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Discussion
Meat Tenderness

J. Regenstein: Bruce, did you try a quadratic fit or some higher fit which may give you more curvature? On computers these days, would some of those fits actually give you a slightly better fit of your data?

B. Marsh: I think that was tried, Joe. Possibly Tom Ringkob could help me out there. I believe they did try up to about the fifth power and this is the better one. This came out as good as any.

Regenstein: Mohammad, on the experiment where you had the three different muscles with the initial Warner Bratzler shear values being very different, the final being all the same; I don't follow the logic of why those three muscles have different levels of calcium-activated protease, or CDP as you call it. It is not clear to me that it fits the explanation you gave, because they all come down to the same level. So the *psoas* would have no activity that is actually related to it. The others have more room to go. It seems to be that there is a bottom, so that the logic of that is what I was a little unhappy with.

M. Koohmaraie: You can't tell your whole life story in 40 minutes. If you noticed, I said at least "theoretically" when I was talking about that. Now there are three different muscles that have three different fiber type compositions. They have differences as far as function is concerned, and there are a lot of things different other than CDP alone. One of the major explanations that I have to come up with, since *psoas major* has only 50% of the activity in the *longissimus* (LD), is how come it doesn't go down? OK? The *psoas major* contains a large percentage of red fibers. The work at Wisconsin from about 20 to 30 years ago showed the zinc content of the red fibers is about 3 to 4-fold higher than that in white fibers. We are working on this problem right now. We have 30 documents which show that zinc concentration of a sub-physiological level would completely inhibit the CDP activity. So simply that's why I said "theoretically." Interpretation of the data by itself is not the whole story. There is a lot to analyze. The zinc content in *psoas major* is expected to be much higher than in the LD and that could be an explanation why you don't get any tenderization with *psoas major*. We are addressing that right now.

Marsh: I would just like to add a point there. I think there are two points that do need to be watched in this sort of comparison, Mohammad. First of all, the *psoas* is tender, exceptionally tender to begin with, largely because it is a stretched muscle on the carcass. The sarcomere lengths are very long, far longer than 2 microns. The other point is that when muscles have tenderized down to a certain value, no matter which muscles we are looking at, they are just not going to tenderize any further, regardless of what enzyme you have present. We find with our beef, which is all *longissimus* muscle, if we have them at the start without aging with 8 Warner Bratzler units, they might come down to 3 units within 3 weeks. Others that started with 4 units were reasonably tender, but also would come down to 3. I think there is a base line at which we hit this sort of background toughness, if you like, which is not going to be affected. So I do feel you should be cautious in transliterating this work among different muscles. The *psoas* is already about as tender as it was going to be and I don't think that a large or small amount of CAP would really make any difference to further tenderization.

Koohmaraie: I kind of disagree and kind of agree, but I have to say that I disagree with you. We find shear data in the literature which goes down to 2. Why doesn't the *psoas major* go to 2? Myofibrillar fragmentation, as far as I am concerned, is the best estimate of tenderness. Shear force value of cooked steaks and so forth has some problems. If you measure myofibrillar fragmentation index, it predicts that *psoas major* is a tough muscle. It does not fragment at all. Why?

Marsh: If that's the case, I suggest that myofibrillar fragmentation index is a very poor determinant of tenderness.

R. Merkel: Has anybody done it (MFI) with rabbits? It doesn't work.

G. Trout: I have a question for Dr. Koohmaraie. Maybe I can't recollect correctly, but when you did the infusion study I thought you used a Tris buffer. Is that right?

Koohmaraie: No, not in the infusion. The slice study was in a buffer. The infusion was just water and calcium chloride.

Trout: So there was no buffer?

Koohmaraie: No, just a .3 molar calcium chloride, neutralized.

Trout: I am talking about the study where you used slices with calcium chloride.

Koohmaraie: That was in a buffer.

Trout: What was the pH of the buffer that you used?

Koohmaraie: The pH was 7.2.

Trout: So then you're using optimal conditions for either of the CAPS.

Koohmaraie: That's right.

Trout: But it's not the optimum for the lysosomal proteinases.

Koohmaraie: That's correct. That's why I said that we were trying to maximize the conditions for CDP.

Trout: Or to eliminate the possibility of lysosomal enzyme activity.

Koohmaraie: Well, it would. That's why I said "under these conditions." You put your eggs in a basket that you believe you have confidence in.

Trout: You could have easily done it at a low pH.

Koohmaraie: Sure I could.

Trout: And obtain the same results?

Koohmaraie: Would I get the same results?

Trout: Would the CAP be so important at the lower pH?

Koohmaraie: Right. We got a paper in food science that shows the activity of CDP under actual postmortem conditions only 24% to 28%. There is a misconception that the protease has to be maximally active to do the damage. Dr. Marsh has mentioned that if you break only one Z line out of every 250, such an undetectable change results in a dramatic improvement in tenderness. So a protease doesn't have to be maximally active to do the damage. If you incubate myofibrils with CDP, all the CDPs are gone and all the Z lines are gone because CDP is maximally active. But you don't see any changes like that as the result of postmortem aging. Z lines are weak and they don't disappear.

Trout: The other thing I was wondering about is that you were saying that CAP is very important in the animal, in the sheep, where you injected the calcium into the sheep. Isn't that also a condition where you have high pH? Don't you have high pH and high temperature conditions which are also typical for CAP optimization, but not for lysosomal enzyme optimization?

Koohmaraie: If you recall, we electrically stimulated the carcasses before we infused them.

Trout: Did you monitor the pH of these?

Koohmaraie: Of course we did. The pH was down to 6.2 at the time we started the infusion.

Trout: So it was moderately high compared to normal beef then?

Koohmaraie: No. Our control started around pH 7 and when we infused the animal, the pH was about 6.2.

Trout: It is still higher than the normal pH 5.5?

Koohmaraie: Sure.

Trout: What I'm getting at is the fact that the conditions you used are not completely identical to those stages where you have pH of 5.5.

Koohmaraie: No, I never said that. I never said that we were simulating what happens under postmortem conditions. We did a whole set of experiments. We wanted to find out what are the changes associated with postmortem aging.

M. Dikeman: My comments may be directed towards Bruce Marsh. If you look at your curve on your tenderness at high and low pH's and so on, I guess I would have reacted almost the opposite of what you did. I would have expected the low pH muscle to be less tender because protein denaturation would reduce water-holding capacity, and at a lower pH a CAP or CDP would not be very active. Then on the other end of your curve, at high pH in the cold-shortening area, you seem to show a fairly definite response to aging in that muscle, a dramatic decrease in shear value. I guess I would not have expected that because I understood that cold-shortened muscle does not respond very well to aging.

Marsh: Those are a couple of astute comments, Michael, and I don't know if I can deal with them adequately here. First of all I know many theories of meat tenderness and toughness have been based on water-holding capacity. I decline that water-holding capacity is supposed to correspond to toughening, but bear in mind that all these samples were of the same low ultimate pH and therefore, we would expect, (they were 3-hour pH values we were looking at; not ultimate pH values) all of these samples did in fact reach the same low range of ultimate pH. I think the extremes were 5.30 and 5.56. In terms of their water-holding capacity, at least with instruments by ultimate pH, we would not expect the water-holding capacity to be very different and therefore, on the water-holding capacity concept of tenderness and toughness, we would not expect a very big range in toughness and tenderness in any of them. I do want to clearly distinguish pH 3 from pH U or if you like 3-hour pH from ultimate pH.

The other point is an important one. You would not have expected so much aging in the highly cold-shortened one, is that right? We were somewhat surprised as well, but I think there are a few points to be taken into account. First of all, regarding the observations or the conclusions that cold-shortened meat does not age (I think that was the work of Damon Gilbert about 1969) I am not denying its validity, but I would point out that they were virtually maximally cold-shortening their muscles. They did get some aging with intermediate shortening. It was only when they got right up to about 40% shortening that they got no aging at all. And when we checked the sarcomere lengths of some of our samples here, we very rarely went beyond 25% shortening. So we were still nowhere near the peak of shortening, as it were. Therefore, we would not have eliminated the aging possibility.

The other point I think that this does bring out is an important point that I just briefly mentioned at one stage. We have to be tremendously careful when we start extrapolating results from isolated systems back to the carcass. Regarding this whole question about cold shortening, we have evidence that it does operate on the carcass and it does make a difference, but under quite different extents. The fact that we have never yet observed the sarcomere lengths in *longissimus* muscle shorter than 1.53 microns, corresponding to about 25% shortening for instance, means that all the

excised muscle work where we talked about 40%, 50%, 60% shortening is totally irrelevant to the carcass situation. So I am glad that you raised that question. It does allow me to speak to the important principle that we can't just extrapolate from excised muscle studies or isolated protein studies straight back to the carcass.

C. Calkins: I have a question for Dr. Koohmaraie regarding localization of CDP and inhibitor and how that might influence their impact on tenderization. I would like to follow that with a question relative to changes that occur postmortem in ionic strength, in pH and how the changes in pH and ionic strength might influence fragility of membranes or release of lysosomal enzymes or detachment of the inhibitor or CDP itself from membranes and so on.

Koohmaraie: I will answer the second question first. I will expand on what Dr. Marsh said. We can observe things for just so long. After a period of time we have to start collecting evidence for it. I guess as you refer to fragility, you are asking if we get release of lysosomal enzymes, is that correct?

Calkins: I am asking: Do you think the changes that occur postmortem, mainly a drop in pH and a change in ionic strength, would that impact on release of lysosomal enzymes?

Koohmaraie: I have heard the theory that the faster you drop the pH while the temperature is high, this causes damage in the lysosomal membrane and that releases the enzyme. That is fine, I have no problem with that as long as it is a theory. But once you get to accepting this hypothesis, I'd like to see some evidence of that, and there is no evidence of that. The only work that has been done is by a French group. They looked at the localization of enzymes with immunofluorescent techniques. They had antibodies to cathepsins B, H and L. They looked at the release of lysosomal enzyme from the lysosomes at different days postmortem, even up to 30 days in the carcasses that we electrically stimulated. They failed to show any release of cathepsin B. The only reason that I did not mention that is because that was only from one animal. That is why I put it up there. We have got antibodies to cathepsins B, H and L. It is a very easy thing to do. Let's go back to localization of CDP-I and CDP-II and inhibitor. CDP-I is definitely cytoplasmic. It is in the cytosol of skeletal muscle cells. As for CDP-II, there is more and more evidence now that it appears to be membrane associated. This may be so for CDP inhibitors, but it is also found in the cytosol.

Calkins: Did you say that CDP inhibitor is in the cytosol, as well as CDP-I?

Koohmaraie: I said CDP-I is cytosolic, CDP-II is membrane associated and inhibitors have been found in the cytosol and membrane, associated with the membrane.

Calkins: Do you think that the presence of the inhibitor within the cytosol would moderate the activity of the CDP-I postmortem?

Koohmaraie: Theoretically, it should. Endogenous inhibitor of the protease somehow is involved in the regulation of the protease activity, but how I don't know at this point.

R. Rogers: Bruce, you kind of indicated that you thought electrical stimulation was for cosmetic effect. I would contend that that is not the case at all. In pre-rigor operations, even from stimulating young, tender-enough meat and from our experiences with a company in Mississippi, if we don't stimulate and wait at least 2 hours before excising the muscle, you simply cannot eat 75% of the meat.

Marsh: I'm sorry, I missed the first part of that.

Rogers: You said that electric stimulation was for cosmetic effects and I contend at least in pre-rigor operations in cow boning plants that it's certainly more than cosmetic, it's essential.

Marsh; This is essentially hot-boning operations?

Rogers: Yes.

Marsh: My statement was obviously not meant to cover the hot-boning situation. The cosmetic effects, etc. obviously only apply to carcass-attached muscles. Certainly in hot-boning operations, electrical stimulation does a pretty terrific job of tenderizing on whole sides that are not boned out for at least 24 hours postmortem. Somebody said to me the other night that if it were not for the cosmetic effects there would be virtually no electric stimulation used in this country at all. It is the cosmetic effect, the setting up of the marbling, the effects on heat ring and so on, which are the principal reasons for use. I think this makes for an unfortunate situation, because if we could only hit this appropriate rate of early postmortem glycolysis, we could make virtually all our beef up to a reasonable standard without the need for any aging at all. It is just that at the moment we are using it in the wrong way, and unfortunately we can't tell you just what is the right way.

S. Smith: I have a question for Mohammad. Wu and I stressed in the paper that you referenced at the end, that there is some importance of the synergism on the interaction between proteases and ionic strength. The question that came to my mind is: Are you considering or have you infused sheep with both sodium chloride and calcium and seen if there is any kind of additivity or synergism? When we mixed trypsin, pre-treated myofibrils and trypsin, and exposed those to high ionic strengths, the myofibrils disappeared. So it looked like things could go to mush in combination with ionic strength and protease.

Koohmaraie: You are asking me if I would consider infusing sodium chloride and calcium chloride together simultaneously? I have not actually.

Smith: Bob, since you referenced some abstracts that are going to be presented this summer, I would just like to mention Ann Schiavett's, only because the data is up front so people can look at it. She did see in her experimental design some changes in lean texture that were very apparent, even after 90 days withdrawal, and in the marbling, a reduction in marbling, that we consider important because it drops a carcass down from a Choice grade down to a Select grade. So we thought it was not a trivial decrease in marbling score and that also persisted after 90-day withdrawal.

Merkel: Is this because of textural properties?

Smith: No, we don't think those are related, we don't know. There were two things that happened. One is a textural change that was very visible and another was that there was a decrease, both in her study and Mark Miller's study, in marbling that resulted in a full decrease in quality grade. This may be important to consider. It just seemed like you gave the impression that maybe these weren't very marked reductions in the marbling.

Merkel: Well I didn't mean to imply that in the ruminant species, if that came across that way I didn't intend it that way. In pigs, I don't think there is any concern for any possible changes in marbling whether it is increase/decrease or whatever. But in the ruminant species that may be another

matter. As I say in the conclusions, to some degree that is probably related to the length of time the agonist is fed. In rats, for instance, in work presented by Romsos and coworkers at the Federation Meetings, the entire effect is over in one week. I think that might even be suggested in some of the work of Don Beermann's in the 3 and 6-week study. So, the prolonged administration may not be necessary to, in other words, optimize quality and still have a tremendous benefit in either production traits or the repartitioning.

Beermann: I would like to add one quick comment, Bob. Regarding effect of somatotropin on marbling, in the pituitary-derived somatotropin trial, we found a very significant linear decline in percent extractable lipids in two hind leg muscles, the *semimembranosus* and *semitendinosus*. It was maximally reduced 50% with the 200 microgram per kilogram dose. In the recombinant form study, we observed again a linear decline in percent extractable lipid in the *longissimus* muscle with increasing dose. In that study we started with pigs that had only 2.02% lipid, and brought it down to .7 and .8%. So I guess in that regard I would express, and have expressed, some concern that when using somatotropin, we may need to keep in mind that response is variable. As an end target, if we reduce lipids below a necessary threshold level that might be considered essential for optimum palatability, we may create some deleterious effects.

K. Killday: Mohammad, in the study where you had the control and then you added the pH 7 buffer to the control, it appeared that there was less proteolysis in the pH 7 buffer system. Since calcium-dependent protease is actually supposed to be most active there, one would expect the opposite, since the lysosomal enzyme has a lower pH maximum.

Koohmaraie: I assume you are referring to the buffer treatment where we kept them in the buffer. You are 100% correct. We saw that in terms of myofibril fragmentation index, it did not go as high as the same magnitude of control. It was also true in the case of CDP's, if you recall. It did not go down to the extent that the control went down. If this is strictly a hypothesis, what we think is happening by putting the slices in such a large volume of buffer is that we could possibly have diluted the concentration available intracellularly. Therefore, if you believe in the CDP hypothesis, you accept that you have less calcium for activation of CDPs and you saw that in the CDP activity decline. Activity was not as much as in the control.

Powell: Regarding the statement, first of all, about beef carcasses. In the domestic scene in our country, cattle tend to dress out carcass weights of 180 to 220 kilos with about 5 to 10 mm of fat. Quite light in relation to your cattle, and these are primarily all fed on grass. The cooling rate is substantially quite quick, and we find that electrical stimulation is absolutely necessary to give out a consistently tender product. Without stimulation on those carcasses, at least 80% of them will eat tough. Electrical stimulation turns that scene all around and we can almost guarantee now with the systems that we have in place, the result is not cosmetic, but does produce a more uniform tender product. I didn't really want to say that, however I want to come back to lamb now.

Lamb hasn't been discussed here, but I would like to suggest that the reason electrical stimulation was used by the New Zealanders was not really to prevent the cold shortening on the cooling side, it was to prevent the rigor shortening when those frozen carcasses were thawed. So that you have the fast glycolytic rate with the electrically-stimulated lambs, such that they didn't rigor shorten when they were thawed. Now, I present that because we were under intense pressure in Australia to recommend electrical stimulation to chilled lambs entering the domestic market. Now I think Dr. Marsh may have given us the explanation why we in fact didn't recommend electrical stimulation for lamb. They cool very quickly and as a consequence they may be covered in the low pH range that he was referring to. They cooled quickly and turned over quickly down to the low pH end and as a result they wouldn't tenderize. He was seeing at 24 hours a tenderization profile in production, for non-stimulated lambs pretty much equivalent to that for the stimulated lambs, and hence we thought there may have been something inherently different between the beef and lamb species. But I think that the explanation may well be in those 3-hour pH profiles.

Marsh: That may come into it. Let me just go back to your first point concerning the cosmetic effect. I was referring there, and I think I said specifically in this country, I am very well aware of the much faster cooling rates that Australia, New Zealand and other countries have to use to satisfy EEC requirements, for instance. Very much more rapid and efficient chillers are used in this country (Australia) and also the leaner and somewhat smaller animals than the 1 to 1½ inch of fat which is what we frequently see in the U.S. So, I wasn't making those remarks specifically there. Countries like Australia and New Zealand, most European countries and certainly South American countries, (if you have ever eaten five-year old Zebu cattle in Brazil for instance, absolutely devoid of any fat cover at all), you'd realize just how essential electrical stimulation can be under some circumstances. Regarding the other point concerning lambs, you could have some good points there. I would just point out, however, one slight correction. When New Zealand started electrically stimulating lamb, it wasn't just to overcome the thaw shortening problem, you were referring to rigor shortening at a later stage, it was also to overcome the cold shortening. If it had been only for the thaw shortening, then they would have applied it only to the lambs coming to North America where it is the usual practice to cook from the frozen state. They wouldn't have bothered electrically stimulating the lamb going to Britain where it's much more usual to thaw the meat out, in which case you wouldn't have any thaw shortening.