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Discussion

M. Dikeman: It appeared in the slides that there were distinct differences in carcass length and I wondered if you had any measures of bone development, such as length of long bones or ossification differences?

D. Beermann: That is an interesting observation. We noticed that the carcasses were shorter in the first study but we made no measurements. In the second, we collected both carcass and metatarsal bone length (lower rear leg). There were no differences in metatarsal bone length but carcass length was shorter in the cimaterol-treated lambs; I interpret that to be the result of the slightly different conformation of the carcass due to the increased muscle. When these carcasses were fabricated into wholesale cuts, we had difficulty making typical blade chop cuts from the front end of the cimaterol carcasses. We did look at ossification and there were no marked differences.

S. Smith: I have a couple of questions, Don. First, where you show a depression in plasma insulin and an increase in FFA. Since insulin is required in nonruminants (rats especially) to depress lipolysis by making more blood glucose available to the tissue, are you certain that the elevated FFA that you see is not due to the fact that you depress insulin levels?

Beermann: We really can't be certain, Steve, but this is a point that needs to be made. Certainly there are species differences; very marked differences, especially for GH effects in rats versus ruminants. We really can't explain why a beta agonist causes these effects.

Smith: We call these repartitioning agents and perhaps that implies what we are seeing in the hypertrophy of muscle and atrophy of adipose tissue is a shunting of energy from one depot to another. Is this a cause or effect? Perhaps cimaterol and clenbuterol are having direct tissue effects and the result is causing more energy to be directed into protein synthesis simply because of increased demand; and, more in my area of interest, decreased adipose tissue accretion because of a direct effect rather than an indirect one.

Beermann: That is certainly a logical explanation for the decreased fat accretion. We would expect a direct lipolytic effect of beta agonists on adipocytes to mobilize FFA. The ruminant is going to depend on FFA as the primary source for energy; so, with the increased metabolic rate, all these are working in the same direction to support the increased rate of metabolism which is associated with protein synthesis and might spare amino acids from being used by the liver for gluconeogenesis, which, of course, is an ongoing process. All this seems to fit in concert, we don't really see any conflicts here.

Smith: Really, the only contradiction we see in our clenbuterol studies is that fat cell size increased while we had expected to see a decrease due to lipolysis. However, we have found no data in the literature indicating changes in fat cell size following lipolysis.

Unknown: What has been the experience in other species with repartitioning agents?

R. Dalrymple: These compounds work in all meat animal species with lambs appearing to be most responsive in terms of repartitioning effect. In finishing swine (50 to 100 kg) we have been seeing increases in longissimus area of 5% to 15% and decreases in backfat of about 10%. One difference from the lamb data is that we see no differences in carcass length. This may be related to the less dramatic effect cimaterol is having in swine as far as increasing musculature is concerned. Cimaterol is also improving feed efficiency in finishing swine by about 5% while having no effect on gain.

Unknown: What about stress susceptibility and meat quality in swine?

Dalrymple: We have measured meat quality in all our studies and have seen no effects on color or firmness and variable effects on marbling. However, none of these hogs have been transported long distances prior to slaughter. When these products are commercially used, we would expect that when marketed following drug administration the animals would have essentially no parent drug or active metabolites in their tissues. Therefore we should not see any ephinephrine-like effects on meat quality in the treated animals.

T. Althen: I have a question about down regulation of beta adrenergic receptors. Don, do you feel that this compound will be effective over a long period of time or are we going to see the effect early on and then a diminishing response?

Beermann: In our studies where we had in-term slaughter data, we saw essentially no diminishing effect or differences with time. The percentage increases in muscle and fat parameters were not different between in-term and final lambs.

R. Merkel: I have a question about the DNA content changes in the muscles of the GH tumor-bearing rats, especially in light of the large changes in muscle mass.

D. Campion: We have looked at the DNA concentration of the soleus and it is actually lower in the tumor-bearing rats. Total DNA is still elevated along with muscle weight. Satellite cell content has been measured and we find about 20% of the muscle fibers of tumor-bearing rats will exhibit satellite cells compared to only 10% in the controls. If we put the tumors into older female rats after they have had their first litter, then we see no effects on satellite cell content. This might imply that maybe with growth hormone, which is significantly elevated, the limiting effect may be the incorporation of the satellite cell daughter nuclei into the muscle fiber as opposed to proliferative action on the satellite cell itself.

E. Allen: I would like to hear some more discussion about the increased metabolic rate and what happens in the animal relative to the energy saved from the standpoint that "nothing is free." What are we giving up here to end up with more muscle? I think you're implying, Don, that we have less fat but how are we increasing metabolic rate that much and yet coming up with a more efficient animal that possibly gains faster? What do you suspect we might be giving up on the other end to come up with the overall net gain?

Beermann: When you think of the energetics of protein vs lipid synthesis, certainly protein requires more energy per se but when carried to muscle growth, where you have a four-to-one water-to-protein ratio, muscle can be more efficiently deposited than adipose tissue.

Allen: When we look in the literature, we see that when metabolic rate has been raised by thyroxine we find opposite effects to those you demonstrated in your lambs.

Beermann: However, there are examples in the literature where thyroxine, when administered at low anabolic levels, will produce increases in gain. We have in fact seen this in rats where T4 has been administered.

D. Cornforth: You have clearly demonstrated effects of beta agonists on young growing animals. Do you think you would see much dramatic effects in adult animals?

Dalrymple: We have done one study in mature female rats and we observed a reinitiation of muscle growth and a decrease in carcass fat. If we can translate this to meat animals, I would have to say "yes, we would expect to see an effect" but how dramatic it would be, I really can't speculate at this point.

R. Cassens: Don, what conclusions do you make from the increases which you saw in thyroid hormones in your cimaterol study? Would you care to speculate if this is a direct or indirect response?

Beermann: There are indications from the literature that catecholamines do in fact increase secretion rates of thyroid hormones. Certainly, the thyroid gland is one of the most vascular and, combined with the increased blood flow, we see we might expect some effect on hormone secretion. We did not really try to establish a cause or effect relationship so it is hard to speculate a direct effect. However, I now feel these effects are significant and are probably worth further study.

P. Bechtel: The differences you are seeing in your cimaterol lambs in subcutaneous fat are not surprising since one would expect a beta-agonist to reduce fat content, but the increases in muscle content are rather striking. What is the mechanism of action causing these changes and have you examined GH, and especially somatomedin levels?

Beermann: With increased metabolic rate, we are perhaps providing the opportunity for increased protein synthesis; at the same time increasing maintenance requirements of the animal but because of the shift in nutrient utilization, maintaining efficiency of growth. There have been rat studies done by Emery and co-workers in the UK which have demonstrated a significant increase in the fractional rate of protein synthesis with clenbuterol in hypertrophied muscles. The literature also indicates that beta-agonists may decrease the rate of protein degradation. There is certainly more work that needs to be done in this area. We did look at GH and saw significantly higher levels at 6 weeks but no differences at 12 weeks. The magnitude of difference at 6 weeks was about 2X (2.3 vs 5.6 ng/ml). Whether this is large enough to be of physiological significance, I really don't know.

M. Bailey: What is the feed efficiency of animals fed a beta-agonist relative to controls? Are they more or less efficient?

Beermann: They were generally more efficient in our lamb studies.

Bailey: Has anyone done any work in pigs?

Dalrymple: In lambs, we have done about 7 or 8 studies and in general see a 10% to 20% improvement in both growth rate and feed efficiency. In swine, we see about 5% improvement in feed efficiency in finishing pigs with no effect on growth rate. In broilers, we have been getting a 2% to 3% growth response and a 1% to 2% improvement in feed efficiency.

R. Kelly: Have you made any observations about animal behavior, hyperactivity, etc., while they were receiving cimaterol in your studies?

Dalrymple: We monitor animal behavior very carefully and have never noticed any signs of hyperactivity during our studies. The only effect that we have ever noticed in this regard was in an earlier clenbuterol cattle study (Ricks et al., 1984) where we saw muscle tremors during the first few weeks of feeding, after which they disappeared for the rest of the treatment period.

Beermann: I would agree, since we saw no real behavioral differences in cimaterol-treated lambs during our studies. In our current studies where we are closely working with individual lambs, we see no evidence of unusual or agressive behavior.

W. Moody: Don, I have two questions. One, I was interested in your fiber-type data and I wonder if you would explain in more detail about what's going on and how it fits with information in the literature relating to muscle hypertrophy? Secondly, I wondered if you observed any difference in the fatty acid composition in these lambs with respect to the subcutaneous fat?

Beermann: I will answer the second question quickly. We have not looked at the fatty acid composition so we don't know if it has changed. Regarding the fiber-type composition, we have used a two-type classification since in lambs it is very difficult to identify the intermediate-type fiber. We saw an equal amount of hypertrophy in both Type I and II fibers in the semitendinosus muscle. In addition to the documented shift in fiber types, we saw a dramatic increase in phosphorylase negatively-stained fibers in the muscles of cimaterol-treated lambs. This is difficult to interpret since I don't believe we are really looking only at the enzyme per se during this staining technique but are probably also measuring the residual fiber glycogen levels. There are, I believe, some major metabolic changes taking place in the cimaterol-treated lambs and these are showing up in the histochemical shifts in fiber type.

Moody: Do you think you can use fiber type as a measure of muscle growth and development?

Beermann: If I had my preference, I would certainly be interested in looking at myosin light chain patterns and other myofibrillar protein subunit compositions. We are not able to do that at this point. An antibody technique would be preferred for such studies.

R. Benedict: Two questions; since clenbuterol is an adrenergic agonist, might you expect the same results with a cholinergic antagonist? Second question; with the fish meal protein, could that be explained by the differences in the lipogenic amino acid content, that is those that might be converted easily to fatty acids?

Beermann: At least as far as the lipid effect i.e. decreased fat accretion, Bob, I would say that we wouldn't expect to see a cholinergic mediated response. Harry Mersmann has clearly documented this where he has seen no effect of cholinergic agents on lipid metabolism. With beta-agonists you see a dramatic effect on lipolysis both in vivo and in vitro. As far as the fish meal effect, maybe the most simple answer is that we expect the fish meal protein to be less degraded by the rumen and provide more available amino acids for uptake. There also may be other compounds, such as biogenic amines and amino acid breakdown products, that may be vasoactive. But as far as I know, there is no other study which demonstrates a muscle hypertrophy response to fish meal in the diet.

L. Orme: A couple of questions. On your analysis on fat and lean, were they chemical or physical separation?

Beermann: We did not do any chemical analysis on these carcasses or cuts. Cyanamid has done extensive chemical composition work on carcasses from animals treated with either clenbuterol or cimaterol. We have analyzed individual muscles for composition but the data have not yet been run through the computer.

Orme: Your data on these sheep here?

Beermann: They were all physical separation, along with carcass measurements such as subcutaneous fat depth, longissimus area, etc.

Orme: Would you describe the cross-sectional picture of these muscles, treated vs untreated, as far as texture of the lean? Was there a difference in it?

Beermann: There was not an appreciable difference in texture but, using the Gardner Color Difference Meter, the treated were less bright and darker red in color; but they were still in the normal range. Shear values on the longissimus and biceps femoris in the second study showed no differences.

Orme: You did not pick up any difference in eye appearance between the two?

Beermann: No, nothing that was discernible. I do want to add that we did do cross-sectional fiber area measurements in the longissimus muscle and found fiber diameter hypertrophy to be only 13%. Which means that, in addition to radial growth, they also had to grow longitudinally to account for the 30% increase in muscle cross-sectional area.

Orme: Was there more water on the muscle surface?

Beermann: No, we saw no difference; if anything, they were dryer in agreement with the higher pH.

Orme: Last question: Don, what would you assume to be the effect of feeding this to replacement females?

Beermann: If you mean the effect on reproductive performance, then I can't venture a guess.

Orme: Have there been any longevity studies done?

Dalrymple: None have been conducted to date in meat animals, but long-term toxicology and several-generation studies will be conducted in rodents as part of the registration program. Longevity and reproduction studies would probably be part of our target animal safety requirements for registration. Another point in this regard is that when we withdraw the drug, the animals tend to return toward their normal adipose tissue content. This reversal also appears to be dose, species- and, of course, time-dependent. More work really must be done to answer if muscle effects are also reversible.

Cornforth: With regard to the percent kidney heart and pelvic (KHP) fat in these lamb carcasses treated with cimaterol, the KHP fat went down. The group from Texas A&M showed that, if anything, their KHP fat did not change or maybe went up a little. Can you explain?

Beermann: I will yield to Ron for this. However, we have used cimaterol in Finn-Dorset lambs and we saw a much smaller response than the data I showed earlier for Dorset and Hampshire-Dorset lambs.

Dalrymple: It may be related to animal age, breed, etc. We have run seven to eight studies with both clenbuterol and cimaterol and have always seen a significant decrease in KHP fat, both on an absolute basis and as a percent of carcass weight. The Texas results were very surprising to me and I can't explain the difference. However, based on my experience, I would have fed a slightly higher clenbuterol level, such as 5 ppm, rather than 2 ppm to maximize the repartitioning response.

Bechtel: Is growth hormone lipolytic?

Campion: That is a good question. Certainly in an in vivo situation in the rat with a high GH level. In the rat, the FFA levels are increased although I cannot say for certain if it is a direct action. In terms of the pig, there is not strong evidence that the FFA levels are elevated, although if you talk to Terry Etherton he would say "Yes, GH has a lipolytic action in the pig, but not to the degree we see in the rat." In the bovine animal (and here I am relying on Dale Bauman's data in older animals), they are not really seeing a lipolytic response except when the cow is in negative nitrogen balance. Would you agree, Don?

Beermann: Yes, I would. In the dairy twin heifer study Bauman conducted with Sejrsen in Denmark starting with 180-kg heifers pair-fed, they found 10% increase in gain with GH administration over a 112-day period and did in fact cause a change in composition, as evidenced by chemical composition measurements on the 9-11th rib section. However, they observed no lipolytic effects in these heifers due to GH administration. I guess I am not convinced that GH is lipolytic in farm animals although I am also not convinced that it is not lipolytic. With the new highly purified recombinant material now becoming available, we may in fact answer this question definitely.

Campion: If I can continue along that line: One of the problems in the rabbit is, for example, if you use pituitary-derived GH, you see a lipolytic response; while if you use recombinant-produced material, you see no response. So I think we have to be cautious of information in the literature where they used pituitary-derived GH and how pure it was. Another possibility of GH (and this goes back to using cell culture, specifically the 3T3-L1 preadipocyte cell line), Howard Green has suggested that GH has a differentiation role or positive effect on the conversion of these preadipocytes to adipocytes. We examined this same question using primary preadipocytes from the rat in our laboratory with Gary Hausman and Roy Martin, and we were not able to demonstrate any effect with GH on the differentiation on process.