Large Southwest Field Trial

	Control	Deccox	Deccox Adv.
Number Cattle	5,108	5,089	
Pens	21	21	
Average Weight (lbs.)	450	450	
Weight Range (lbs.)	400-500	400-500	
Pulled for Treatment	1,087	880	18.4%
Diagnosed Cocci	12	11	
Scours (Non-specific)	189	130	30.9%
Retreats (Mostly Respiratory)	310	267	—1.8%
Deaths	113	90	29%
Realizers	80	62	22.2%
Economics		$2,700	$12,000 (Estimate)
Dead $200			
Realizer $175			
Treated $ 12 (3 days x $4)			

Conclusion

1. Low level (sub clinical) coccidiosis constitutes a serious economic loss.
2. The sub clinical disease of coccidiosis interferes with optimum performance.
3. The severity of the coccidiosis symptoms is affected by stress and can as a disease, contribute to stress.
4. Medication with a coccidiostat can remove one of the stress associated with cattle husbandry, and as a result help reduce the severity of some other disease syndromes.

Suppression of Neutrophil Function in Bovine Respiratory Disease

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During the last seven years or so I have been doing research on bovine respiratory disease at Iowa State University. We have been trying to define the immunosuppression that occurs with the animals susceptible to the bacterial pneumonia, with the ultimate goal of identifying or finding drugs that can be used to reverse immunosuppression. I will go fairly rapidly through our data, just hitting the high points and probably not giving you time to digest all of the data, but I'll try to hit the high points as I go along. As you all are aware I am sure, of the bovine respiratory disease syndrome, or shipping fever, the hypothesis for its pathogenesis is that it requires three things—stress, plus a nonbacterial infection, and these two components suppress the host defense mechanism sufficiently to allow the bacterial infections to produce a severe pneumonia. And the economic losses are primarily due to the bacterial infection.

This talk is going to focus on neutrophil function primarily, and I will talk about how each of those things can suppress neutrophil function. First of all neutrophils are white blood cells found in the blood stream. Polymorphonuclear leukocytes are very active in phagocytosis and destruction of bacteria. They ingest bacteria in the blood stream or in the lungs, wherever they come across them and take them internally. The lysis zones within that neutrophil are little sacks with enzymes that merge with the phagozone containing the bacteria. The enzymes then attempt to degrade and destroy the bacteria and control the infectious process. Neutrophils have several potent mechanisms for killing bacteria, in addition to these enzymes. Neutrophils take oxygen and convert it to hydrogen peroxide, as well as other oxygen radicals more potent than hydrogen peroxide. They also cause a generation of aldehyde, and you are all familiar with the bacteriocidal properties of hydrogen peroxide and aldehydes like formaldehydes and gluteraldehyde. Neutrophils figured out long before man did that you can kill bacteria with these things. So they have mechanisms for generating these products.

When we do these experiments, the first thing that we do is isolate neutrophils from the peripheral blood. We bleed the cattle and go through some steps to get pure neutrophils and we evaluate their function. We use a series of tests. Neutrophils go through a lot of steps in killing bacteria and we can assay most of those steps. So we use a battery of functional assays. We don't really have time to get into the specific assays. I'll be doing a lot of generalization in this talk.

First of all we want to look at stress because that is an important component. One thing that universally occurs with stress is an elevation of cortisol levels, plasma cortisol levels. An animal under stress releases ACTH from its pituitary gland, which causes the adrenal gland to release cortisol. These are conditions that have been reported to cause increased cortisol concentration in cattle. You will recognize

that several of these, for instance, weaning, transportation, handling, castration, dehorning, forced exercise, are all components, things that occur in association with bovine respiratory disease when an animal first arrives in the feed lot. So what we want to look at is the effect of glucocorticoids, specifically cortisol, on the neutrophil function. To summarize several experiments, we find that dexamethazone is a very potent glucocorticoid and it alters every neutrophil function that we can measure. ACTH administration to cattle causes increased cortisol. Cortisol is not as potent. You know there is a couple of the neutrophil functions that we can measure. So we know that stress through cortisol depresses certain aspects of neutrophil function.

We also wanted to look at viruses. What do some of the viruses do in neutrophil function? The one we have chosen to look at is the BVD virus. It is a very interesting virus. There is quite a bit of clinical evidence that it is immunosuppressive and it leads to problems with bacterial infection and other virus infections. So we took two strains of BVD virus, a field isolate and a NADC challenge strain, one cytopathic, one non-cytopathic, infected some antibody-negative steers, and looked at their neutrophil function, as well as several other parameters in the immune function. I will only discuss the neutrophil function right now. When you infect healthy cattle with the challenge strain of BVD almost nothing happens. They run a little bit of a temperature spike, they'll have a mild neutrophenia and lymphophenia, and they might go off feed a little bit. So when you look at neutrophil function, you can see an ionization reaction in neutrophils, one of the important killing mechanisms, was depressed for about three weeks after infection. These cattle didn't get very sick, but their neutrophils were not working right for three weeks after infection, so they were presumably more susceptible to bacterial infection for that whole period. We also wanted to look at the modified live BVD virus. As you are aware, there is a controversy about the modified live virus and some reports of clinical problems after vaccination, so we chose the Singer strain of virus and we gave a group of cattle one dose of modified live BVD, right out of the vaccine bottle, intramuscularly, and we gave one dose intranasally, so we cheated just a little bit on the recommendation from the bottle.

We used 9 treated and 9 control cattle. We wanted to combine the effects of stress with vaccination to try to mimic stress during vaccination. So we had another group that received ACTH that caused increased cortisol. A third group was given ACTH plus the BVD vaccine, to mimic vaccination during stress. We looked at several immune parameters. Neutrophil function in cattle that received the modified live virus was suppressed for a total of thirty days. They did not even come back to normal after thirty days after receiving the vaccine virus.

In the group of cattle that was given the vaccine virus plus ACTH, their neutrophil function was suppressed below that of either the ACTH alone or the vaccine strain of virus. So we get fairly profound suppression of neutrophil function in these cattle with high cortisol and the vaccine strain of virus. That group also had suppressed function for about thirty days.

To summarize the results of our BVD experimentation on neutrophil function, we see that the virulent BVD virus suppressed one aspect of neutrophil function, the modified live strain of virus suppressed two aspects. One of those we did not measure with a virulent virus because presumably it would also be suppressed. When we used the killed BVD vaccine we did not see any suppression of neutrophil function which we would not really expect because the virus is not replicating. ACTH alters two functions. One of those, migration, is enhanced by ACTH. It sounds like that is an improvement but actually that is a detrimental effect. Cattle that get the vaccine virus plus the ACTH have all of the effects. So the BVD virus suppresses neutrophil function and if virus is present in cattle with high cortisol they have more profound suppression.

The third component of bovine respiratory disease is a bacteria. We've shown that at least one of the viruses suppresses neutrophil function and stress suppresses neutrophil function. So the three bacteria that are of primary importance in bovine respiratory disease are *Pasteurella haemolytica, Pasteurella multocida,* and *Haemophilus somnus. Pasteurella haemolytica* has been shown by other work to have a cytotoxin which is toxic for bovine lymphocytes, neutrophils, and macrophages. So these bacteria elaborate a poison that is toxic for these important cells of the bovine immune system. We have looked at *Pasteurella multocida* and have isolated a heat stable surface component from them that is a large molecule that suppresses neutrophil function by impairing the neutrophil's ability to ingest that area and inhibiting the ionization reaction, one of the important killing mechanims. So *Pasteurella multocida* does not kill the neutrophils but it stops some of their functions, or inhibits some of their functions, thus allowing the *Pasteurella* to evade killing by the neutrophil a little more easily. *Haemophilus somnus* also has a heat stable surface component of large molecular weight that inhibits the ability of neutrophils to inject the bacteria. And it has a small heat stable surface component, very small molecule, less than a thousand molecular weight, that inhibits neutrophil ionization reaction, a bactericidal mechanism. So all three of these bacteria have means for getting around the neutrophil. And in general, any pathogenic bacteria that you want to look at extensively has some means to escape being killed by neutrophils. Neutrophils are very efficient killers of bacteria. There are thousands of species of bacteria living in the soil, water, and on skin surfaces, that can't escape killing by neutrophils, and they don't cause disease because neutrophils kill them so efficiently. These pathogens have mechanisms for escaping neutrophil killing.

I've shown you that stress, viruses, and bacteria can inhibit neutrophil function. They also do other things to the immune system. The big question is, what can we do about it? Is there any way that we can intervene with this immunosuppression

and get the neutrophils working better and maybe keep the animals healthier when they are under stress or experiencing virus infection? These are some of the drugs we've looked at in our model system. We've looked at levamisol, thiabendazole, ascorbic acid, Vitamin E-selenium, and then some interesting experimental compounds.

Our typical protocol is to have 20 head of cattle. We have a group of four controls, a group that gets the dexamethasone. We've chosen dexamethasone to be our immunosuppressant because it is easy to do, it is easy to reproduce. We use a dosage of dexamethosone that is recommended on the bottle, 10 mls for a thousand pound animal, that would be about .04 milligrams per kilogram. Then we have three groups that receive the dexamethasone plus whatever drug we want to evaluate. In this experiment, the first one we were using thiabendazole. We repeated this several times until we had looked at a total of seven dosages of thiabendazole, because there are reports of the literature in mice that thiabendazole might be an immunomodulator. So before treatment they should all be up at 100% of the control value. After treatment, dexamethazone value is the lower bar, so we have the striped bar, which is dexamethazol and the top bar, which is the solid bar (slide projected on screen). If our immunomodulator were effected it would bring that striped bar up to 100%. And as you can see, none of these seven dosages was able to do that. So thiabendazole didn't do anything in this experimental situation. And we looked at several parameters of neutrophil function. I am just showing you the one right now. To summarize, thiabendazole didn't do anything to neutrophil function. It did have some effect on lymphocyte function, but not very dramatic, at low dosage, much lower than the worming dose. We looked at six doses of levamisole in this same experimental situation and levamisole didn't do anything for neutrophil function. We looked at doses from .05 milligrams per kilogram up to 8 milligrams per kilogram and we could not get levamisole to do anything. We looked at ascorbic acid, three dosage levels of ascorbic acid, 10 milligrams, 20, and 40 milligrams per kilogram, on top of the dexamethazone. We found that ascorbic acid does reverse one of the things dexamethazone does, and that is antibody-dependent cell-mediated cytotoxicity, the very bottom line. You can see the dexamethasone inhibits to about 50% of normal. Ascorbic acid in normal cattle with no dexamethasone enhances that activity up to 150% of normal, and if we look at the high dose of ascorbic acid, 40 milligrams per kilogram, which is a lot of ascorbic acid, we can see that it raises it from the 50% that dexamethasone has to about 80%. So there is some mild immunonormalizing effects of ascorbic acid. Very high doses. Probably not going to do a great deal clinically for a stressed animal, but it did have some effect, not very dramatic.

Vitamin E-selenium had some effect (this is without dexamethasone in normal cattle) and it went the wrong direction. It suppressed some of the neutrophil functions below normal. That is the ADCC phenomenon that was enhanced by ascorbic acid, suppressed by Vitamin E selenium. These are levels on top of what a normal animal has. Our cattle apparently had normal levels of Vitamin E selenium, they weren't deficient. So we're giving them excessive amounts. The dosages are what comes BO-SE, the commercial amounts. These are the amounts that are in that product. And it suppressed that one function below normal. When we gave it in addition to dexamethasone, it suppressed it lower than the dexamethasone effects. So Vitamin E selenium went the wrong direction. Basically we looked at these four drugs that are on the market and available now to see if they could do anything for immunosuppression. They really don't do very much. Ascorbic acid did just a little. But we did look at an experimental compound that looked very good and that's averdine, also called CP2961. It's a product that Pfizer has, an experimental product. They finally gave it a name so we don't have to use a number. It's now called averdine. It's a lipoidlamine compound, an experimental drug. It's supposed to be an interferon inducer. When we gave this to cattle, it reversed four out of the five effects that dexamethasone has. The blue bar is the control value. The green bar is the dexamethasone, and the yellow bar is dexamethasone plus averdine. We look at the first function which is random migration. Dexamethasone enhances that which is detrimental. Averdine brought that right back down to normal. The second set of bars, in this case dexamethasone did not suppress *Staph aureus* ingestion in this particular group of cattle. But if we gave averdine plus dexamethasone it enhanced it. It improved it above normal. The MBT reduction assay, dexamethasone dropped it down, averdine brought it back again above normal in the face of dexamethasone. The ionization reaction in the fifth set of bars, that is the one that averdine did not help. Dexamethasone dropped it down and it stayed down, even though we gave averdine. So averdine didn't help that particular parameter. The fifth one, ADCC, dexamethasone dropped it back down and averdine brought it up just a little bit above normal. So averdine has some very impressive effects here. After all the negative results we've seen with the other compounds we're beginning to get encouraged by these results. The problem with this drug is that it induces tissue swelling at the site of injection, so it is not ready to go on the market. They are going to have to develop some analogs that have a beneficial effect without inducing the tissue swelling, the inflammation at the site of injection. Averdine is an interferon inducer so we were interested in looking at some compounds that might contain interferon to see if it's due to the gamma interferon that is present. So we looked at lymphokine *in vitro*. There is a group of lymphokines secreted by lymphocytes when they meet up with an antigen. To summarize, we found that lymphokines produced from bovine cells from cattle infected with IBR virus did essentially all of the things that averdine did. They had all the beneficial effcts *in vitro*. Now this was *in vitro*, the averdine was *in vivo*. The lymphokine we know contains gamma interferon, so the next question was, could this activity be due to the gamma interferon in the lymphokine? Very recently gamma

interferon has become available. A company in California has recombinant, genetically engineered bovine gamma interferon, and they are doing a lot of research on it. If it is effective it will probably be on the market in the next few years for use. They can produce it very cheaply. We looked at gamma interferon *in vitro* and we see that several of the effects of averdine are mimicked by the gamma interferon. This is *in vitro,* we haven't given it *in vivo* yet. So we think there is some potential for the gamma interferon to be an immunomodulator. I should mention that some of you probably know there is a product called agritheron that is coming on the market in Texas very soon which is an alpha interferon. It's a human alpha interferon. This is bovine gamma. Human alpha interferon has antiviral activity in cattle, but it may not have these immunomodulating activities that we see with gamma. I don't think it will have the immunomodulating activity. The gamma interferon should be treated as a totally separate sort of substance than the human alpha. The human alpha is not working too well, we shouldn't give up interferon totally.

That was a fly through our last seven years' research. It is kind of sad when you can summarize seven years research in fifteen minutes!

Potassium & Sodium for Dairy Cattle

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Stress may be defined as a condition adverse to the well-being of an animal. It can originate from the climatic environment (i.e. heat or cold stress), from the physical environment (i.e. shipping stress or limitations of confinement), from nutritional deficiencies or toxicities or from social interactions. An animal's environment is the aggregate of all external and internal conditions and influences. Environmental stressors, to name a few, may include altitude, wind, disease organisms, ectoparasites, endoparasites, soil pH and fertility, rainfall and humidity, temperature, light and radiation.

Lactation is also a stress placed on a producing cow. If lactational stress is associated with heat stress, the potassium requirement for the cow appears to increase. This is due to decreased feed intake and also increased loss of endogenous potassium through sweating.

In general potassium is important for the following functions:

1. Osmotic balance between cells and extracellular fluids;
2. Acid-base regulation;
3. Ionic balance controlling cellular excitability and activity;
4. Water balance;
5. Activation of several enzyme systems;
6. Oxygen and carbon dioxide transport in blood.

Also, potassium is the mineral element present in highest concentration in milk. Milk contains 0.15% potassium as compared to only 0.11% calcium and 0.08% phosphorus. A high producing dairy cow secretes 25 to 40% of daily potassium intake in milk, depending on level of feed intake and milk yield. In higher producing cows, potassium may become the limiting dietary factor for milk production. Since the cow has little storage capacity for potassium (such as bones for calcium and phosphorus), she must consume adequate amounts to supply maintenance and production requirements on a daily basis.

Research interest in potassium nutrition has been intensified during the past several years for several reasons. The trend in recent years toward the use of complete blended rations has reduced the feeding of roughages which contain high levels of potassium. There is also more feeding of by-product feeds, corn silage and cereal grains, all low in potassium content.

Dr. David Beede, a University of Florida nutritionist, has conducted several studies in which he investigated the relationship of potassium and sodium to milk production and heat related stress.

In the first experiment conducted by Dr. Beede, he placed one group of cattle on a potassium adequate diet (0.96% K) and a second group on a potassium deficient diet (0.11% K). Based on earlier research reports, it was expected that 3-4 weeks would be required to see signs of potassium deficiency. However, the response to the potassium deficient diet was much more dramatic than expected. Within 3-5 days of the start of the experiment:

1) Dietary potassium deficiency resulted in precipitous declines in feed and water consumption, milk production and blood plasma potassium concentration;
2) The death of three cows occurred and others suffered from nearly complete inanition and pica;
3) Cows could detect absence or presence of potassium (as potassium chloride) in the basal diet. They would consume feed containing potassium, refuse feed with no potassium;