$ , ##:  $P<0.01$ , ####:  $P<0.001$  vs CE. FF: fiber-free. CE: cellulose, CH: chitosan.