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Effect of Calcium Chloride and Sodium Bicarbonate Inject Solutions on Tenderness and Organoleptic Properties of Bovine Rumen Viscera

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Abstract: To examine the contribution of 2.2% $CaCl_2$ or 3% $NaHCO_3$ to tenderization and organoleptic properties, rumen viscera (*Saccus ruminis*) samples were injected with a volume equal to 10% of the sample weight. After the injection, rumen viscera samples were held at 4°C and taken on 24 and 48 h to measure pH, water holding capacity, cooking loss, shear force and extractable protein. Results indicated that 3% $NaHCO_3$ treatment reduced cooking loss and shear force, while elevating pH. On the other hand, the 2.2% $CaCl_2$ treatment did not show any difference from those of the control (no injection). There were no significant differences on water holding capacity and extractable protein among treatments ($p>0.05$). Evidence in this study indicated the advantage of using 3% $NaHCO_3$ for improving tenderization and organoleptic properties of rumen viscera.

Key words: Calcium chloride, sodium bicarbonate, tenderness, organoleptic properties, rumen viscera, Thailand

INTRODUCTION

The acceptability of meat after purchase is determined by tenderness (Jeremiah, 1982). The inadequate tenderness in meat resulted in lower satisfaction of consumers for the quality of meat. Meat tenderness can be related principally to the connective tissue and myofibrillar protein components depend on part of organ or carcass location of the muscle. The variation in tenderness of different muscle or organ in the same animal had also been reported by Lawrie (1985).

In Thailand, cattle after slaughtered have been segregated into carcass and variety meats. Taylor and Field (2004) defined variety meats as the edible products originating from organ and body parts other than the carcass. Liver, heart, tongue and stomach are among the typical variety meats. Rumen viscera (*Saccus ruminis*) is an edible product originating from the fore-stomach of cattle. However, rumen viscera is known as a variety meat-associated with tenderness problem and long time cooking needed to lower the degree of toughness.

It is know that postmortem meat tenderization is influenced by alteration in myofibrillar proteins (Goll *et al*., 1974). Proteolysis of myofibrillar proteins is reported to be a key event in meat tenderization during postmortem storage of meat at refrigerated temperatures (Goll *et al*., 1983). It has been shown that a calcium activated enzyme (calpains) degrades certain myofibrillar protein (Olson *et al*., 1976). Calpains are proteinase enzymes having an absolute calcium requirement for proteolytic activity (Koohmaraie, 1996). Infusion of lamb and mature cow carcasses with 0.3 M calcium chloride accelerated tenderization and proteolysis of myofibrillar proteins (Koohmaraie and Shackelford, 1991; Morgan *et al*., 1991). The hypothesis is that calcium chloride injection increases intracellular calcium concentration, which activates calpain and in turn improves meat tenderness (Dransfield, 1993). Not only has calcium chloride been used for improving meat tenderness but also sodium bicarbonate. Sheard and Tali (2004) reported the reduction in shear force in the injected pork loin with sodium bicarbonate. Offer and Trinick (1983) described the mechanism responsible for the increased tenderness and juiciness of meat with sodium bicarbonate, which is connected with higher water holding capacity and swelling of myofibrils. Bicarbonate also reduces drip loss and shear force (Wynveen *et al*., 2001), presumably because of improved water holding at elevated pH (Bouton *et al*., 1973).

Water Holding Capacity (WHC) is an important characteristic attributed to the quality of meat. WHC determines meat's ability to retain water during storage.

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Water in meat is bound with protein. Denaturation of meat protein contributed to loss of its water-holding capacity and causes meat to lose some fluid or weep. During meat conditioning, the proteins of the myofibril and of the sarcoplasm denature due to the declination of meat pH. The minimum water-holding capacity of meat exists around pH 5.3 since, this is near isoelectric point of the myofibrillar proteins (Honikel and Hamm, 1994).

According to Honikel *et al.* (1986), changes in WHC are very sensitive indicators of changes in the charge and structure of myofibrillar protein.

The increase in tenderness, observed on conditioning, was found to be associated with an increase in water-soluble nitrogen. This could be explained by the denatured protein in meat are liable to attack by proteolytic enzymes, which break-down protein molecules to smaller unit such as peptides and amino acids (Lawrie, 1985). Thus, the proteolysis of myofibril protein could be reflected by a significant release in soluble nitrogen substance that extractable at high ionic strength solution. While, injected meat with calcium chloride and sodium bicarbonate is recognized as a mean of enhancing meat tenderness; however, the application of the solutions on tenderness and organoleptic properties in rumen viscera has little information.

The present study was conducted to determine whether $CaCl_2$ or $NaHCO_3$ injection improved tenderness and organoleptic properties such as pH, WHC and cooking loss of rumen viscera. To determine the effect of $CaCl_2$ and $NaHCO_3$ injection on changes in protein structure, the soluble protein extracted from bovine rumen viscera samples after incubated according to the storage period was also studied.

MATERIALS AND METHODS

Source of sample and treatment preparation: Six of rumen viscera samples were collected on different days from local market in Kamphaeng Phet Province, Thailand. Each sample was divided into four sections. One of them served as the control at 0 time. The other three were then randomly assigned to 3 treatments consisting of, non-injected control, 2.2% $CaCl_2$ (feed grade) injection and 3.0% $NaHCO_3$ (feed grade) injection. Samples were injected using hand held injector (1 mL disposal syringe) to a target of 110% of initial weight. Each injection had 2.5 cm separation apart. After the injection, each rumen viscera section was sub-divided into 2 pieces and then allotted to either 24 or 48 h storage time treatment.

In each treatment, sample assessed for cook loss and shear force was cut from the piece of rumen viscera, covered with plastic film and held at 4°C in refrigerator. At the end of each storage time, the sample was blotted, weighed and vacuum packed, then stored at -20°C, until the determinations. The remaining sample used to examine the treatment effects on pH, water holding capacity and extractable protein was kept in the refrigerator and done immediately at the end of each storage time.

Water holding capacity determination: The centrifugation method is applied for evaluating the Water Holding Capacity (WHC) of treatment sample. The procedure was followed according to Honikel and Hamm (1994). In brief, a weighed of sample treatment (3-4 g) was placed in centrifuge tube, which bedding at the bottom with glass beads. The samples were then centrifuged at 6,000 g for 20 min. After the centrifugation, the sample was removed by forceps and weighed immediately. WHC (%) was calculated as follows:

$$\text{WHC (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

pH value determination: The pH of the sample, taken before and after the storage times, was determined by homogenizing 2 g of the sample at 23000 rpm with 20 mL of 5 mM Na-iodoacetate in 150 mM KCl (Bernthal *et al.*, 1989). The slurry was filtrated through Whatman paper No. 4 and the pH of the resultant filtrate was measured with standard pH meter (Model S20, Mettler-Toledo, Switzerland) equipped with a pH electrode (Model Inlab 413).

Cook loss determination: Cook loss was determined as outlined by Sheard and Tali (2004). Vacuum pack of rumen viscera samples were thawed overnight in refrigerator, then placed in a bath with preheated water at 80°C for approximately, 1.5 h and cooked to a center temperature of 70°C, checked using needle thermocouple thermometer (Temp JKT, Eutech Instruments Pte Ltd., Singapore). After being cooked, the samples were then removed from the pack, blotted and left to cool down at room temperature for 2 h. Samples were then weighed and cook loss was calculated as follows:

$$\text{Cook loss (\%)} = \frac{\text{Weight loss after cooking}}{\text{Raw sample weight}} \times 100$$

Shear force determination: Following the cook loss determination, rumen viscera samples were prepared for assessment of tenderness. Two 1.3 cm in diameter cores were removed from the center portion of each sample using a cork borer. Thickness of each core was measured and then sheared perpendicular to the axis fiber with

Texture Analyzer (Model TA-XT plus, Stable Micro Systems, UK) equipped with a Warner-Bratzler type shear blade using a cross head speed of 200 mm min^{-1}. The results were expressed as kg, the means of two shears values were used for statistical analysis.

Extractable protein: Prior to and after each incubation time, the treatment samples were examined for total and extractable protein. The procedure for determining extractable protein was a modification of the procedure used by Bernthal *et al.* (1989). Briefly, 5 g sample of each treatment was diluted to 20 mL with 1 M NaCl. Samples were homogenized at 23,000 rpm for 10 sec and allowed to stand for 3 min. The slurry was then centrifuged at 6,000 g for 12 min. After centrifugation, the supernatant was decanted and analyzed for protein concentration by the Kjeldahl nitrogen method. It was assumed that solubilized myofibrillar proteins (which, presumably had dissociated from the myofilaments) remained in the supernatant. Total protein in the rumen viscera sample was also determined by the Kjeldahl nitrogen method. The percentage of extractable protein was calculated as the protein concentration of the supernatant divided by the total protein concentration.

Statistical analysis: Data were analyzed as a split-plot design using General Linear Models Procedure in SAS (SAS Institute, Inc., Cary, NC). Replication was based on the six different rumen viscera samples and the experimental unit was a piece of rumen viscera after sub-dividing. Replication and injected treatment were included in the model as whole-plot factors and the effect of injected treatment was tested with replication x injected treatment as the error term. The split-plot factors were storage time and injected treatment x storage time. These factors were tested with residual error. Additionally, in the shear force data analysis, sample thickness values were used as covariates for the shear force variables. For all statistical analysis, an alpha level of 0.05 was used to determine statistical significance. And whenever significant, the means were separated using Tukey's studentized range (HSD) test.

RESULTS

Water holding capacity: The effects of calcium chloride and sodium bicarbonate on WHC are shown on Fig. 1. Mean values of percentage WHC for calcium chloride and sodium bicarbonate treatment at the two incubation times were higher than control; however, their difference did not reach significant ($p>0.05$). It was also found that WHC did not differ between calcium chloride and sodium bicarbonate at both of incubation times.

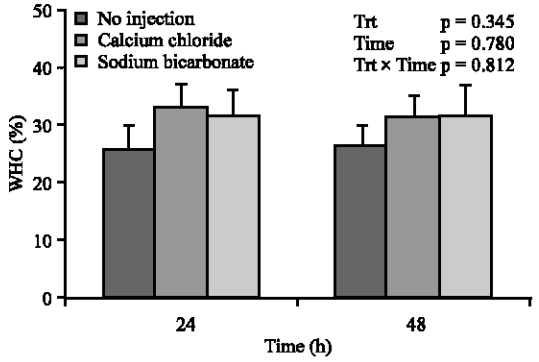

Fig. 1: Effects of control treatments on percentage Water Holding Capacity (WHC) of rumen viscera samples at 24 and 48 h. Each mean is based on 6 samples with duplicate determinations; bars on the graph indicate standard errors (Trt: treatment; Time: Storage time; Trt × Time: treatment by time interaction)

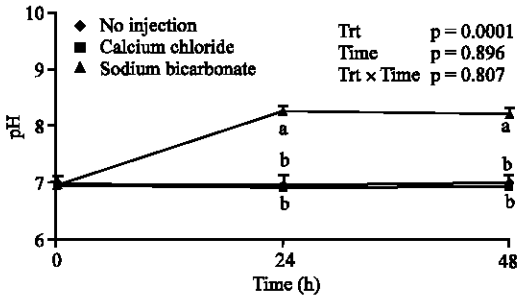

Fig. 2: Effects of control treatments on pH of rumen viscera samples at 24 and 48 h. Each mean is based on 6 samples with duplicate determinations; bars on the graph indicate standard errors (Trt = treatment; Time = storage time; Trt × Time = treatment by time interaction). $^{a, b}$: Mean values of treated samples, within the same storage time, bearing different superscripts are significantly different ($p<0.05$)

pH values: Prior to the injection, pH of the treatment solutions were measured. The injected solution pH was found to be 6.1 for $CaCl_2$ and 8.9 for $NaHCO_3$. The changes in sample pH during the storage times are shown in Fig. 2. Samples treated with $NaHCO_3$ solution had increased in pH from the initial pH value of 7.0 to the pH value of 8.25 at 24 h and then held at this pH for the remainder of the storage times. On the contrary, no changes in pH were found in the control and $CaCl_2$ injected samples. Evidences in this present study indicated the ineffectiveness of $CaCl_2$ on the sample pH which, did not differ significantly from the controlled pH.

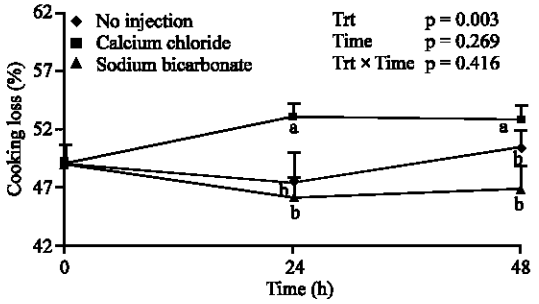

Fig. 3: Effects of control treatments on percentage cooking loss of rumen viscera samples at 24 and 48 h. Each mean is based on 6 samples with duplicate determinations; bars on the graph indicate standard errors (Trt: Treatment; Time: Storage time; Trt × Time: Treatment by time interaction). [a, b]: Mean values of treated samples, within the same storage time, bearing different superscripts are significantly different ($p<0.05$)

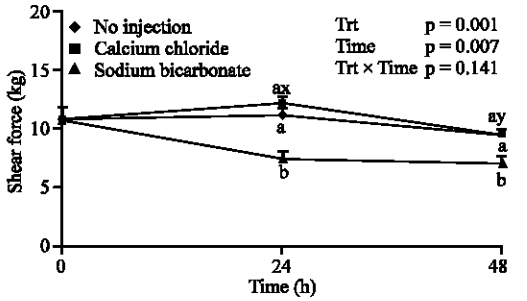

Fig. 4: Effects of control treatments on shear force of rumen viscera samples at 24 and 48 h. Each mean is based on 6 samples with duplicate determinations; bars on the graph indicate standard errors (Trt: Treatment; Time: Storage time; Trt × Time: Treatment by time interaction). [a, b]: Mean values of treated samples, within the same storage time, bearing different superscripts are significantly different ($p<0.05$), [x, y]: Mean values of treated samples, within the same treatment, bearing different superscripts are significantly different ($p<0.05$)

Cooking loss: It was found that the loss for $CaCl_2$ solution treatment was greater than the control and $NaHCO_3$ solution treatment ($p<0.05$) (Fig. 3). No significant difference in cooking loss was found between the storage times in each treatment. This result indicated the lower yield in the $CaCl_2$ injected sample than those of the $NaHCO_3$ treatment.

Shear force values: Figure 4 shows, the shear force values of treatment effects for rumen viscera samples. The

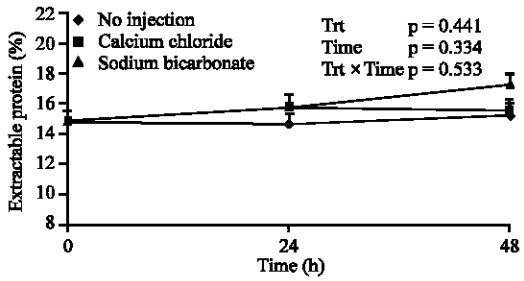

Fig. 5: Effects of control treatments on percentage extractable protein of rumen viscera samples at 24 and 48 h. Each mean is based on 6 samples with duplicate determinations; bars on the graph indicate standard errors (Trt: Treatment; Time: Storage time; Trt × Time: Treatment by time interaction)

mean value and standard deviation of rumen viscera core samples thickness used in this experiment were 0.54±0.12 cm. When, the data was analyzed using sample thickness as a covariate, it was found that the $NaHCO_3$ solution treatment revealed lower shear force values than $CaCl_2$ solution treatment and control at both storage times ($p<0.05$). There were statistic differences between the storage times in the calcium chloride treatment. At 48 h, sample injected with calcium chloride showed lower shear force value than at 24 h, however, both values were not difference from those of the controlled sample. Result indicated that $CaCl_2$ treatment had no effects on tenderness of rumen viscera.

Extraction protein: Results of protein extraction are shown in Fig. 5. It was found that the percentage of extractable proteins revealed no treatments difference at both of the incubating period even though the $CaCl_2$ and $NaHCO_3$ had higher percentage extraction proteins than the control treatment, however the difference did not reach significant ($p>0.05$). It should also be noticed to the extractable protein in control sample which, explicated unchanged even though, the incubation time increased from 24-48 h. This could be suggested that the structural protein did not have any proteolytic degradation during the incubation time.

DISCUSSION

Results on the percentage WHC of treatments sample demonstrate that calcium chloride and sodium bicarbonate do not improve WHC in rumen viscera. Earlier research has already shown the positive effect of injecting sodium bicarbonate on beef WHC (Bouton *et al*., 1973). Sodium bicarbonate injection increases pH and swelling muscle

fiber (Offer and Trinick, 1983). This led the free water to be immobilized and the intact tissue could able to hold water (Honikel and Hamm, 1994). The result on WHC of sodium bicarbonate treatment did not support to these observations. Since, most of the water present in muscle is present in the myofibrils (Lawrie, 1985). A possible explanation may be that rumen viscera, compared to the meat, has more connective tissue and consequently, a lower content of myofibrils, then a lower capacity to bind water. If such was a case, then the small extent of swollen myofibril due to sodium bicarbonate injection may limit WHC to achieve. However, to support this suggestion, more work is needed to define the contents of myofibrils and connective tissue in rumen viscera.

The pH data presented here demonstrated the stability of pH samples injected with calcium chloride. Koohmaraie *et al.* (1989) reported that calcium chloride had no effect on the final pH value of meat. Evidence in this present study also supported the ineffectiveness of calcium chloride on the sample pH, which did not differ significantly from the controlled pH. However, it should be noticed to the stabilization in pH of the control and calcium chloride treatment samples. Normally, the post mortem pH in meat fall as influenced by glycogen converted to lactic acid (Lawrie, 1985). The unchanged in pH of controlled treatment (no injection) during storage indicated that rumen viscera may have different bio-chemical alteration from that of muscle. The bio-chemical pathway responses to the stability of pH in rumen viscera post mortem could not be elucidated in this study and need further investigation.

The high ultimate pH of pork injected with 3% of bicarbonate marinade solution has been reported by Sheard and Tali (2004). Thus, injection with sodium bicarbonate elevated pH in the rumen viscera sample was expected in this study. However, the pH values from those reports were less than the value reported herein. Due to the unaltered in pH of the controlled treatment sample thus, the elevated of pH in the sodium bicarbonate sample could be reflexed solely to the high pH in sodium bicarbonate solution.

Heat during cooking can cause the muscle protein denatures, which allow bounded-water to release from the meat. Although, the injected solution treatments did not have an effect on WHC during the storage, the $NaHCO_3$ injected sample revealed lower percent in cooking loss than the $CaCl_2$ injected samples. Injected meat with $NaHCO_3$ solution resulted in lowering cooking loss and improvement in percentage yield has also been reported by Wynveen *et al.* (2001). The lowering in percentage cooking loss in $NaHCO_3$ injected sample compared to the $CaCl_2$ injected sample may due to the disruption in cellular structure of sample during heating. Sorheim *et al.* (2004) found that $NaHCO_3$ generated carbon dioxide gas during cooking and altered the structure of muscle fiber. Sheard and Tali (2004) demonstrated the air-filled pockets around fiber bundles and longitudinal splits of cooked pork loin treated with 3% bicarbonate. Therefore, in the present study, it is possible to suggest the porous structure probably induced by heating could be responsible, in part, for the ability of $NaHCO_3$ injected samples in retaining the excess water released.

Numerous reports indicated that the addition of exogenous Ca^{2+} results in an enhancement of meat tenderness (Koohmaraie *et al.*, 1990; Morgan *et al.*, 1991). This tenderness improvement has been through the activation of the calpain proteinases, which degrade the myofibril protein such as titin, desmin and troponin (Goll *et al.*, 1983; Whipple and Koohmaraie, 1991). However, in the present study, injection of $CaCl_2$ to rumen viscera sample was found no improvement in tenderness. The reason other than the small extent of myofibril protein in rumen viscera as has been suggested previously may be attributed to the enzyme inactivated. The stabilization in pH of the treatment samples during the storage may respond to this effect. Dransfield (1994) stated that action of the inhibitor of calpain, calpastatin, was pH dependent. As the pH meat declines, the binding of activated calpain to calpastation is reduced and the level of (free) activated calpain rises, then tenderization increases. Normally, the post mortem pH in meat fall as influenced by glycogen converted to lactic acid (Lawrie, 1985). However, in this study the pH sample of control and $CaCl_2$ treatments did not alter with progressing time. The stabilization in pH may prevent calpain to be activated. Data on the extractable protein supported to this suggestion. The results on the extractable protein presented here showed that $CaCl_2$ injected did not cause any apparent solubilization of the proteins in the rumen viscera samples. Therefore, it is possible to suggest that the structural protein was not degraded and hydrolyzed by calpain.

There was little information about the effect of sodium bicarbonate on protein degradation. So, it is difficult to compare the result with limited reported finding. Anyway, evidence on the extractable protein of sodium bicarbonate treatment, which did not differ from the control lead us to suggest that the myofibril protein was not affected by sodium bicarbonate injection.

The improvement in tenderness of rumen viscera sample had been seen in the $NaHCO_3$ infusion treatment. This may due to the porous structure generated by carbon dioxide gas during cooking as previously described. The porous structure could damage the structural myofibrils and connective tissue and pocket air-filled could dilute the load-bearing in sample, allowing the blade of Warner-Bratzler to penetrate more easily, thus reduce the shear force value. Sheard and Tali (2004) also, reported the pork samples treated with sodium bicarbonate has an unusual porous structure, which may

have contributed to the reduction in toughness. So, the observed improvement in tenderness according to $NaHCO_3$ infusion could presumably due to structural changes in the myofibrils and connective tissue of rumen viscera sample. It should be also noted that the tenderization as a result of $NaHCO_3$ injection to rumen viscera samples have been completed within 24 h storage time, thus the storage beyond this point would not be necessary.

CONCLUSION

The results of this study indicated that the $CaCl_2$ infusion had no effect on the improvement of tenderness in rumen viscera. This could be due to the stabilization in pH of rumen viscera, which determined the calpalstatin, the calpain-inhibitor, activity. On contrary, the $NaHCO_3$ infusion decreased cooking loss, which in turn improved juiciness and had success in reducing WBS values. The observed improvement in tenderness and juiciness of sample injected with $NaHCO_3$ could be due to the structural changes that disrupt myofibril and connective tissue integrity.

IMPLICATION

In this study, $NaHCO_3$ injection showed the advantage in improving tenderness and juiciness of rumen viscera. The procedure eliminated the need for high temperature cooking to maximize meat tenderness. However, further studies will have to be conducted to investigate the effect of this procedure on other important characteristics (e.g., flavor and sensory panel ratings) before any recommendation about application can be made.

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