OVERVIEW OF THE NUTRITION OF FREE-RANGING AND CAPTIVE BLACK RHINOCEROS

Ellen Dierenfeld

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Dierenfeld: ... Mean retention time that has been measured in controlled studies is anywhere from 50 to 60 hours for lucerne or alfalfa hay to approximately 60 hours for grass hay.

Bolin: If alfalfa hay is a better forage, why would they eat less? Why would they eat less grass hay than alfalfa hay?

Dierenfeld: I am not sure that it is better forage, but...

Blyde: Alfalfa hay should have a higher protein percentage than grass hay.

Dierenfeld: The energy sources for the rhino come primarily from soluble carbohydrates first, through digestion and also lipid digestion, which takes place in the stomach and small intestine; and secondarily from the volatile fatty acids from the fermentable carbohydrates that are in the browse and/or forage that they are consuming. In the fermentable carbohydrates I am talking about both hemicellulose and also cellulose, the unlignified portions of cellulose only. Lignified cellulose or the lignin portion of lignocellulose is totally unavailable for fermentation for any animal. It is only broke down by aerobic processes.

Hemicellulose is an interesting fiber fraction. It is partly digestible and partly fermentable. In other words, part of it becomes available through acid and/or alkaline treatment. So some it actually disappears before it even gets to the main fermentation site. But both the acid treatment from the stomach and the alkaline treatment in the small intestine can make more hemicellulose available further down the track. I want to argue that hemicellulose has become a real important *phytofraction* that really has not been looked at.

I would like to talk a little bit more about rhino browse. While there have been a number of studies on food consumption of black rhinos in the wild, there have been very few that include any information at all on nutrient composition. As I say, we can rarely duplicate ingredients, but we can duplicate the nutrients. Furthermore, the studies that are out there are done using different assay techniques, so it is really limited scattered data that I am trying to pull together here, so bear with me a bit. All the information on the future slides come from these studies done in Kenya, Namibia, South Africa, and Zimbabwe. It has been accepted for publication, but it is in the rewrite process.

Now as I said, there may be some very different analytical techniques that are not necessarily directly comparable. The studies from South Africa and Kenya looked at crude fiber as the fiber entity. The South Africans also did cellulose and glucose separate from crude fiber. In our study we looked at also some browses taken in Texas from animals that had been imported from Zimbabwe. We were actually comparing the browse composition eaten in Texas versus that from the field sites that they were collected. So we looked at hemicellulose, cellulose and lignin, which broke dawn the fiber constituents quite differently than just a crude fiber assay does. Work that has been down by Tom Foose with zoo rhinos digestion of grass hay versus alfalfa hay shows substantially better overall digestion of alfalfa hay than grass hay by the black rhino. The study from Paul Martin in South Africa, where lignin was measured both in feces and in food, the lignin could be used as a marker to estimate digestibility of the natural diet. It is approximately 50%. So even in the wild, their diets are not necessarily that highly digestible.

Blumer: From other species, is there much difference in digestion of different grass hays, when you look at coastal, bermuda versus timothy or other kind of hays?

Dierenfeld: There is some, but there have not been real controlled studies done with the same species under the same conditions to do that. There are certainly some differences in chemical composition of tropical grass hays versus temperate grass hays as a group. The nutrient composition does vary, which does influence their composition and digestibility.

Blumer: Because just for example, we could not even get our animals to eat our traditional grass hay. They would not touch it. I think they had a little trouble here [White Oak] initially with their coastal hay and then when they suddenly 458 timothy hays, they had better acceptability. Is that correct?

Lukas: Yes, and just when he was sick we would try a combination of a timothy/alfalfa mix. He ate that pretty well. The timothy/alfalfa mix came that way.

Dierenfeld: The point being here is that there are very few studies that have been done, controlled studies of digestion in black rhinos. When you compare alfalfa and grass hay there is definitely a difference, but it looks like from the one study that we could put together from natural foods that it is kind of in-between grass and alfalfa, in terms of overall digestibility. Also Tom Foose's work compared Indian, white and black rhinos. There is a difference in the ability of the grazing species versus the browsing black rhino to digest both hemicellulose and cellulose in alfalfa compared to timothy. The bottom line of this is that there is no difference in the hemicellulose digestibility between alfalfa and timothy, even in the black rhino, compared to the cellulose digestibility. So in fact, that is really where a lot of that digestibility difference falls out between black rhinos and some of the other rhinos.

So what does this mean? When you look at cell wall constituents or at the fiber, crude fiber is not a good term because it is really not a chemically identifiable entity. Which is why we break down fiber into hemicellulose, cellulose and lignin, which are much more chemically definable fractions. If you compare legume species, these are typical forage species that are used in livestock agriculture in North America. You have got five temperate grass species compared to six tropical grass species, compared with 34 browses that we looked at in Texas and Zimbabwe. You see much less lignin in grasses in general, although there is a big difference between tropical and temperate grasses. Tropical grasses are more lignified, they have more structural carbohydrates, so they are less digestible in general. But the thing that really shows up is this hemicellulose fraction. There is a distinct difference in the hemicellulose fraction between grasses and legumes in general. And many in fact become really important, because it is made up of totally different kind of sugars than the cellulose fractions. The browses that we looked at for the rhinos, while they are higher in lignin and therefore less digestible overall, the hemicellulose kind of falls between the hemicellulose content of legumes versus grasses. The cellulose fraction really much more closely matches that of the grasses that we have a *good idea*.

When you look at the soluble sugars in these typical forages that we feed in livestock agriculture... We keep having to fall back on livestock agriculture, because this is where the work has been done. This is a separated entity from the fiber fractions that we were just looking at. This is the soluble sugars that come out in the *stomach contents*. Simple sugars are higher in grasses than in alfalfa. And a lot of these four and five carbon sugars are higher in grasses than alfalfa. This may really be important for black rhino nutrition, because these sugars are pretty much available to an animal as soluble carbohydrates go through the digestion process through the stomach and small intestine. These are not fermentable carbohydrates. We have already seen that there is a difference in the fiber carbohydrate composition. There is also a difference in the soluble sugar carbohydrate composition. It has not been looked at in the browses at all from the wild.

Crude protein levels in browses eaten by black rhino... There has been shown to be some distinct seasonal differences in crude protein levels in the field studies that have been done, with a higher protein level in spring, or following rains. Which is typical. Just from a general range, we are seeing these kind of crude protein levels. This is actually a mean of seven species, so the rest of them are ranges of all the species that were 544. The bottom line here is that these crude protein levels in browses that have been measured much more closely duplicate grass protein levels than some of the legumes that we typically feed. Many of the alfalfas, that are feed in North America at least, contain a minimum of 15% and even up to 25% crude protein. So maybe again, the rhino browse is falling halfway in-between.

One of the groups of secondary compounds that can effect crude protein levels are tannins. Particularly the condensed tannins are those that are known to bind up dietary protein to make it unavailable. We have measured available versus unavailable protein in the 34 browse species we have looked at. About 2% overall of the protein becomes unavailable. So with the Zimbabwe and Texas browses you can drop those levels by two percentage points, and that is really the available protein for the animal. There is another group of tannins, the hydrolyzable tannins which actually get absorbed intact into the body, and can go to the liver and cause other kinds of toxic insults. But, those are not the ones that are concerned with protein availability. This is just looking at overall crude protein and how that relates to forages we typically feed.

Jessup: Do you know if these were taken at one time of the year, Ellen? Or were they sampled across the season...

Dierenfeld: It depends on the study. Some of the studies actually... I can not remember now. As I say it is a clumped summary, but none of them were very excessive in protein, and none of them were really really low in protein in summary.

Morkel: Were they weighted at all in terms of popularity to the rhino? Because a *subselective* browser is going to go for...

Dierenfeld: No. It is a good point. That is one of the reviewers comments on our paper, is that you can not just look at the overall composition. But by the same token can we argue that if they walk from bush to bush, taking a bite here, a bite there, does the *grandme* of everything really more or less represent what *they are eating*. I do not know. I mean those are some of things we need to tease out.

Morkel: Raoul, do you have any comments on that?

du Toit: I think it does. You know when we did the sampling for this, we wandered around as though we were rhinos doing surely well what a rhino would do. Walking down the 597 taking the plant species we know that were good and taking the part of the plant that we know they go for, and taking roughly a bite size piece of that plant. So I am of the mind that we have got something that 601 obviously there would be seasonal variation if you do not take it into an account. Obviously it needs more work.

Dierenfeld: Actually, I forgot something. When you brought up natural behaviors... Something Raoul [du Toit] said last night triggered me. I knew it at the time when we were collecting, but I did not put it together, is that a lot of the browsing goes on at night. Many many xeric adapted plants, or desert adapted plants have *can*-metabolism or night photosynthesis that goes on. And that *can*-metabolism, *crysalinacid* metabolism, actually is for the production of five carbon sugars. So in fact, what is going on at night is very different and has been shown in a number of other studies to have a very different impact on the chemical composition of plants collected in the daytime versus night. This may be really really important for these animals if five carbon sugars become essential. It is something we need to take into account.

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OK, another thing that we looked at that is summarized here, are the lipids in browses eaten by black rhinos. Lipids are not something that we look at a lot in animal agriculture, they really are not as important for our animals. Most of our diets contain 3% or 4%, who knows, crude fat. It has even been a real crude measurement. But in fact, the studies that have looked at crude fat in black rhino browses have shown in some instances up to 24% of the dry matter of the plant is crude fat. There is one preferred plant, euphorbia, that we measured in Zimbabwe that is just filled with a milky latex. That is very high in fat. So lipids may become really important for these guys too, particularly in regards to energy sources.

Munson: Do you know what your essential fatty acids are for them and if this preferred plant contains them?

Dierenfeld: One of the papers that was sent around for reading, Ghebremeskel and the guys in London do really good fat work, is looking at the fatty acid composition in rhino browses. The main fatty acids that they found in the Kenyan browses that they looked at were these three, palmitic which is a saturated fat, as well as linoleic and linolenic. How does that compare to the fatty acid composition in the forages that we typically feed in animal agriculture? There are some interesting differences, I just put six of the main fatty acids, this is a percentage by weight. For the most part, the rhino browses fall in-between grasses and alfalfa again. With the exception of stearic, where rhino browse may have a little bit more of stearic acid, but many of your grains would have stearic acid. So if you are feeding a forage plus grain diet, you are probably covering your stearic acid needs. What stearic acid is good for is membrane stability. Giving coconut oil to primates increased their red blood cell membrane stability to cut down the hemolysis in one study. And that was a direct effect of stearic acid in the diet.

Stuart: Are these fresh grass and alfalfa samples or are these hay?

Dierenfeld: That is a good question. Actually these were fresh forages. So it is a real comparison, sort of. The rhino browses that have been looked at were collected and taken to the lab in London, and within 48 hours they were extracted. So while there might be some changes, it might be as good as you get in terms of a direct comparison. The other one is oleic acid where it looks like the rhino browse may have more than grasses or leguminous hays, as alfalfa. Again, seeds would probably make that up, seeds or grains that we typically use in a feed formulation.

There are some real differences between lipid production, or lipogenesis, in ruminants versus nonruminants that really may factor in this whole thing for the rhino, particularly if lipid is a source of energy storage as it is in other species. In ruminants, the main carbon molecule comes from acetic, *when the* volatile fatty acids in the rumen fermentation goes through the citric acid cycle with fat production. In nonruminants, glucose becomes the main source of energy of the carbons for fat generation. It goes preferentially through the pentose phosphate shunt which may tie into a lot of body systems. That is why I am real interested in some of the enzyme work that Dr. Paglia has done with the red blood cell and see if it carries over to other cell systems; the liver for example, to see if some of the enzymes that are limiting the red blood cell may in fact be limiting in the system in other organs.

Another aspect comes in some enzyme work in the hydroxylation of organic substances, which includes desaturation of fatty acids by the genesis of linoleic and linolenic acids; as well as steroid production, maybe even cholesterol production. Hydroxylation pathways that are used need these two enzyme systems, superoxide dismutase and catalase. Now, Ghebremeskel and his co-workers in their paper that was circulated, suggest that both copper and zinc may be limiting in the mineral composition of the browses that they looked at. They hypothesize that maybe that was impacting on superoxide dismutase activity. Talking to Dr. Paglia last night, he said that when he looked at superoxide dismutase it did not seem to be...

Paglia: It is one of the few enzymes that the black rhino has that is every bit as active as it is in human red cells. It is quite 060. We had that confirmed in Mike Scott's lab up at Children's Hospital 061. He got the same results.

Dierenfeld: So if you have got something going through this part of the cycle, and stopping here at peroxide, you would have a build up of peroxide because there is no catalase. Is that potentially possible?

Paglia: It certainly is, and it is not just the catalase that is deficient in that shunt region. They have virtually no detectable *glutathionese* transferase for example, which is one of the main pathways for detoxifying a lot of plants and antibiotics.

Jessup: It is detoxifying what?

Munson: Antibiotics.

Paglia: It is a pathway for a lot of intermediates of plant breakdown products which might be oxidants in and of themselves, [they] are detoxified by conjugation with reduced glutathione catalyzed by *glutathionese* transferase. And we can not really detect significant activity, either in the black or the white rhino path however. If anything, the white rhino has less *glutathionese* transferase than the black rhino. But the white rhino has catalase in significant amounts. That is the only major difference we have found so far between the blacks and the whites in this area.

Dierenfeld: But, from several different systems it is looking like that pentose phosphate shunt may be a really really important energetic pathway, if it applies to all the cellular systems in the body. All of these five carbon and odd carbon sugars would gear into that cycle as well. So it is something that really needs to be looked at in a lot more detail.

The studies that have looked at minerals in black rhino browses include 42 different species, from two studies. What I have got here is the National Research Council requirement for horses. While I am not saying that a rhino is a horse, I am saying that I have to use something as our model at this point. From the digestive tract anatomy and physiology perspective, the horse is the best we have got; although the rhino may in fact be a new entity within that group. But, again I have got ranges up here. It does not look like from the ranges of the studies that have been done calcium is ever deficient compared to horse calcium requirements, nor potassium or even magnesium. However, the 42 samples that we have got sodium contents of, look like they certainly could be deficient in sodium. This really does not pose much of an ecological problem, because a lot of animals seek out salt sources. There are natural salt licks that can make up the deficiency of sodium in many plants. So that is important in rhino management to make sure that at some time there is a salt lick in nature, or in a captive field situation or in the feed itself.

The other thing that becomes interesting to look at is phosphorus level in a lot of these browses. It has been looked at. It is on a low side to marginal, especially if it is compared to very high calcium in the diet. Perhaps that imbalance naturally is causing some kind of a mechanism shut down through feed backup and decreased efficiency of absorption at the gut level possibly. I really do not know.

The last aspect of the minerals is just some trace element work that has been done, again looking at 39 species from Kenya, Zimbabwe and Texas combined. For these, the selenium content was looked at by Ghebremeskel and his co-workers. In the ten browses they looked at from Kenya, it looked like selenium content could certainly be deficient. As Raoul [du Toit] said last night, the soil underlying any of these browses becomes the primary source of minerals for any of the plants grown on that soil. So this can not be looked at independent of the habitat from which it is collected.

Sadler: So you know if the phosphorous were looked at phytate as well as total, or were those just totals?

Dierenfeld: That would have just been totals. So they are probably even lower than 109. Plus a lot of desert adapted plants are very high in oxalic acid which may even further decrease the availability of phosphorous.

Munson: Do they eat those plants though, with the high oxalic acid?

Dierenfeld: Seem to. I mean they are eating very high calcium plants. Whether people have looked to see if it is calcium oxalate or not, has not been done. But in a lot of desert plants that have high calcium loads, it is in the form of calcium oxalate.

Munson: Most species try to avoid those, don't they?

Dierenfeld: Some of them seem to be preferred, at least in the Australian desert when I was working there.

Blyde: The other thing about plants, is that they have different 116.

Paglia: Ellen, I am not sure I understood Raoul's [du Toit] comment about the selenium. Is it low, is soil selenium the rule?

du Toit: There are areas affected in Kenya. *It seems to be the issue that is most* considering in Kruger National Park, because Nancy did some soil there. That is what is the problem in cattle. Richard is better qualified to talk about that.

R. Kock: That is where in fact we got the intense evidence of massive 122. They found it from the grasses. They do not follow the soil, in fact they just seem to be better, some plants better at picking up what is there. They concentrate it.

Dierenfeld: They certainly accumulate in plants, in any ecosystem. So, again just looking at a range like this may not give you a complete picture.

Paglia: I think one good indicator, that if it is brought up, I am talking about glutathione peroxidase, which is a selenium derivative enzyme. I believe from Australian human data they indicate if there is a selenium deficiency in the soil that people will have a lower glutathione peroxidase activity. There is a good correlation in the human literature. Glutathione peroxidase is one thing that the black rhino has in great abundance, more than humans. So it would not be reflecting a selenium level like that.

Dierenfeld: Ghebremeskel [et al]. said in their paper that they had done some blood work and found marginal selenium levels in the blood of the animals they looked at. But that was just a 132 comment after discussing the selenium levels of the plants they were looking at, so I am not sure how much work has been done on selenium nutrition overall in black rhinos.

Paglia: They are using it to alter their peroxidase, whatever they have; assuming that their peroxidase is the same as other.

Dierenfeld: That certainly could be. That is the pathway for breaking down the hydrogen peroxide, correct?

Blumer: Your measures of peroxidase are based on captive animals?

Paglia: 139 the ones that just came in from Zimbabwe. I guess I need your definition of captive. There is a ring, it is one of the higher activities among the enzymes of the pentose phosphate pathway. It is maybe two to five times more than what the human levels are.

Stuart: There seems to be a long gap between selenium supplementation and glutathione peroxidase activation, 20 to 30 days. This is in dairy cattle.

Miller: In your comment 147 selenium liver or serum levels on wild rhino, in rhino themselves.

du Toit: Almost all.

Sadler: The selenium is an integral part of the enzymes, it is made when the red cells are made. So if you have a bunch of red cells, and did not see any selenium, it kind of 150. It is used in cattle to determine selenium adequacy.

Stuart?: It is a spot test. 152

Dierenfeld: Ghebremeskel and his colleagues also have suggested from their blood work that zinc might have been marginal in the animals that they were looking at. But again, with this kind of range, it is really going to depend on what you are eating, if 40 is a requirement in the diet. The reason I have got an asterisk by the copper requirement for the horse is that there is some discussion right now among the horse nutritionist that that is too low for many horses. They are thinking of raising the copper requirement up to 40 mg/kg.

I want to talk a little bit more about some of the fat soluble vitamins that we have been doing quite a bit of work on for the past several years. 163 Myself, Eric [Miller], and Raoul [du Toit] looked at vitamin E levels in captive versus free-ranging black rhinos from Zimbabwe. At that time the 11 animals that we had looked at had a mean alpha tocopherol level of 0.2μg/ml compared to I believe it was 39 free-ranging rhinos in that paper of 0.8μg/ml. We said, well this may be linked into the hemolysis that we are seeing. Subsequent to that original report, we have had samples come in from Namibian animals, a bunch more Zimbabwe animals, South African and some in from Kenya. As you can see, it really depended on the population that we were comparing to our captive population, what the mean level of alpha tocopherol was in the black rhino. Because circulating levels of vitamin E do reflect absorption of this nutrient from the diet, it to me suggested dietary difference in vitamin E levels in some of these populations in Africa.

M. Kock: Do you know where that Kenya population came from?

Dierenfeld: It was the *Soleo* translocation. I do not know if it was before or after, or anything about the handling either. That is why we need the details from the people that were probably there.

R. Kock: We need more samples.

Stover: What about cholesterol in those animals?

Dierenfeld: We did not get cholesterols on all of them. But I think when we looked at cholesterols between free-ranging and captive populations, that in fact was the stuff that you guys published last year, showed similar cholesterol levels between free-ranging and our captives. So that comparison and the cholesterol to [vitamin] E comparison is probably a fairly good comparison. In fact, I broke out the samples from Zimbabwe and of #31, #40 and #15 over three different sampling years. We actually saw a decrease in the circulating levels of vitamin E even in Zimbabwe in populations of rhinos. So it could of just been one of those flukes of sampling, the numbers that we got, to start this whole cascade.

M. Kock: We must check the seasonality and handling.

Dierenfeld: Yes, exactly, there is seasonality, there is handling differences. We were already talking this morning about differences in immobilization techniques and time of stressing the animals before you are getting them down. All that may in fact have something to do with the differences we are seeing. At one time I thought perhaps the free-ranging animals that we were looking at had all been anesthetized, compared to zoo animals which may not have been anesthetized. Maybe we were seeing an affect of anesthesia. I have spoken to a couple of equine people at Oregon State and Cornell, and they do not think that anesthesia has an effect on circulating vitamin E levels, but the studies have not been done. So I do not know.

Smith: That looks like your anesthesia 198.

Dierenfeld: I know!

Jessup: With lower vitamin E they go down faster!

Dierenfeld: And so the current updated numbers for the four populations... The Namibians are the high end of the spectrum compared to the Kenya guys at the moment.

R. Kock: I think you need more samples.

Dierenfeld: I think so too. We have also looked at vitamin E levels among different species of zoo rhinos. This is not from 112 individual [black rhinos], but it is 112 samples from individuals that were not in the middle of a study, where their vitamin E could start really up there. So what it is showing is that there is some real wide variability in these numbers, but it looks like the circulating level of vitamin E among black, white and Indian rhinos, granted the Indians are two, do not seem to differ. Actually, what we have seen in the zoo black rhino group in North America is a trend towards increasing levels over the past ten years, aiming for this free-ranging blue stripe; if free-ranging animals are the normal we are looking for in terms of vitamin E levels.

M. Kock: What happened in 1993?

Jessup: What happened in 1987?

Dierenfeld: I am not sure, I think what we did... Here, this is when we said it looks like there might be a link with Vitamin E and ...

Jessup: So those are your supplemented animals?

Dierenfeld: I think there are some supplemented animals in this one, or maybe the one that Scott [Citino] gave the injection to might actually be included. I would have to look back and look at that particular year. I thought I had pulled out all of those.

Sadler: But they all would have been supplemented probably through a diet anyway, to some extent.

Dierenfeld: Because everybody said, oh, this is it.

Sadler: So the question is you may not have seen any additional supplementation, and the diet could have changed five fold because somebody changed...

Dierenfeld: At the feed formulation end of things. I honestly do not know why this is down there. I do not have the enzyme *assays* so that could be part of it too. Overall it seems to be on the uprise. The thing that is interesting about vitamin E in black rhinos is the study that Scott [Citino] did with a single animal that was going to be euthanized. We were trying to look at an injectable dose of vitamin E to see if it behaved the same way as vitamin E injections did in other hoofstock. It appears to follow the exact same pattern of peaking in the blood in about two days after the injection, then trailing out after about a week. So it looks like from a number of studies, injection and oral studies, that there is really not particularly inhibited metabolism of this nutrient provided in the diet at proper amounts. Rhinoceros and elephants do something different than other perrisodactyls with vitamin E though, because when we look at normal plasma vitamin E levels in zoo equids, tapirs, elephants and rhinos, this is the kind of difference we see. These are all on animals that are fed basically the same diet concentration of vitamin E.

There is definitely a difference in cholesterol levels too 246 cholesterol ratio. One of the things that may account for that is the difference in lipoprotein composition among these four groups of perrisodactyls. Actually, elephants are not perrisodactyls, but I lumped them in there just from a gut physiology perspective. In fact, in the studies that have been done by Lee and also my colleague 251 at NYU, we can find no high density lipoproteins in the plasma of zoo rhinoceros. I do not know if this is real in free-ranging animals. These samples have all come from captive animals. The fact is, high density lipoproteins are the main carrier for this nutrient in animal systems. So if the lack of high density lipid protein fractions is normal, then you really would expect low circulating levels of this nutrient, because there is no carrier mechanisms for it.

Sadler: Ellen, are you aware of any studies on any wild tapirs, elephants, you said not rhino, on HDL's? Do these match, do you have any clue?

Dierenfeld: Not in tapirs, equids yes, I think there is one in elephants from back in the seventies, but I think the techniques were different such that you can not really do a comparison.

Sadler: So any of those animals need to be probably looked at as to whether they are actually...

Dierenfeld: To see if this is real or just an effect of captivity.

Munson: When you get the serum, is it from healthy ones, or are these the sick ones with the dysfunctional livers? It is a part of the function of livers to make these things.

Dierenfeld: No, that is another issue. It is an important distinction. We have gotten very few animals, because they have to be healthy and the plasma can not be frozen, it has to be separated and refrigerated. So it is a very different handling protocol. So this is really from about six animals total.

Sadler: Is that just blacks, or is that all that you have looked at?

Dierenfeld: That is both white and black. We did not find high density lipoprotein in rhinos. So we did look at the dietary vitamin E content in the plants in four different locations in Kenya, and also the samples from Zimbabwe from the Zambezi Valley; just to give us an idea of what kind of dietary vitamin E level these animals are consuming. Because we saw such differences in the population's circulating means, I thought perhaps it would relate, a real nice correlation, with the plant levels. In fact, it is a negative correlation. One interesting point is that the samples we got from the Ol Ari Nyiro Ranch, is the same location that Ghebremeskel group sampled, and their numbers got 76. So it is a really comparable value using totally different collection and analytical techniques. I was pretty impressed that it came out like that. Nonetheless, this gives us at least some idea, and these are non-weighted means of all the plants, of the vitamin E content in the diets of these animals.

How does it compare to forages that we may use in the zoo? Well the hays, and I am throwing in mixed alfalfa/legume, grass, and alfalfa together in the dry forages category; five different browses that we fed at our zoo; green produce including kale, romaine, and other dark green leafy produce; compared to some of the levels that we were seeing in the wild. So just using a dry forage in the zoo simply is not supplying the levels of vitamin E that these animals would consume in the wild.

We looked at plasma vitamin A levels in black, white, and Indian rhinos, and they are a magnitude lower in some instances than what you would expect as normal for other captive animals. Not a magnitude, but normal retinol levels for most animals is between 0.2 and 0.7 and maintained fairly level. There is not a lot of fluctuation unless you have got vitamin A deficiency or vitamin A toxicity. It does not look like there is a real difference among the species of rhinos.

Stuart: Did you look for other esters?

Dierenfeld: Yes, they do not have esters.

Stuart: What about other toxic high levels of retinol, do you see any *esters there*?

Dierenfeld: We have only seen one rhino that has got a really high level of [vitamin] A that had a high retinol level. I will have to look back and see if we looked for esters, because the high level for the rhino is like *0.4*. I am not sure with my technician if that would have triggered a response to do the ester assay, because that is a typical level for other animals. Again, when comparing the retinol values in free-ranging populations we see really low retinol values. I think this is a normal retinol level for rhinos.

Looking at tissue tocopherol levels in rhinos, and I have got all four species on this slide so it is a little bit busy, there is only an "n" of two for the Indian, and the Sumatran rhino. So really we can discount those at this point. There looks to be some difference in adipose tissue vitamin E levels between black and white rhinos. We have an "n" of nine for complete tissue sampling in the blacks and an "n" of seven for the whites. Now, again I am using horse normals as my comparative value, but it does not look to me like the metabolism of vitamin E storage in horses is a good model for vitamin E storage in rhinos. Horses have apparent adipose tissue storage of vitamin E that they can take back and use in their body, up to 25μg/g or so. We are just not seeing it in the adipose tissue of any of the rhinos we are looking at. We are seeing the opposite in the liver. The horse normal would be right here, and the zoo rhinos are having much higher levels.

To think about it ecologically, the domestic horse may have evolved in a temperate climate such that adipose tissue becomes a more important storage organ for something that might be seasonal, that they can draw upon when there is less fresh forage out there. The adipose tissue storage component may be very different for tropical evolved species or desert species. I do not know.

Breaking down the liver vitamin E levels by individual rhino species, we are seeing a real increase in some individual animals. These are all black rhinos on the upper end here, which may be from more recent supplementation of vitamin E. And again, if you remember, the normal for horses is down here. So if we are assuming that the horse is a good model for this, a lot of our whites and many of our black rhinos are way over that. Some of these animals did have the green liver syndrome, I will admit that freely. So in fact, there was not normal liver metabolism going on. If there was stasis, vitamin E supplements would have stayed in the liver also. So that is where I need some input from people as to which data points to throw out of something like this.

Miller: Did any of those high data points include injectable animals, because many of the animals got, I mean as part of the "shotgun therapy," got vitamin E before.

Dierenfeld: I do not know. It is partial histories on a lot of these animals. We just get the tissues in our lab after these animals die, so burying out some of that information becomes really important. I did put some individual numbers of animals on this slide.

Blumer: #9101 and #9104 were both animals that died of liver disease and were both supplemented.

Dierenfeld: With injection?

Blumer: Yes, I do not know when these samples were from. I am not sure what samples you have are. And #9109 is "Chifumbi."

Jessup: If you throw out your outliners then you are down around ten.

Dierenfeld: Which seems like a more normal level. #180 was "Princess" at Cincinnati.

Miller: I would be surprised if she did not get [vitamin] E before death. That animal was agonal for a long period and received about every therapy that could be thought of.

Dierenfeld: This is just a picture of the green liver syndrome while we are doing the vitamin E extractions on liver tissues. They are really a different color than normal liver tissues of these guys.

We have also looked at liver vitamin A levels in zoo rhinos. And again, you have got to start getting some medical histories behind this to really understand or interpret what is going on. But, I would guess that we were not giving any [vitamin] A injections, that is not necessarily so. But if we had impaired liver function, I would expect these high data points to show up just from the stasis in the liver. Normal vitamin A level for horses is less than 1.0, so it is really low. Just looking at black rhinos, these were the two highest ones. Actually the fax that I got yesterday is right in here, so it is high also. That was from Oklahoma City, I do not know if it had liver problems or not.

Miller: Definitely 391 that is interesting, because that animal has been chronically *ill*.

Dierenfeld: Interesting. This is #180, that animal from Cincinnati, we had a blood sample from her in January. At that time, her retinol level in her blood was high, it was really excess It was ten times higher than the means in rhinos. I was out of the country and did not see it, or I would have called them 396 right away, and asked for another sample just to repeat it. I do not know if it is a correlation or not. But any time you see a high retinol in the blood for these particular animals I think we really need to take a serious look.

So, what does all this mean? I have got some indications or future thrusts that I think might be applicable for feeding black rhinos in managed feeding situations. And one of those might be to use *mixed* forages. I do not think this applies in the same way at all in the boma feeding situation, because you are already doing it. You are feeding browses, you are feeding sugarcane, as well as alfalfa. I think those browses and probably the sugarcane contain a lot of available five carbon sugars that may go into the energetic pathways in a different manner than the fermentable carbohydrates. I think it is a real important thing to consider when we are trying to find suitable substitute forages for these animals. It worked for our Sumatran rhino. We had a really hard time with her. She was really sick when they just had her just on alfalfa. And I said, I do not think you are going to like this, but can we try her on mixed hay? It took along time to wean her over to it, but once she did, she straightened right up. It may have something to do with that soluble sugar in the grasses; especially when you consider there is sugar in browses. So that all ties into obviously the insoluble sugars and the soluble sugars and also the fatty acid composition. We know what they are, at least for some limited browsers. I think we need to try to duplicate it in our diets.

I think we need to look at the mineral status of a lot of our animals, the same way we have with vitamin status--see what normal is, so we can get some background data, just baseline information. We may even want to reevaluate vitamin E and A supplementation. It does not look at all like blood levels of alpha tocopherol are telling us anything about tissue levels. It is just not a good indicator of body status of this nutrient. What blood levels of [vitamin] E are telling us are the availability from the diet. But again, we have got some good levels to at least start with for diet formulation. Vitamin A I am getting a little bit concerned about. No herbivore would normally be consuming pre-formed vitamin A in the wild. They would be making their own from carotenoid 436. So we may want to consider replacing retinol in manufactured diets with beta carotene or some other mixed carotenoids such as the animals can do what it is they do best. There is also good links with beta carotene and immune function in other animal species. It may link into some of the health problems we are seeing. I would really like to know what normal tissue levels of these fat soluble vitamins are for free-ranging animals. So if we have got any tissues or any possibility of getting fresh tissues in the field, those are some of the priorities that I can see for interpretation of some of the data that we have got.

Blumer: I know that you have been going around a bit with Rosalina 448 Bill's colleague about the vitamin A level in the other two animals at Fossil Rim. These are animals that are on this new diet that we are working on, in fact they are also animals that are at this group. Does that send up major flags for you in terms of liver disease?

Dierenfeld: Maybe. I can not be more definitive than that. The only times that we have been able to do a correlated blood sample with a tissue sample, has been from something... It has never been at the same time, and it has always been a terminal case. That is the only time we ever get tissue samples, so of course it is going to correlate with death or disease. Most of our blood samples are not from the same animals that we have gotten any tissue samples. It is really hard to make the link on status. I am looking at the retinol levels in a lot of rhinos, both in the free-ranging groups we have sampled and also in the zoo populations. There is more than 200 rhinos we have got in fact. They are all ten times lower than the levels in some of your animals 468. I think it is something to consider. The other physiological factor is that these animals do not get retinol normally in a wild feeding situation. They would manufacture their own vitamin A. We also have to consider that some of the things that we are doing to enhance absorption of some of the fat soluble vitamins, maybe enhancing absorption of other fat soluble compounds, including pharmaceuticals, including vitamin A from the diet, things like that; in ways that we are not even considering at this point. So all of the fat soluble stuff goes to the liver. If you have got an at risk group already, then you are just adding more potential insult to injury. I do not know.

Stover: Evan, were they high before you started the diet?

Sadler: Yes, I will show you that [vitamin] A in a minute, but yes.

Blumer: To answer your question, they are high but they are basically normal for most other herbivores, it is just that compared to the other rhinos that they are high.

Jessup: Have you looked at the vitamin A levels on those animals when they were captured, compared to what they were when you bled them when they got here, and the ones that survived what they are since?

Stover: You did the [vitamin] A's on when they came in, on the April bloods?

Dierenfeld: If we got samples we would have done them. I have got all the raw data.

Blumer: I do not know if they have any from arrival.

Jessup: Those were after they had been on lucerne and...

Blumer: Yes, they had been in captivity for nine months at that point.

Jessup: We have samples from when they were caught too.

Blumer: But if that becomes a priority, than that is something we should try to use those samples for.

Dierenfeld: Actually, one of the papers that was summarized, had a typo in it, because it said 0.4 for normals when they were caught for retinol. I went back to the original data, and it was a decimal point error. They were 0.04. I was going to mention that, I forgot.

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