CHAPTER V

DISCUSSION

In the present study during the P_i infusion a marked increase in the plasma P_i concentration was apparent while it did not affect to the mammary A-V difference and the mammary extraction ratio of P_i in each group (positive or negative EtOH testing group). It may be related to the capacity of mammary gland in the utilization of P_i. However, in consideration of P_i uptake by the mammary gland before P_i infusion, the mammary A-V difference and the mammary extraction ratio of P_i in the positive EtOH testing group showed a lower values. The release of the P_i from the mammary gland in the positive EtOH testing group was apparent. It indicates that the difference of the mechanism in the utilization of P_i during milk synthesis will be occurred in animals between the positive and the negative EtOH testing group. During milk synthesis, more Pi transport inside the golgi vesicle and the cytosol of secretory cell by both diffusion and active transport has been noted (Holt,1985). The process may occur the positive EtOH testing group for a lower utilization of P_i during milk synthesis in which coincided with a low P_i concentration in milk .

During the experiment, the plasma electrolyte concentrations for Ca, Na, K, Cl and Mg were not affected by P_i solution infusion. It indicates that the homeostasis of animals which controlled normal function, blood constituents,

and metabolic production (Rothbauer, 1994) were still kept constant in the present study.

In testing of the precipitation of normal milk by measuring the optical density showed no differences of the optical density between before and after given P_i solution infusion in each group. However, the precipitation test of normal milk in the negative EtOH testing group showed lower values of the optical density than that of the positive EtOH testing group. These results imply that the higher of casein taken into micelles causing a lower of free forms would be apparent in the negative EtOH testing group. In the present results are agree with those reports of McKenzie (1971) Abbassy and Wahba (1986) that high stability of milk was more precipitates which lower of the optical density was determined.

During testing the stability of milk with EtOH the occurrence of precipitation in the positive EtOH testing group was more than that of the negative EtOH testing group. It indicates that the maintenance of stability in milk of negative EtOH testing group would be due to a higher formation of casein micelle. It is probable that in the positive EtOH testing group the process of casein micelle formation would be interfered hydrogen bonds by ethanol within casein molecules. Ethanol can replace the intermolecular hydrogen bonds of caseins and disturb salt linkage (McMahon and Brown, 1984) which make much of the casein precipitates from the dispersion instead of being taken into micelles.

In the negative EtOH testing group, milk was not affected by the different levels of EtOH 68, 75, 80, 85, 90 and 95 % during P_i solution infusion. In contrast, the positive EtOH testing group, a shift to higher stability from 68% EtOH to 75 or 80% EtOH was apparent after P_i infusion. It indicates that an

increase in the stability of milk was dependent on P_i level, the transport of P_i from blood into milk can support in the casein micelles formation and the stability taken place. It is also confirmed by a higher value of mammary A-V difference and mammary extraction ratio during Pi solution infusion in the positive EtOH group. It generally accept that the phosphorus incorporation into casein is derived from the P_i of blood (Bingham and Farrell,1977).

During P_i solution infusion, the P_i concentration in milk markedly increased in both groups. It indicates that there was transportation of P_i from blood into milk during milk synthesis in mammary gland. Other compositions, Ca, Na, K, Cl, Mg, citrate, lactose, fat and protein concentrations were not affected by Pi solution infusion particularly in the negative EtOH testing group. The constant of the concentration of lactose, Na, K and Cl in milk may be related that milk shall have an osmotic pressure close to that of blood and to accommodate this constraint a family of correlation exist involving principally these compositions (Peaker, 1977 ; Holt, 1985). Milk citrate, Ca and Mg concentrations would be kept to a constant level for forming Ca-citrate and Mg-citrate complexes. In addition, Ca ions also involve the casein micelle formation in proper concentration (Holt, 1985). The concentration of fat and protein in milk were not affected by P_i solution infusion because these compositions were upon hormonal regulation (Dils, Clark and Knudsen, 1977) and their genetic breed (Sharaby, 1988). After Pi solution infusion, milk compositions of the positive EtOH testing group were in normal range as in the negative EtOH testing group exception for the milk Na concentration. It is possible that the transport or across the cell membrane of Na ions in secretory cell into milk for maintenance for overall electrical neutrality (Holt, 1985).

P_i solution infusion did not affect the fraction of total casein and casein concentration during the experiment. The α-casein , β-casein and κ-casein concentrations were maintained in constant level in comparison between before and after P_i solution infusion. However, in consideration between groups, the negative EtOH testing group had significant higher concentration of κ-casein than the positive EtOH testing group. It indicates that milk from the negative EtOH testing group would be higher than that of the positive EtOH testing group, since κ-casein would modify the overall structure of casein micelle through the formation of surface coat by κ-casein (Robitelle, Hang and Monardes, 1991 ; Farrell,1973), and the κ-casein will act as the stabilizer of casein micelle (Brunner, 1981; Robitelle, Hang and Monardes, 1991 ; Farrell,1973).

The soluble salt balance of milk (Ca + Mg / P_i + Citrate) were not affected by P_i solution infusion in both groups. However, the soluble salt balance of the negative EtOH testing group showed significantly lower ($P<0.01$) when compared with the positive EtOH testing group. The present results support the interpretation that a lower value of the soluble salt balance of milk would be an index for high stability of EtOH test (Donnelly and Horne, 1986).

The results in *in vitro* study show that addition of P_i in milk sample at 0.1mg P_i/ml, 0.5 mg P_i/ml and 0.9 mg P_i/ml would affect the stability of milk to EtOH test. After P_i addition in all levels had significant increase ethanol stability which the proper level of P_i addition would be 0.5 mg Pi/ml. The negative EtOH testing group did not affect from P_i addition to EtOH concentration at 80,85,90 and 95 % while 68 and 75 % EtOH P_i addition at 0.9 mg Pi/ml decrease EtOH stability. It may be related to the P_i concentration which was higher than the requirement of casein during formation of micelle and it would decline the stability

of milk (White and Davies,1958). In addition of P_i in milk sample *in vitro* caused formation and growth of casein polymer to submicelle size and then promoted micelle formation. It is possible that more addition of P_i would compete and reducing the amount of calcium bound to the micelle causing low net charge on micelles which made them reducing the stability (Horne and Parker,1981b).

The present study can concluded that P_i affects to the stability of milk. The higher level of P_i concentration make more higher the stability of milk. Highly level of P_i concentration influences to milk stability and physicochemical properties either in alveolar lumen or in the secretory cells in the mammary gland. According to the report of Beery, Hood and Patton (1973) using electron microscope showed loose forming of casein micelles in golgi vesicle and partially tight forming in the lumen of golgi vesicle. Thus the incorporation of P_i to stabilize the micellar structures and physicochemical properties of milk would be occured mainly extracellular site of the secretory cell, i.e. in alveolar lumen, while small part of Pi on the milk stability involving in the intracellular site of the secretory cells. The physicochemical properties of milk and micelle forming are dependent on the property of each casein formation undergo in golgi apparatus of individual animal.