CHAPTER IV

RESULTS

The arterial plasma inorganic phosphorus (P_i) concentration during continuous infusion of P_i solution.

Cows in both groups showing either positive or negative EtOH test were alert and responsive during P_i infusion. After 30 minutes of P_i infusion, the plasma P_i concentration gradually increased and it maintained the level approximately three folds of the normal plasma P_i concentration at 90 to 120 minutes of infusion (Figure 6).
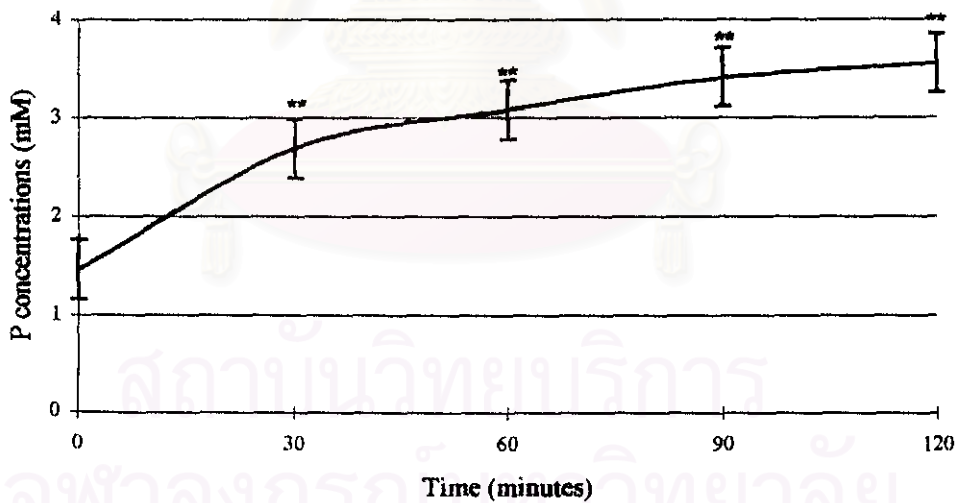
Figure 6. Changes the arterial plasma concentrations of Pi during infusion of Pi solution (28.5 mg/min) of both the positive or the negative EtOH testing group.

P- values by paired t-test : ** (P<0.01) with respect to the control value.

Effect of inorganic phosphorus solution infusion on the mammary arterio-venous (A-V) difference and the mammary extraction ratio of P_i and Ca.

An infusion of inorganic phosphorus solution into animals showed no changes in the mammary arterio-venous difference and the mammary extraction ratio of P_i in compared to the control period in either positive or negative EtOH testing group. The P_i infusion produced significant increase in mean arterial plasma P_i concentration from 1.37 ± 0.44 to 3.59 ± 0.40 mM/L, (P<0.01) in the positive EtOH testing group and from 1.55 ± 0.52 to 3.49 ± 0.49 mM/L, (P<0.01) in the negative EtOH testing group. In the positive EtOH testing group before given P_i infusion, mammary A-V difference showed a release of P_i from the mammary gland. After P_i infusion, it showed slightly increase in mammary A-V difference and the mammary extraction ratio of P_i in compared with that before P_i infusion. By contrast, slight decreases in mammary A-V difference and the mammary extraction ratio of P_i were apparent after P_i infusion in the negative EtOH testing group.

During Pi infusion, there were no significant changes in mean arterial plasma Ca concentration, the mammary A-V difference and the mammary extraction ratio of Ca in compared with pre-infusion period in both positive and negative EtOH testing groups.

Before P_i infusion, the mammary A-V difference and the mammary extraction ratio of either P_i or Ca did not significantly change in comparison between the positive and the negative EtOH testing group (Table 1).

Table 1 Effects of inorganic P solution infusion on the plasma concentration, the arterio-venous difference and the extraction ratio of P_i and Ca by the mammary gland.

	Positive EtOH group		Negative EtOH group	
	Before infusion	After infusion	Before infusion	After infusion
Inorganic phosphorus				
A (mM/L)	1.37 ± 0.44	3.59 ± 0.40**	1.55 ± 0.52	3.45 ± 0.49**
A-V (mM/L)	-0.07 ± 0.19	0.02 ± 0.18	0.10 ± 0.35	0.04 ± 0.30
(A-V)/A (%)	-8.98 ± 16.43	0.64 ± 5.11	3.16 ± 26.14	0.87 ± 9.01
Calcium				
A (mM/L)	2.37 ± 0.33	2.31 ± 0.42	2.21 ± 0.41	2.15 ± 0.46
A-V (mM/L)	0.01 ± 0.12	-0.01 ± 0.02	0.03 ± 0.11	0.01 ± 0.13
(A-V)/A (%)	0.05 ± 6.20	-0.76 ± 1.51	1.88 ± 6.29	0.37 ± 7.36

Value are mean±SD. Abbreviation: P_i , Inorganic phosphorus; Ca, calcium: A ,concentrations in arterial plasma; A-V, mammary arterio-venous difference : (A-V)/A , mammary extraction ratio

P- values by paired t-test : ** ($P < 0.01$) with respect to the control value.

Effects of inorganic P solution infusion on the arterial plasma Na , K , Cl and Mg concentrations.

There were no significant changes for Na , K , Cl and Mg concentrations in arterial plasma between before and after Pi infusion in the either the positive or the negative EtOH testing group. The Na , K , Cl and Mg concentrations in arterial plasma of prior P_i infusion did not significant change between the positive and the negative EtOH testing group.

<u>Table 2</u> Effects of P_i solution infusion on arterial plasma concentration of Na, K, Cl and Mg

	Positive EtOH group		Negative EtOH group	
	Before infusion	After infusion	Before infusion	After infusion
Na (mM/L)	130.40 ± 3.31	130.20 ± 3.33	130.10 ± 4.25	131.62 ± 5.85
K (mM/L)	4.89 ± 0.18	4.89 ± 0.20	4.90 ± 0.16	4.95 ± 0.25
Cl (mM/L)	99.40 ± 5.21	100.30 ± 4.85	98.20 ± 5.67	99.25 ± 2.81
Mg (mM/L)	1.20 ± 0.13	1.28 ± 0.15	1.15 ± 0.14	1.14 ± 0.13

Value are mean $\pm$ SD. Abbreviation : Na , Sodium concentration; K, Potassium concentration; Cl , Chloride concentration; Mg , Magnesium concentration

Effects of P_i solution infusion on the precipitation of milk

The effects of P_i infusion on the precipitation of milk which gives the result form the *in vivo* studies, by measuring the turbidity of supernatant values using the optical density are shown in table 3. After P_i infusion, the optical density of supernatant did not significant change from that of before P_i infusion in both groups. The precipitation by measurement of the optical density of the supernatant slightly decreased in the negative EtOH testing group when compared to positive EtOH testing group.

Table 3 Effects of P_i solution infusion on the precipitation of milk.

	OD	OD	n
	Before infusion	After infusion	
Positive EtOH testing group	0.37±0.06	0.36±0.06	10
Negative EtOH testing group	0.33±0.08	0.31±0.07	10

Values are mean ± SD. Abbreviation: OD, Optical density; EtOH, ethanol ; n= the number of cows

Effects of P_i solution infusion on the EtOH stability of milk

In the positive EtOH testing group after inorganic phosphorus infusion, the EtOH stability of milk by measuring the optical density significantly increased (P<0.05) at 68%, 75%, 80% of EtOH. The optical density of milk sample of the negative EtOH testing group did not significant difference from those of samples collecting after P_i infusion. The EtOH stability of milk by the optical density at the pre infusion period of negative EtOH testing group showed significantly higher (P<0.01) at all levels of EtOH test than those of milk form the positive EtOH testing group. (table 4)

Table 4 Effects of P_i solution infusion on EtOH stability of milk by optical density measurement.

Conc of EtOH (%)	Positive EtOH group		Negative EtOH group	
	OD	OD	OD	OD
	Before infusion	After infusion	Before infusion	After infusion
68	0.40 ± 0.18	0.54 ± 0.40*	0.68 ± 0.12 $$	0.66 ± 0.13
75	0.22 ± 0.07	0.34 ± 0.08*	0.55 ± 0.11 $$	0.57 ± 0.16
80	0.14 ± 0.04	0.29 ± 0.08*	0.44 ± 0.12 $$	0.51 ± 0.17
85	0.10 ± 0.04	0.12 ± 0.06	0.36 ± 0.10 $$	0.31 ± 0.05
90	0.05 ± 0.03	0.05 ± 0.02	0.21 ± 0.13 $$	0.23 ± 0.06
95	0.03 ± 0.03	0.03 ± 0.51	0.11 ± 0.04 $$	0.12 ± 0.04

Values are mean ± SD. Abbreviation : EtOH, Ethanol ; OD, optical density

P- values by paired t-test : * (P<0.05) with respect to the control value.

P- values by unpaired t-test: $$ (P<0.01) with respect to differ among groups.

Effects of P_i solution infusion on the concentrations of P_i, Ca, Na, K, Cl, Mg, Citrate, lactose, fat and protein in milk.

The results in the table 5 show that the P_i concentrations in milk after P_i infusion significantly increased (24.68 ± 6.70 mM/L) ($P<0.05$) in compared to period before P_i infusion (23.35 ± 6.37 mM/L) in the positive EtOH testing group . In the negative EtOH testing group, P_i concentrations in milk after P_i infusion significantly increased (30.42 ± 5.18 mM/L) ($P<0.01$) in compared to before P_i infusion period (29.47 ± 4.95 mM/L). Ca concentrations in milk after Pi infusion did not change from those before P_i infusion in both the positive and the negative EtOH testing groups. The Na concentrations in milk after P_i infusion significantly decreased ($P<0.01$) from that before P_i infusion in the positive EtOH testing group, while the negative EtOH testing group did not change the Na concentration. The K, Cl and Mg concentrations in milk did not significant differences from those before P_i infusion in both group. After P_i infusion , the citrate concentrations in milk slightly increased when compared to that before P_i infusion in both groups. There were no significant differences of the lactose, fat and protein concentrations in milk between before and after P_i infusion in both groups.

Table 5 Effects of P_i solution infusion on concentrations of P_i, Ca, Na, K, Cl, Mg, Citrate, lactose, fat and protein in milk

	Positive EtOH group		Negative EtOH group	
	Before infusion	After infusion	Before infusion	After infusion
P_i (mM/L)	23.35 ± 6.37	$24.68 \pm 6.70**$	$29.47 \pm 4.95\$\$$	$30.42 \pm 5.18**$
Ca (mM/L)	28.30 ± 3.91	27.28 ± 2.02	28.38 ± 3.05	28.30 ± 2.12
Na (mM/L)	29.00 ± 18.29	$27.50 \pm 16.06**$	25.00 ± 21.63	22.00 ± 24.61
K (mM/L)	34.34 ± 2.24	33.48 ± 1.85	34.75 ± 2.23	37.35 ± 2.13
Cl (mM/L)	48.43 ± 11.65	48.26 ± 7.41	48.23 ± 11.98	49.00 ± 12.13
Mg (mM/L)	4.28 ± 0.91	4.27 ± 0.88	4.76 ± 1.64	4.36 ± 1.07
Citrate (mM/L)	8.45 ± 1.92	8.93 ± 2.06	12.02 ± 5.75	14.03 ± 6.89
Lactose (gm%)	4.07 ± 0.76	4.03 ± 0.47	4.21 ± 0.64	4.23 ± 0.72
Fat (gm%)	3.68 ± 1.66	3.34 ± 1.18	3.87 ± 2.01	3.35 ± 1.61
Protein (gm%)	3.46 ± 0.32	3.38 ± 0.37	3.27 ± 0.50	3.28 ± 0.51

Values are mean $\pm$ SD. Abbreviation : P_i, Phosphorus ; Ca, Calcium ; Na, Sodium ; K, Potassium; Cl, Chloride; Mg, Magnesium.

P- values by paired t-test : ** (P<0.01) with respect to the control value.

P- values by unpaired t-test : $$ (P<0.01) with respect to differ among.

Effects of of P_i addition (0.1, 0.5 and 0.9 mg P_i/ml) on EtOH stability of milk from the *in vitro* study

The results in table 6 show the ethanol stability of the milk which give the results from optical density of the supernatant in the positive EtOH testing group. After P_i addition in all treatments of the optical density significantly increased (P<0.01) from the control at using 68, 75, 80 and 85 % EtOH concentrations . At 90 and 95% of EtOH concentration using the P_i addition in treatment 2 (0.1 mg P_i/ml) showed significant increase (P<0.05) of the values of optical density whereas in treatment 3 and 4 showed significant increase (P<0.01) when compare to the control

<u>Table 6</u> Effect of of P_i addition (0.1, 0.5 and 0.9 mg P_i/ml) on EtOH stability of milk from the *in vitro* study in the positive testing group.

Conc.	Treatment			
EtOH (%)	1 Control	2 (0.1 mgP$_i$/ml)	3 (0.5 mgP$_i$/ml)	4 (0.9 mgP$_i$/ml)
68	0.40 ± 0.18	0.59 ± 0.22**	0.56 ± 0.25**	0.58 ± 0.20**
75	0.22 ± 0.07	0.51 ± 0.28**	0.53 ± 0.27**	0.51 ± 0.19**
80	0.14 ± 0.04	0.36 ± 0.14**	0.45 ± 0.22**	0.41 ± 0.13**
85	0.10 ± 0.04	0.19 ± 0.09**	0.34 ± 0.12**	0.30 ± 0.05**
90	0.05 ± 0.03	0.17 ± 0.14*	0.18 ± 0.07**	0.21 ± 0.08**
95	0.03 ± 0.03	0.07 ± 0.02*	0.12 ± 0.06**	0.15 ± 0.06**

Values are mean ± SD. Abbreviation: EtOH, Ethanol concentration; OD, optical density
P- values by paired t-test : * (P<0.05) . ** (P<0.01) with respect to the control value.

The results in table 7 show the ethanol stability of milk which give the results from optical density of the negative EtOH testing group. After P_i addition, in treatment 2 and treatment 3 the value of optical density did not show any differences from control whereas it significantly decreased (P<0.01) in treatment 4 when testing with the 68% EtOH concentration. At 75% EtOH concentration, P_i addition milk caused significant changes (P<0.05) in treatment 3 and treatment 4 as compared to control. There were no significant differences of optical density in testing with 80 , 85 , 90 and 95% EtOH concentrations treatments on compared to the control milk sample.

Table 7 Effects of P_i addition (0.1, 0.5 and 0.9 mg P_i/ml) on EtOH stability of milk from the *in vitro* study in the negative EtOH testing group.

Conc	Treatment			
	1	2	3	4
EtOH (%)	Control	(0.1 mgP/ml)	(0.5 mgP/ml)	(0.9 mgP/ml)
68	0.68 ± 0.12	0.69 ± 0.15	0.67 ± 0.18	0.53 ± 0.10**
75	0.57 ± 0.16	0.60 ± 0.17	0.63 ± 0.18*	0.49 ± 0.10*
80	0.51 ± 0.17	0.55 ± 0.10	0.52 ± 0.10	0.44 ± 0.11
85	0.36 ± 0.10	0.42 ± 0.13	0.38 ± 0.09	0.37 ± 0.11
90	0.21 ± 0.13	0.25 ± 0.11	0.28 ± 0.03	0.22 ± 0.09
95	0.11 ± 0.04	0.15 ± 0.09	0.18 ± 0.07	0.13 ± 0.07

Values are mean $\pm$ SD. Abbreviation: EtOH, Ethanol concentration; OD, optical density

P- values by paired t-test : * (P<0.05) , ** (P<0.01) with respect to the control value.

Effect of P_i solution infusion on milk α -, β - and κ -casein concentration

Effects of inorganic phosphorus infusion on the concentration of α -casein , β -casein and κ -casein are given in table 8 and Figures 7-9.

During P_i infusion into animals, there were no significant affect the concentrations of α -casein , β -casein and κ -casein in the both groups.

The concentrations of α - casein and β - casein did not significant difference in comparison before the positive EtOH testing groups. However, the mean concentration of κ - casein in the positive EtOH testing group (1.05 $\pm$ 0.28 μg / 10μg CN) was lower than that of the negative EtOH testing group (1.41$\pm$0.70 μg / 10 μg CN).

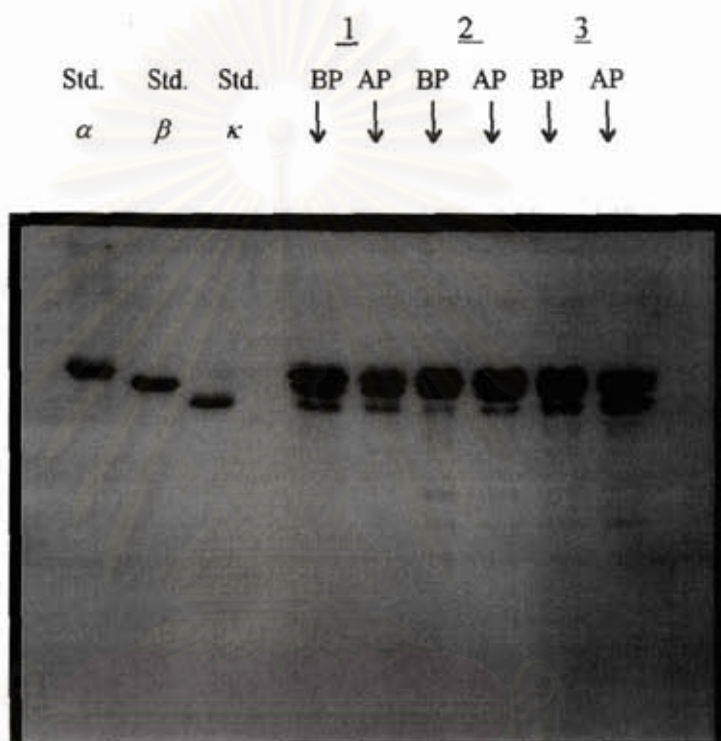The molecular weight of all caseins showed no did not significant differences between before and P_i infusion.

Figure 7 . Photograph showing the casein separation to α - casein , β - casein and κ - casein by SDS- PAGE in the positive EtOH testing group.

Abbreviation : BP, Before P_i solution infusion ; AP, After P_i solution infusion ; SDS-PAGE sodium dioctyl sulphate polyacrylamide gel electrophoresis

Figure 8 . Photograph showing the casein separation to α - casein , β - casein , κ - casein and standard MW by SDS- PAGE in the positive EtOH testing group.

Abbreviation : BP , Before Pi solution infusion ;   AP, After Pi solution infusion;   SDS-PAGE   sodium   dioctyl sulphate polyacrylamide gel electrophoresis; MW, molecular weight.

Figure 9 . Photograph showing the casein separation to α - casein , β - casein and κ - casein by SDS- PAGE in the negative EtOH testing group.

Abbreviation : BP , Before Pi solution infusion ; AP, After Pi solution infusion; SDS-PAGE sodium dioctyl sulphate polyacrylamide gel electrophoresis

Table 8 Effects of inorganic phosphorus solution infusion on the α-, β-, and κ-casein concentration.(n=10)

	Positive EtOH group		Negative EtOH group		Negative BP VS Positive BP EtOH group
	BP$_i$	AP$_i$	BPi	APi	
α-casein (μg/10μg)	3.57 $\pm$ 1.83	3.91 $\pm$ 0.73	3.60 $\pm$ 1.24	3.87 $\pm$ 0.91	NS
β-casein (μg/10μg)	2.19 $\pm$ 0.87	2.41 $\pm$ 0.28	2.19 $\pm$ 0.65	2.58 $\pm$ 2.46	NS
κ-casein (μg/10μg)	1.05 $\pm$ 0.28	1.52 $\pm$ 0.43	1.41 $\pm$ 0.70	1.54 $\pm$ 0.39	$$
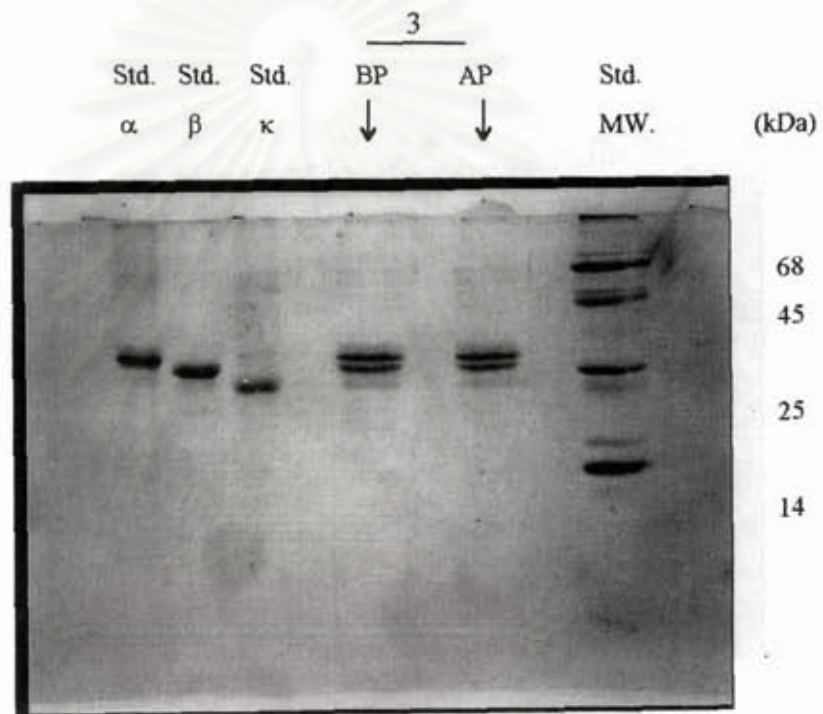
Value are mean $\pm$ SD. Abbreviation : α-casein , alpha -casein ; β - casein , beta - casein κ - casein , kappa - casein ; μg , microgram ;BP , Before P$_i$ solution infusion ; AP , After P$_i$ solution infusion.

P- values by unpair-t-test : $$ (P<0.01) are differed among groups.

Effect of P$_i$ solution infusion on the soluble salt balance in milk

After inorganic phosphorus solution infusion, no significant changes of the soluble salt balance were apparent between before and after P$_i$ infusion. It slightly decreased in the period after P$_i$ infusion in both positive and negative EtOH testing group.

There were significant decrease (P<0.01) in the soluble salt balance in the negative EtOH group in comparison with the positive EtOH group in the period before P_i infusion. (table 9).

Table 9 Effects of inorganic phosphorus solution infusion on the soluble salt balance of milk.

	SSB		Negative BP VS Positive BP EtOH group
	BP	AP	
Positive EtOH testing group	1.06 ± 1.08	0.96± 0.86	NS
Negative EtOH testing group	0.82 ± 0.81	0.76 ± 0.97	$$

Values are mean ± SD. Abbreviation: SSB, soluble salt balance (Ca(Mm) +Mg(Mm)/ P(Mm)+citrate(Mm) ; EtOH, ethanol ; BP. Before Pi solution infusion ; AP, After Pi solution infusion

P- values by unpair-t-test : $$ (P<0.01) are differed among groups