Peck Farm Research Report

Phase 3

Title: Identifying Nutritional Characteristics' of Whitetail Deer under CWD Quarantine

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Hypothesis: A Cervid supported with optimized feed, forage and water along with its genetics can stave off an initial or continuing disease process that could lead to the onset of a disease process like a neuro-degenerative disease called Chronic Wasting Disease (CWD).

Whitetails of Wisconsin's' Cervid Farmers Foundation (WCFF), North American Deer Farmers Association (NADeFA) , Deer Breeders Corporation (DBC) and Agricultural Omega Solutions LLC have continued to collectively fund this research investigation into the understanding of an initial disease process' based on nutrition or lack of nutrition. This continuing investigation will help the members of the Captive Cervid Associations as well as other Wildlife Agencies in the understanding compounding disease processes or nutritional processes that could lead to a progressive neurodegenerative onset and subsequent mortality of cervids involving a detectable Chronic Wasting Disease. Our test farm continues to exist under quarantine conditions in a CWD endemic area as the wild deer population continues to increase in CWD detectable contamination in post mortem deer.

Phase 3 research proposal is to best utilize the past investment of the research findings, to date, as a continued effort into understanding what organisms are responsible in degradation of the deer's immunity leading to how CWD develops to a clinical case resulting in death in cervids.

To review or refresh yourself with the complete background information (Phase 1, Phase 2 & 2.5) of this study please visit www.whitetailsofwisconsin.com

Background

Phase 1 summary review in later 2016 and early 2017 noted the deer farm under quarantine for CWD had been provided a historical feed ration. When tested, the ration was considered to be nutrient deficient to the deer on this farm as opposed to other farmed deer. Though this ration was fed to all ruminants on this farm historically, the first positive CWD whitetail deer was detected January, 2016.

Further, upon initial rectal biopsy testing, the deer on this farm have presented no initial or clinical signs of CWD to date. The current ages of the deer on this farm are from 1.5 to 7 years. They are expected to convert to CWD positive status, given the confirmed status of the CWD prion in the lymph nodes and brain tissue of the first detected deer from this farm.

Nutritional and bacterial status of the feed, water, and deer from this quarantined farm, in addition to 2 control farms, provided foundation information for future follow up health assessments. In the event any deer on the quarantined deer farm successfully reproduced fawns, they will be recruited in the study. If current deer show clinical symptoms of disease, a follow up investigation can be initiated with the use of the previously collected baseline data and/or review of necropsy information in the event of any deer death.

Summary interests gleaned from the Phase 1 review include deer farmers paying attention to farm activities including water quality, nutritional feedstuffs, animal transfers and sanitation practices. This also includes developing and embracing an on farm Bio-Security program to prevent deer from being affected by negative organisms.

Phase 2 summaries further provided supporting information, from Phase1, to the deer farming community, in an effort to understand a more in depth health perspective of raising your deer.

The primary objective in Phase 2 was to determine the CWD status of the quarantined deer by conducting a 3rd rectal test in a 2 year timeframe from the first index case. Results showed that two of the 6 deer were found to be rectal positive as detected by IHC via NVSL. Additional pre-clinical samples provided by AOS resulted in further refinements to samples studied in Phase 2. These provided a more accurate assessment of health changes in a disease process in deer.
Other results concluded that feed, water and hay do harbor negative organisms. These negative organisms were shown to transfer from feed to saliva showing up in other areas of the deer's digestive process (blood, fecal). Hay or other forage products produced from fields that use livestock manure for landspreading nutrients were confirmed to show additional negative organisms in forages even when pelleted. These should be considered a risk factor and causal to negative health consequences to your deer.
Though the 2 positive deer (genotype 96GG / 96GS) did not have what is consider a "resistant" genotype, the span of time was 980 days since the first index CWD case in January 2016 on the quarantined farm.

Progress made in the Phase 2 study design for collecting samples for analysis continues to show a direct correlation and understanding of where negative bacteria reside in the deer (AOS) with potential to create negative health consequences.

Phase 2.5 was an in-depth review of two rectal positive deer (Orange 1 / Yellow1) that had died in the fall/winter of 2018 on the quarantined farm. This review sought to define specific negatively associated organisms associated these deer that were not common to healthy deer (without CWD on board).

The overall findings of Phases1, 2 and 2.5 were helpful in determining the course of future proposed research, specifically in sample collection and testing as a positive refinement. These refinements will help in expediting detection of negative environmental organisms (impacting disease status) and, more importantly, identify potential intervention strategies for any deer in this study.

Phase 3 will seek to continue to follow the evolving health status (rectal biopsy 4rth) of the remaining 4 deer on the quarantined farm (1 buck, 3 Does). Since only Does on the quarantined farm died from negative health conditions, 1 Doe from each control farm will be added to the Phase 3 study tracking both sexes in comparing deer health status.

As we continue to investigate live deer under quarantined conditions, it is important to understand how any disease progresses begins in deer and to be able to potentially counter any health complications moving forward.

Left to right - While Dr. Amy Robinson and Jerome review final strategies / rationales for live deer sample collections with State Representative Jeff Mursau, Brad lines up a deer to sedate for another biopsy.

Results:

Rectal Biopsies

This year with 4 deer left on the quarantined farm (Buck Red 1, Purple 1, Yellow 2 and Pink 1) we proceeded in early April 2019 to collect our rectal biopsy samples along with blood, nasal, saliva,urine and fecal samples.

Red buck 1 was an inconclusive finding since his rectal follical sample was not sufficient for the lab to have enough tissue with follicles for testing. Purple 1 was considered a non - detect with both Yellow 2 and Pink 1 tested rectally positive by NVSL using the IHC method.

From Left to Right: Red 1 Buck, Purple 1 Doe, Yellow 2 Doe and Pink 1 Doe on June 13, 2019.

The deer's ages are as follows - Yellow 2- born on 5-20-16 - genotype 96/GG. Pink 1- born 5-20-12 - genotype 96/GG. Both deer were born on the same day but 4 years apart. Red Buck 1-born on 6-4-15- genotype of 96/GS. Purple 1 - born on 7-8-12- genotype 96/GS. Both deer are about 3 years of age difference.

In past observations, deer detected as rectal positives took a longer time to see hair coat changes to the typical summer coat color. This was noticed more in rectal CWD positive deer as compared to the non- detected CWD deer. This slower haircoat changeover to a full winter coat from a summer haircoat was observed to take longer as well.

Ultrasound examination noted that both Yellow 2 and Purple 1 were pregnant. Pink 1 was negative upon ultrasound however, she deliverd a fawn last year on July 20th so if she were deliver a fawn this year would be consistant to the prior year.
Now, this leaves us with an interesting query. In the first year, we had no pregnancies due to a poor dietary ration fed on this quarantined farm. The second year, after rectifying the ration with an industry ration, we had 1 pregnancy though the fawn did not survive past day 1 of life. This year (3rd year) we have at least 2 known pregancies with a 3rd Doe being a possibility.
This provides a great opportunity to follow a pregnant deer being detected with CWD and a pregnant deer with non- detected CWD. One couldn't order up on a menu the opportuntity presented for continued research efforts in the study of disease progression by the farming community.

Water

In the past we only tested the water source from the quarantined deer farm so we expanded our testing to include water samples from all 3 farms. This was done so we could build the review of comparable farms for any negative organisms (Table1) that could impact deer health.

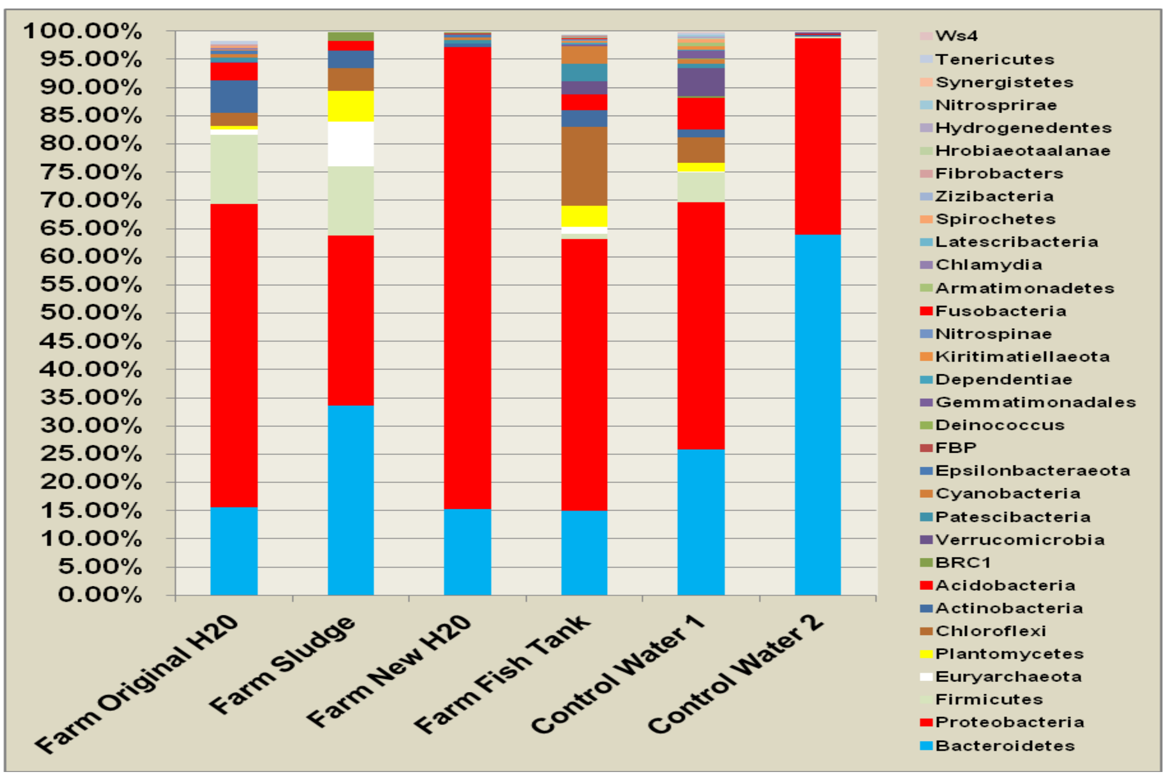


Table 1. Quarantined farm original well water, sludge and fish tank water compared to Farm new water. These are compared to the water tests from both control farms in this study.

The original farm water source noted many gram negative proteobacteria (red bars) in the original water test of the quarantined farm. After investigating the source of these negative bacteria we decided to replace piping from the well head that supplies the deer drinking water.

Replacement of an ascending water pipe (green) to a decending water pipe (white) after it was determinend that years of sludge build up supported negative associated organisms in the water.

The original water supply from an artisenal well at 4 gallons / minute providing water to this farm since 1990. When installed, the water supply piping had an asending angle upward to a pair of qoi fish stock tanks that doubled as the deer drinking water source. When replacing this pipe we collected some sludge for testing heavy metals and presence of bacteria. The heavy metal content showed high levels of iron (138,000+ppm), calcium (586 ppm), mangenese (183 ppm), copper (72 ppm) and cobalt (7.5 ppm). Though these levels were part of the sludge in the pipe, it is unknown how much would be suspended in the deer drinking water, which, over time, could have a negative effect.
It is noted though that the liver of both deer (Orange 1 / Yellow 1) that died this past year on this farm had elevated iron levels in their liver (360 – 960 ppm) respectively where as a normal range is considered 120-300 ppm.
Currently, dietary minerals are typically supplied through feed(s) providing deer their nutritional needs. By identifying what is in your water, you are able to repair and / or adjust accordingly. Decreasing the excessive mineral / bacterial content will increase the potential for healthier deer. We changed the upward supply angle to a decending angle to eliminate the potential of air or sedimentation collection inside the water supply pipe. We believe the ascending angle was allowing negative associated organisms or mineral build-up found on the first water testing. After replacing the water supply pipe from the well head, we took another water test 1 month later to see "how clean was our new water supply"? The result of this effort was unexpected. Now we had more biologically dirty water (Table 1 Farm New H20) reflecting more gram negative proteo-bacteria. After further review, we found water flowing from a deep aquafer through a well can have changes to the biological make up of water bacteria seasonally as well as geographically. Bacterial testing in the 2 control farms (Table 1) water sources had lower gram negative proteobacteria which represents 2 different geographical locations in the state. Another note regarding Control Farm 2 water (Table 1); this farm practiced washing out their water drinking receptical on a bi-weekly basis. This shows a benefical support to the deer drinking water and the opportunity for those to develop this sanitary practice if not already done so on your farm.

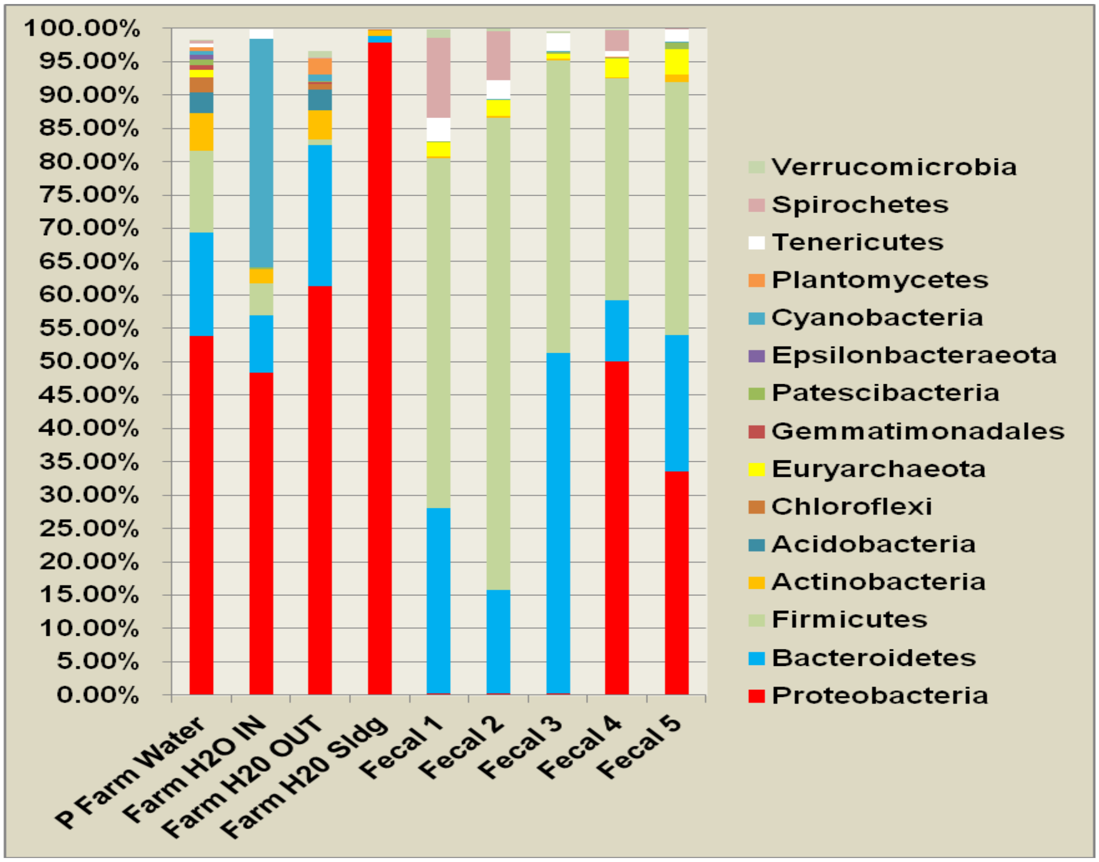


Table 2. A farm not in this study shows contaminated water with gram negative proteobacteria passing orally through to the deer's fecals (4 & 5) but not deer fecal 1, 2 & 3 possibly affecting deer health.

Farm Water Report			DWS	PWS	PWS	Lifetime
Unit	ug/L	mg/L	ug/L	ug/L	mg/L	ug/L
Aluminum	n/d	n/d	200			
Arsenic	n/d	n/d	10			
Cadmium	n/d	n/d	5			
Calcium	50.2		no / standard			
Chromium	n/d	n/d	100			
Cobalt	n/d	n/d	40			
Copper	n/d	n/d	1,300			
Iron		0.933			0.3	
Lead	n/d	n/d	15			
Magnesium		35.9	no / standard			
Manganese	17.1	n/d	50			
Nickel	n/d	n/d	100			
Strontium	40.4		25			4,000
Vanadium	n/d	n/d	30			
Zinc	n/d	n/d		5,000		
Hardness		273	very hard			
Drinking Water Standard (DWS)	Public Welfare Standard (PWS)				Lifetime Health Advisory	

Tables 3. New water testing this spring on the quarentine deer farm still shows high levels of iron. Since water is an important nutrient for animal it is important to review the possibility that water could be a nutrient source that could have a negative impact on the deers performance values.

Feed

The feed reviewed in this study (Farm 3, Phase 1) noted that the original ration on the quarantined farm was deficient in certain nutrients as compared to a commercial ration. Of the two control farms, one used a common commercially available pelleted feed whereas the other used a custom farm mixed feed. With the discovery of the deficiency of nutrients on the quarantined farm, it was decided to replace this deficient feed for the deer. Since one of the control farms used a home recipe that showed a lower amount of macro / micro mineral support as compared to an industry supplied feed, the replacement feed chosen for the quarantined farm was as the same commercially available feed as used on one of the control farms. This was done as to assess whether the commcercial feed has a positive / negative / no change impact on these deers health as compared to their deficient feed in the disease process. This review could provide helpful information to farmers since other farms in the industry, that have sucumed to at least one deer testing positive for CWD, might have been using different availalable feed and or supplementing other feedstuffs of unknown nutritional values. When buying feed / hay / other products, farmers may not know the origins of their feed purchase. Did it come from an area where CWD is in the wild or an area (affected area / non-affected area) where CWD is or not present in the wild deer, or from a fellow farmer that provides such feedstuffs?

Pathogenic and anti-microbial resistant (AMR) bacteria can be introduced into agricultural operations through wildlife feces causing illness in livestock and contaminating food products harvested and prepared for human consumption. The ability to detect and diagnose diseases is key to monitoring and preventing their spread. Wildlife* in general are increasingly recognized as carriers of antimicrobial resistant bacteria and for their abilities to disseminate the pathogens across agricultural landscapes. This can negatively impact agriculture, public health, and food safety.
(Title: * FY 2018 Annual Report on Technology Transfer, June 2019, https://www.ars.usda.gov/ARSUserFiles/ott/FY2018%20USDA%20TT%20Rpt.pdf).

One example of this potential includes insect pathogen transfer. One farm that maintained honey bees was having a colony death of unknown origin. Upon review using the same techniques for the deer in this study showed that these bees were carriers of many gram negative proteo - bacteria including e-coli bacteria and pseudomonas (Table 4).

Other examples of environmental exposures are noted in a wildlife agency document titled: Technical Report on Best Management Practices for Prevention, Surveillance, and Management of Chronic Wasting Disease produced and embraced by Association of Fish and Wildlife Agencies, Washington D.C. (AFWA). This document notes in section 7 regarding under baiting the allowance of feeding or baiting wildlife notes while natural aggregations of animals exist due to a variety of behavioral, seasonal, and resource factors, human-associated aggregations related to baiting and feeding can greatly increase the risk of disease transmission due to increased animal numbers and concentrations over extended time periods. One concern notes that sales of wildlife bait and feed provides markets for surplus agricultural commodities considered unfit or unmarketable for human or livestock consumption. Feeding substandard feed products to wildlife or farmed population supports promotion the increased potential for disease.
Another area of concern is the interest in composting CWD infected carcasses as a method of disposal vs. the recommendation by APHIS in approved landfills in Section 14 – Carcass Disposal.

Infected CWD carcass disposal needs to be in approved landfills that are properly licensed and operated. The landfill disposal method offers one of the most economically feasible options for disposal of carcasses and parts, particularly in high volumes. While disposal via landfill may not eliminate infectious prion, carcass parts disposed of in a landfill would be inaccessible to cervids and may functionally contain the CWD prions. It is important that carcasses are properly covered after disposal in a landfill to prevent scavenging or the opportunity of creating more insect populations (flies, beetles, etc…) on the landscape to expand the opportunities of spreading disease promoting organisms.

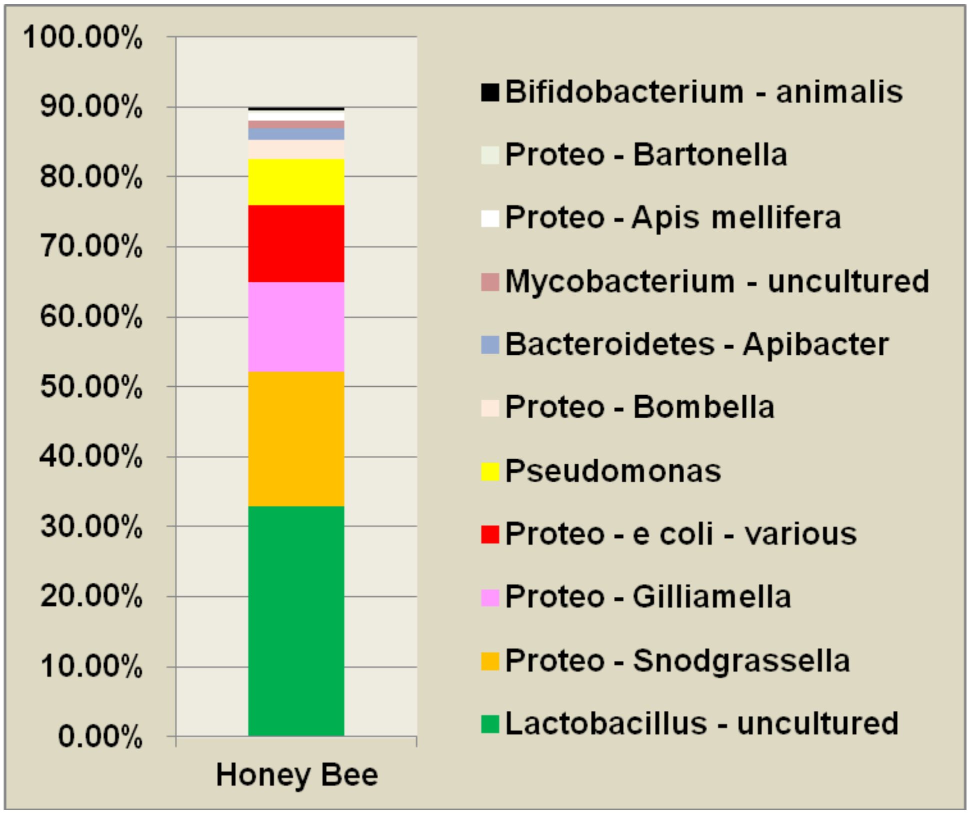


Table 4. Bacterial review of honey bees from a non –surviving hive shows many proteo - bacterial organisms including e-coli and pseudomonas.

If a farmer does not know where the feed is originating from, along with not testing to verify feedstuffs, this could have a negative impact on deer health (Table 3-6 Phase 2). Two deer on the quarantined farm being fed a commercially available feed for over a year eventually did develop rectally positive (IHC) for CWD. These deer subsequently died, but from other causes (Phase 2 & Phase 2.5) as determined upon their respective necropsy. Though they showed some typical anatomical signs (loss of body fat) and testing positive for CWD, death was not attritubuted to CWD. Deer on both control farms consuming their respective commercially available or custom feeds did not show negative associated organisms or other observable body conditions of being detected with CWD (Phase 2.5) as was on the deer that died on quarantined farm.

In the history of the study we had 3 farms on 3 different rations with 3 different health signatures from the deer. This helps lay the ground work for what we were dealing with in the initial health assessment of deer on the quarantine farm and the deer on each control farm. This is important to know since current data show deer from different farms on different diets in different geographic areas tend to signature differently due to these differences. When the quarantined farm was determined to be nutritionally deficient we then fed a commercial ration so now we had 3 farms consuming 2 ration types but still with 3 different different health signatures on all 3 farms though the quarantined farm showed improvements to health over it's original deficient feed. Even though improved health was evident we still saw 2 deer test positive for CWD from their rectal biopsies.

Deer aquire a disease state leading to an end stage disease process we have come to know as CWD. As we continue to fully understand this disease process it would be important to have all 3 farms on the same ration. To bring all 3 farms into a common nutritional compliance, a ration preparation was designed (Table 5) to reduce any potential primary negative associated health conditions found in deer.

Historically, inflamation is considered to be the most prevalant disease state diagnosed in any mamalian system. Inflammation can be exasperated by negatively associated environmental organisms or contaminants. Since the 3 farms are separated by a large geographical area in the State of Wisconsin, having the same ration being fed on each farm would prove beneficial to the study in review of controlling any inflammatory state of the deer which could lead to a potential negative health condition irregardless of geografical location.

Deer, like cows, are ruminants and have a basic process of digestion for various feedstuffs which can have a positive or negative health effect. As compared to what wild deer eat on the landscape (geographically) farm raised deer are fed various feed(s) or a ration that can vary in the industry for many reasons. Unlike farmed raised deer, wild deer eat what they can get which could be nutritionally deficient in relation to seasonal change geographically. Farm raised deer rations are controllable with enhanced nutrition if one pays attention to details. To review a disease process in ruminants one needs to understand some basic priciples of nutrition during the disease process. Deer are set up to ruminate by biohydration of the feeds they consume. That means they "ferment" their foods with microbes in their rumen to digest their feed properly. This method of "rumenation" is based off of these microbes in their rumen to do the work aiding in digestion. Any negative association to upset this digestive process can lead to improper fermentation,reduced performance, getting sick and even dying from the wrong things they may eat.

An example of a wrong nutrient for a ruminant to consume is too much fat in the ration. A higher fat usage such as vegitable fats in a liquid form or a high grain fat (corn) percentage in a deer ration can be cause for concern. The quality of fat used is also important to know as to the make up the percentage ratios of saturated, mono-unsaturated and poly-unsaturated fats. Too much of the wrong kind of fat in a deer's ration can kill off the helpful micro-organisms in the aiding of optimum rumination and performance. Too much fat also allows for negative organisms to flourish and put the deer's body under a stress condition. This stress condition in turn will diminish the deer's immune status affecting performance.

With a continueing suppressed immune status the deer will now be set up for environmental contamination (geographically) of more opportunistic negative organisms. This contiuing stress will continue to degrade the deer's immune system while increasing the chances of more infections. Left unchecked over time the deers body functions are redirected to fight off this onslaught of infections and eventually could lead to death. For this portion of the study we will be reviewing the basic fat component the feed in relation to any pro-inflammatory process with deer.

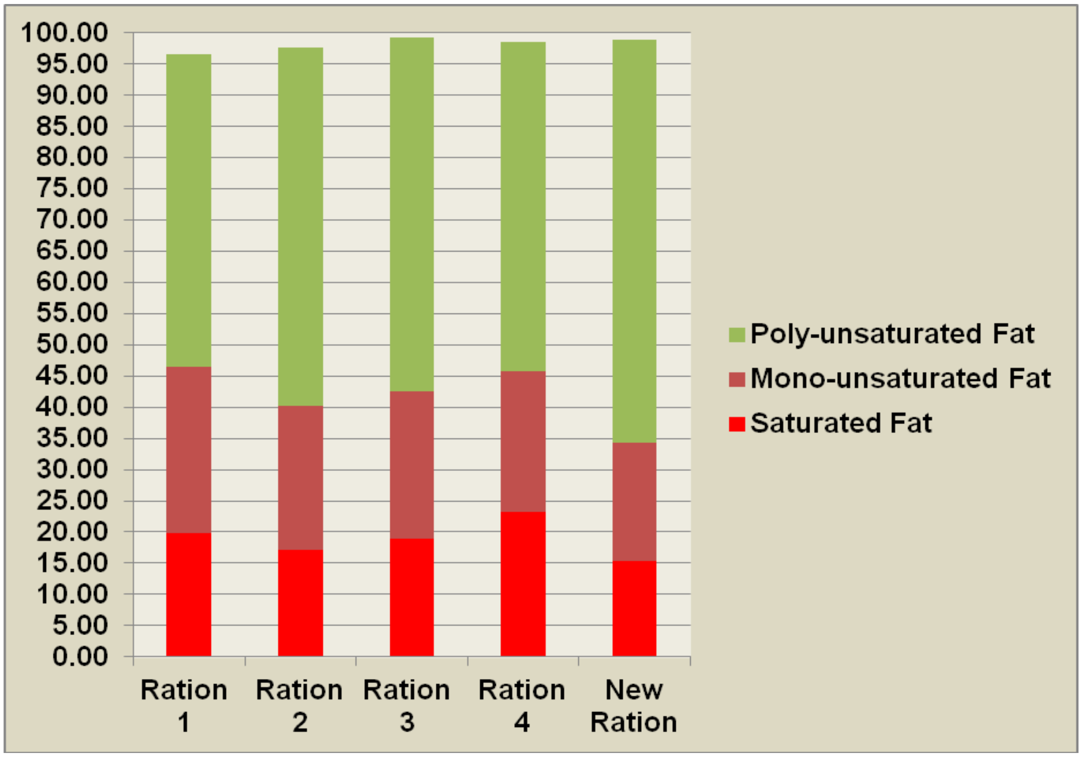


Table 5. Basic fat component types of rations used in this study. (1) original quarentine farm,(2) industry ration cntrol farm,(3) custom textured ration control farm, (4) querentine farm recovery ration, new ration all 3 farms.

In review of the fat components of the ration it is hard to discern one from the other on a generic basis but it is more about the quality of the fat along with the volume percentage of fat types in a ruminant ration. Too much of certain types of fat from different sources could lead to lowering the pH in the rumen of the deer called acidosis. This lowering of the pH in the rumen allows for negative associated organisms to take over making the rumen sick. Certain fat types are also important to know their sourcing and preparation for the ration as fats have the ability to oxidize (turn rancid) in the digestive process that can also make your deer sick.

Rations that are comprised of mostly grains contain a higher amount of a polyunsaturated fat. Higher amounts of certain types of poly-unstaurated fats can lead to a pro-inflammatory state in deer. This inflammatory process can lead to stress that can be further exasperated by the deers gram negative organisms load aquired by consuming untested water and feedstuffs including pellets,hay or forages.

Left to right - A leaf doesn't last long as Purple 1 enjoy's a snack then a cool 54 degree drink of water on this warm afternoon. Later Yellow 2 joined in for some fresh green grass / clover mix. Purple 1 is rectal negative where Yellow 2 is rectal positive. Both deer are pregnant and due to fawn soon.

In review of the dietary inputs for this study (Table 6) the major bacterial organisms present in feeds presented are listed from the quarantined farm, control farms as well as the new study ration. Other farm rations (not in this study) are used to show differences for negative associated feed organisms as compared to feeds involved in this study. As one can see, the first three original rations have some common basic organisms found in the feed though they do differ in organism concentrations. These original feeds were made at 3 different mills geographically.

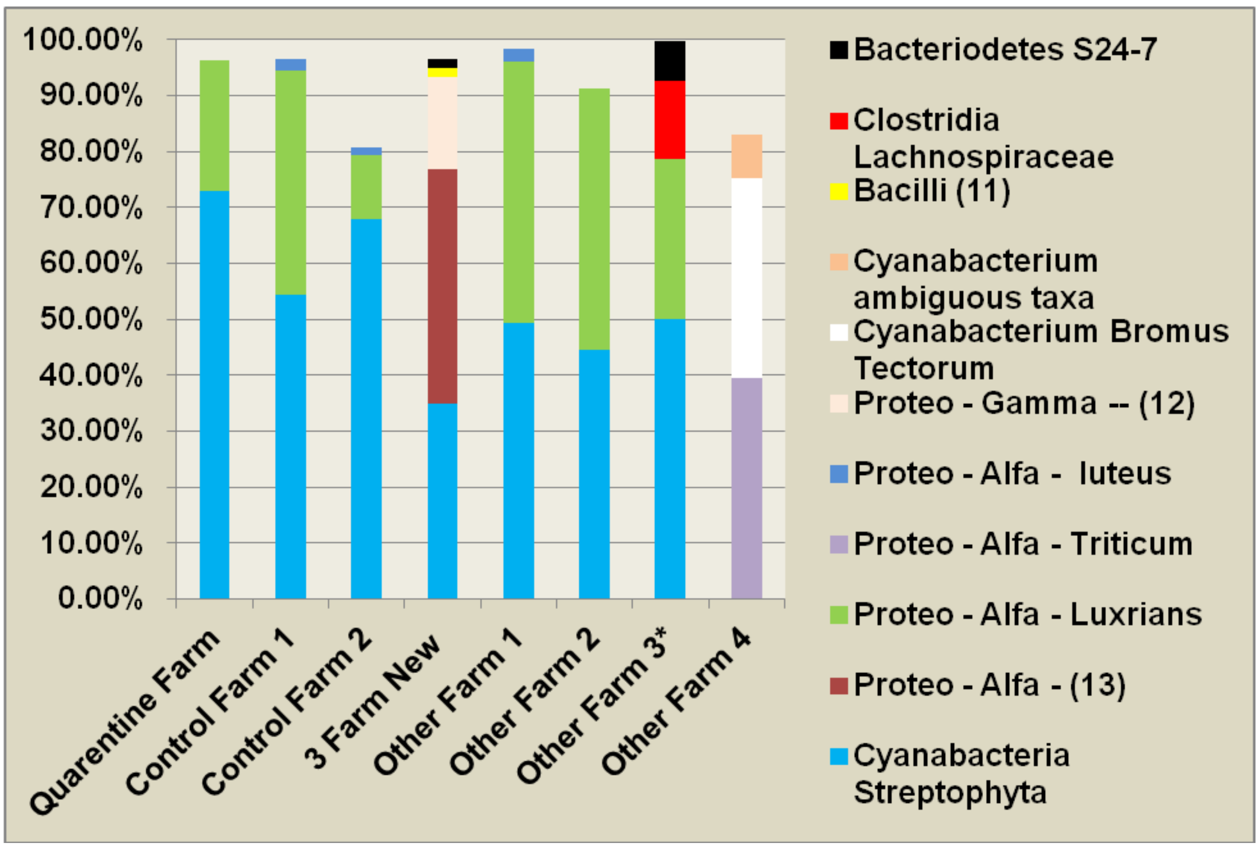


Table 6. Left to Right - Feed bacterial signitures of the original 3 farm rations in the study with the new ration (3 Farm New) being fed on all 3 farms for Phase 3. Other farm ration signitures show feed organism diversity.

Other rations that were not in the study (other farm 1,2,4) show differences in bacterial organisms from different feed type sources or from feed on a farm (other farm 3*) from the feeder itsself. Normally one shouldn't find Clostridia organisms in feed but testing shows how deer carrying these organisms can, and do, leave these organisms in a feeder for the next deer to consume. This is a form of verticle transmission of an organism, where, as part of developing a bio-secure feed program, review of how oftern the feeder is cleaned would be warranted.

Nasal / Oral

Past bacterial organisms directly connected to the quarantined deers' oral pathways showed differences (Phase 2 Table 7) when comparig the 2018 sampling to the changes found during the bacterial disease assessment (Phase 2.5 Table 1) in the final days of Orange 1 and Yellow 1. These changes are expected to be different in each deer since their continued immune suppression would allow more opportunistic organisms to occupy these areas (nasal /oral) due to increased stress and disease progression. Water samples from the quarantine farm (Table 7) as well as a water source from another cervid farm in the area were used in comparing the current water conditions to any organisms found in the current deer nasal and oral samples taken this spring.

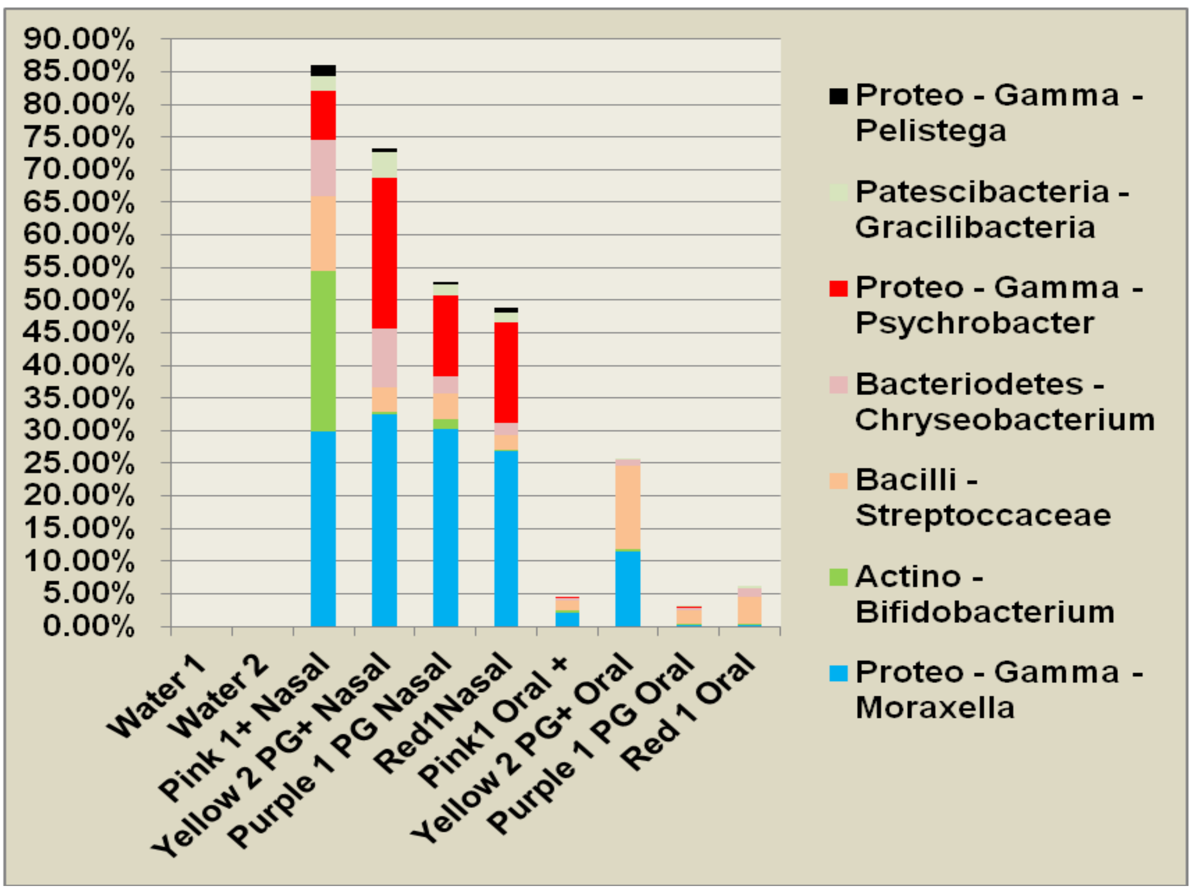


Table 7. Bacterial organisms compared from nasal and oral samples on the quarentied farm did not originate from the water source on the farm or another well supply close by the querentined farm.

The only bacteria that seemed to be consistant was the Psychrobacter from the Gamma – Proteobacterim group year to year from the nasal swab of the deer that were either positive and or considered non-detected with rectal CWD . The bacilli – Streptoccaceae was ony found in Pink 1 to any great degree in last years testing that is now seen in more deer. The Actino- Bifidobacterium present is considered a healthier organism not found before in past samples.
Nasal and oral samples collected for a fungal assessment of the 2 deer that died with CWD (Orange1 / Yellow 1) for various available samples are reviewed (Table 8,9) for comparable organisms.

Buck - Red 1 and Does Pink 1 and Yellow 2 enjoy the shade during a warm afternoon. The Buck was inconclusive on his rectal test but Pink 1 and Yellow 2 were both found to be Positive detects.

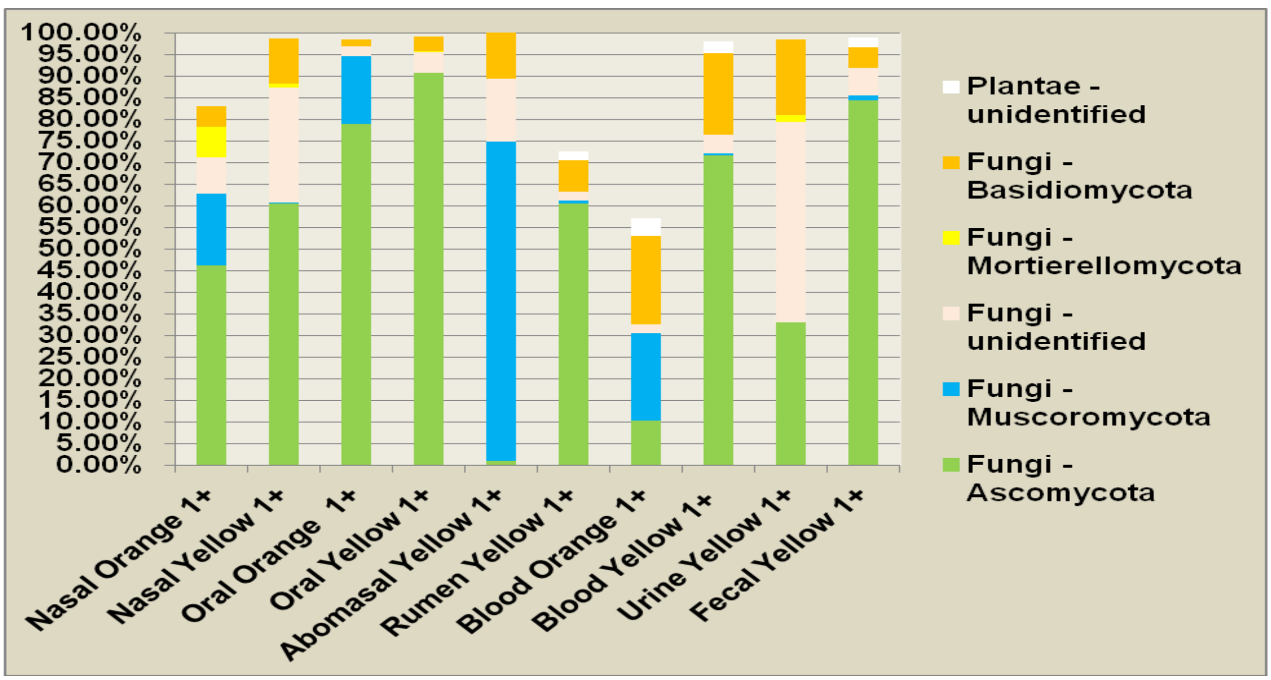


Table 8. In the Fungal assessment Fungi – Ascomycota was the most ocupying phylum shared in various samples between both deer (Orange1 / Yellow 1) in review of comparable organisms. Of all phylums presented in samples there were a total of 59 Fungi – Ascomycota

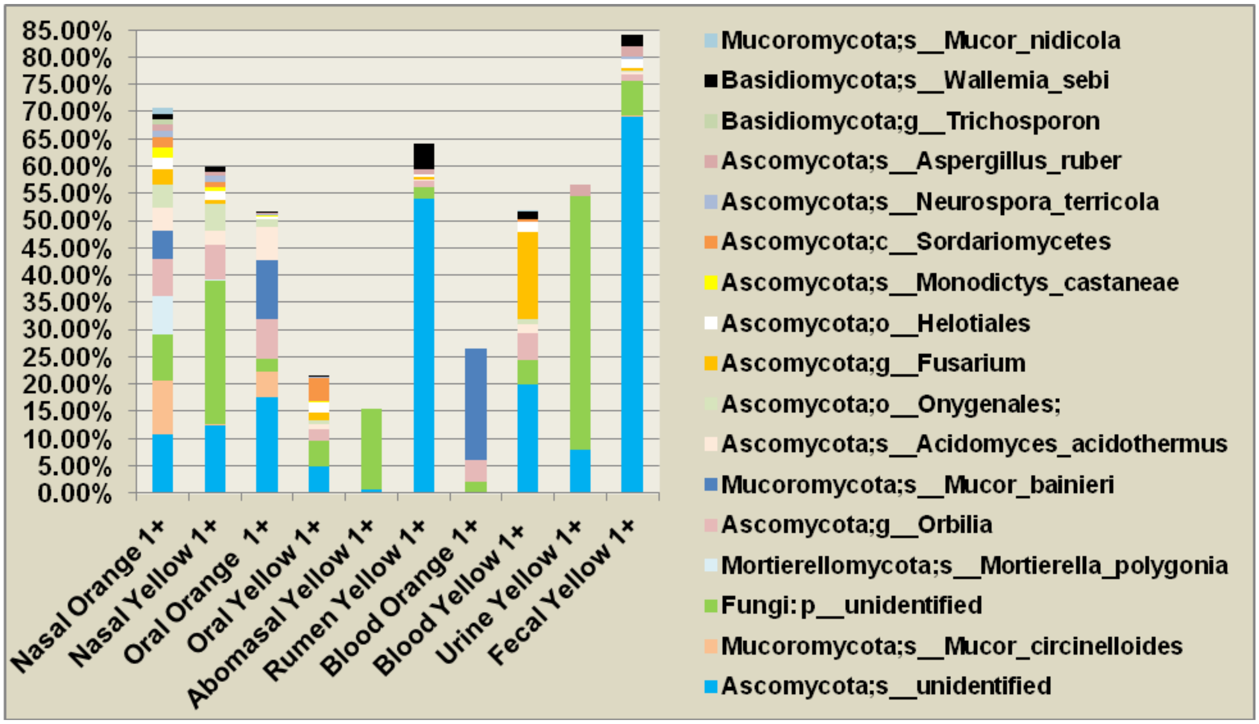


Table 9. Fungi (p-phylum, s- species) organisms and dirtribution show differences in concentrations as they pass or survive through the deers digective process'.

Current fungal assesments from sick / non sick deer or the environment provides a timestamp basis of organisms for the comparrison to future samples. Though it is currently unclear how fungal exposures plays an intergative role of deer health (positive / negative) it is important to capture samples from deer exposed to or testing positive to a disease in understanding the disease process.

Ray discussing the importance of targeted research with Representative Jeff Mursau on live deer in a controlled setting as Dr. Robinson preps another Doe for an untrasound to determen pregnacy.

Bloods

Pre-clinical and follow up blood samples from deer (Phase 2 Table 16 &17) show the difference of bacterial organisms between the deer on the quarantined farm compared to control farms. These deer along with 3 other bucks from other farms were fed the same industry ration for comparison. The two most common organisms of concern in prior blood samples were ecoli – shigella and mycoplasma. These organisms were diminished greatly (Phase 2.5 Table 2.) in the bloods of Orange 1 and Yellow 1 taken upon their untimily deaths. It seems that as time progressed for these two rectally positive deer they seemed to lose diversity of these organisms in their bloods.
This may provide us with an early clue -- negative organisms associated with deer detected with or whithout being found rectally positive for CWD.

The current years' blood work shows a more positive outlook on bacterial organisms that were present as compared to the last 2 years of sampling on these deer (Table 10).

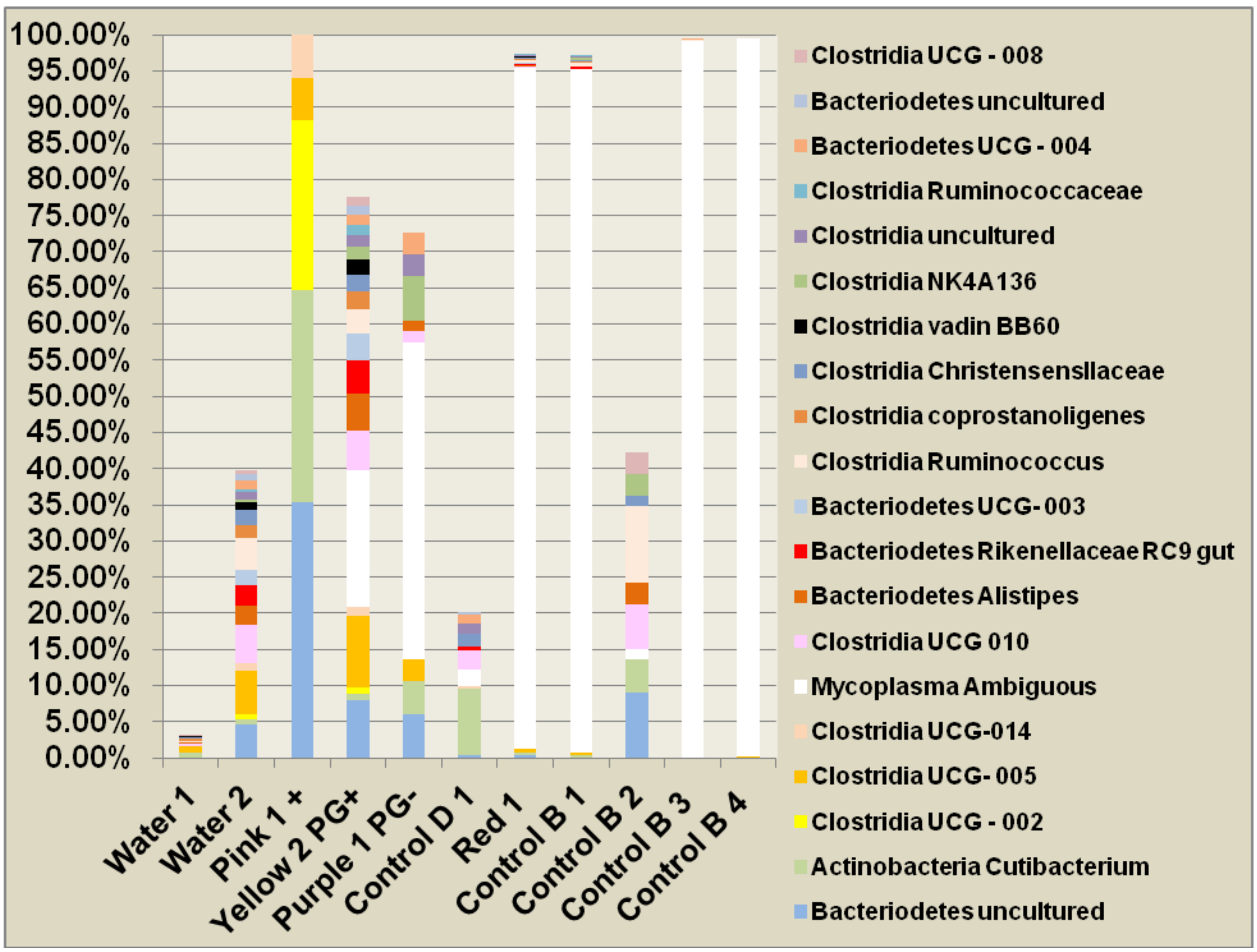


Table 10. Water source 2 is in close proximity of the quarentined farm but shows the organisms can be found in the bloods of the deer geographically across the state.

There was an absence of the ecoli - shigella organism in the deer bloods this spring though the mycoplasma organism was still present. In Phase II (table 17) deer blood samples, mycoplasma was shown in all deer but more prevalant in bucks vs. Does. Also absent are other negative organisms such as the ones found in both Orange 1/Yellow 1.

This could mean that either the 2 current deer with detectable CWD are in an earlier phase of a disease process, or have not progressed to a later clinical state as last years deer Orange 1 and Yellow 1. This is importnant for the industry to know as no one has ever nailed down the approximate timeline of contraction to the onset of a clinical case with no return towards death.

Currently Yellow 2 was born on this farm after the first index case of CWD was found. By continuing to follow timelines (age, disease status, death) of when 2 past deer were found dead (September / December), the next time point to watch this year, for Yellow 2 and Pink 1, would be in line with fall seasonal changes. During this seasonal change the deer are hormonally active and preparing for the coming winter. Fall is also when undersirable environmental organisms are at their highest levels in the environment and animals promoting stressfull conditions. This added stress comes at a time when seasonal changes are demanding a thicker haircoat and building of body fat stores for the winter. Deer that are rectally positive with CWD last year had a slower development of a haircoat and both lacked building any appreciable body fat stores and neither were bred by the buck.

Blood Values	Unit	Pink 1+	Yellow 2+ PG	Purple 1 PG	Red 1	Average
Glucose	mg/dL	307	206	256	229	247
AST(GOT)	U/L	107	64	85	87	86
SDH	U/L	14.8	11.6	16.9	10.8	12.4
Total Bilirubin	mg/dL	1.4	2.1	0.9	0.5	1.3
Cholesterol	mg/dL	47	31	45	36	38
Total Protein	g/dL	6.1	6.1	5.8	6.4	6.2
Albumin	g/dL	3.4	3.5	3.2	3.3	3.4
Urea N	mg/dL	31	33	28	31	31.6
Creatinine	mg/dL	1.7	1.6	1.5	1.2	1.5
Phosphorous	mg/dL	1.6	7.5	4.2	5.5	4.8
Calcium	mg/dL	8.4	7.9	7.7	8.3	8.2
Sodium	mmol/L	140	141	139	134	138
Potassium	mmol/L	4.1	4.7	4.4	4.5	4.4
Chloride	mmol/L	105	103	104	99	102.3
Bicarbonate	mmol/L	24	23	22	25	24
CK	U/L	274	63	146	95	144
Gamma-GT	U/L	42	20	44	43	35
Anion Gap mmol/L	mmol/L	15	20	17	15	16.6
Globulin g/dL	g/dL	2.7	2.6	2.6	3.1	2.8
A/G	Ratio	1.3	1.3	1.2	1.1	1.2
Urea/Creat	Ratio	18	21	19	26	21.6
Sodium / Potassium	Ratio 32	34	30	32	30	31.3
Nonesterified Fatty Acids	mEq/L	0.592	0.62	0.571	0.499	0.57

Table 11. Metabolic Blood Panel of the 4 remaing deer on quarentined farm. Elevated NEFA above 0.8 mEq/L in plasma may be antagonistic to the immune system.

Additional blood test were submitted for the 4 current deer to review their metabolic status (Table 11) this past spring. Interest in the prospects of the exisiting deers overall health was developed via a concern of the past 2 deer that died were lacking appreciable body fat (Phase 2.5) upon necropsy. The metabolic panel did not show any blood perameters out of sorts from normal.

This would be interesting since two of these deer tested rectally positive for CWD for which at least 1 deer is preganant. With the metabolic panel showing a healthy condition in the deer one might wonder whether the rectal biopsy positives are an idicater of earlier stage of detection vs. a later stage of the disease process. Only time will tell if this could be the case.
In reviewing pro- inflammatory conditions a Non-esterfied fatty acid test (NEFA) is most frequently done in camelids (which develop hepatic lipidosis), other ruminants (e.g. cows, sheep, goats) to determine if they are at risk of ketosis, and horses (equine metabolic syndrome). The Non-esterfied fatty acid testing was performed as to review the health status in the deers blood that would be consistant with lipid (fat) mobilization / oxidation process present in deer that would show signs of potential negative energy balance. Negative energy balance increases the potential of a disease process as seen in Orange 1 and Yellow 1 noting a loss of body fat. Negative energy balances in camelids particularly pregnant females are at risk of developing hepatic lipidosis. Measuring NEFA levels in blood can determine negative energy balance. Camelids with NEFA > 0.8 mEq/L (1. Tornquist et al., 2001) are at increased risk for lipidosis. The 4 deer on the quarantined farm were found to be below the 0.8 mEq/L level as noted in this reference for camalids (Table 11).

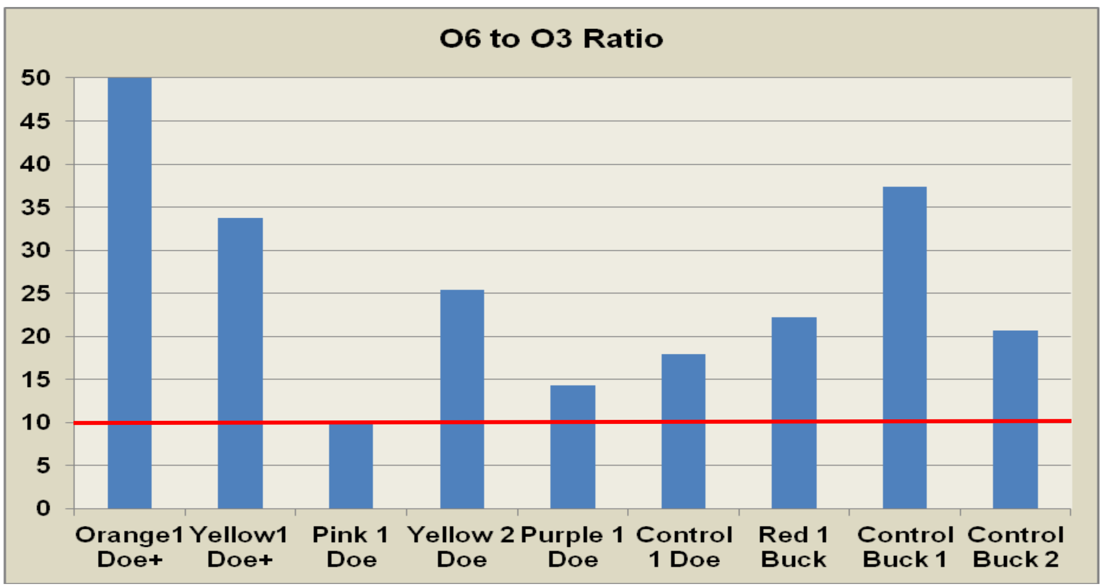


Table 12. Review of the Omega 6 to Omega 3 ratios in deer blood for quarentined and control deer for inflamatory status. Above a 10 to 1 ratio (red line) is considered inflamatory.

In further review of a what is considered a pro-inflamatory state , blood samples were collected in review of the essential fatty acid content of the deer in this study. Essential fatty acids are fat types in the blood that can demonstrate concerns of the inflamatory process’ in animals. These essential fatty acids are generally referred to as an omega 6 and an omega 3.

These essential fatty acids cannot be made by the deer body process' perse and must be provided to the deer through their feed ration.

When referring to a pro-inflamatory state vs. a less inflamatory state in blood lipids (fats) it is typically refered to as the omega 6 to omega 3 ratio. The higher the ratio of inbalance with the omega 6 being high to an omega 3 being low supports an inflamatory process in the ruminant such as Orange 1 with a 50 to 1 (Table 12) omega 6 to omega 3 ratio being excessively pro-inflamatory.

An inflamatory process in the body generally leads to increased cellular stress. Cellular stress can then alter how a cell functions normally in an animal. This cell stress could be further deminished by having unwanted gram negative organisms onboard as observed in the bloods of Orange 1 and Yellow 1 (Phase 2 and 2,5) that could collectively diminish their ability to fight off the increased disease organisms contracted from the environment.

Typically , an omega 6 to omega 3 ratio should be at or below a 10 to 1 ratio value as depicted in Table 11. This is typically where rations are formulated for deer but can vary depending on rations provided. There were some rations I tested in the past that had ratios as high as 25 to 1 values.
At the time that Orange 1 and Yellow 1 when found to be rectally positive in April 2018 they had ratio of 50 to 1 and 34 to 1 respectively. This was very high considering the feed ratio was only 10 to 1. This high ratio ment these deer had a very high inflamatory process actively working against them in trying to maintain their normal body functions. As one can see the other deer on the quarentined farm as well as the control farms were also signituring higher than the ration of a 10 to 1 ratio with the exception of Pink 1.

This could have been because she was pregnant at this timepoint and rectally tested as a non-detect for CWD in 2018. This year, this Doe has tested positive detect for CWD rectally.This Doe is an older deer originating on a poor feed ration early on which has reduced the proper development of a normally functioning rumen. This process shows us later on in life that she would be a good candidate to be at risk of eventually having a deminished immune sytem leading to other health issues.

A higher inflamatory process over a period of time can overwork the deers' body function leading to a challenged immune system. This process could allow for potentially more undesirable organisms to continue to compromise the deers immune defenses to fight off disease process. This also would keep your deer from performing the normal functions of growth , proper immunity and reproductive status in your herd.

Past and current research has shown that cells infected with certain negative organisms prevent cells from their normal functional duties in the creation of conformational (normal) proteins. Continued inflamation and increased sustained cell stress leads to a more cascading cellular stress and dysfunction leading to creation of non- conformational (misfolded) protein such as prions in deer. Since these deer in our study have been exposed to these higher inflammatory proceses it provides the opportunity to see how long it will take to unwind these inflammatory process back to near normal with all 3 farms on the same ration. By reducing the inflammatory process (Table 13) in the same deer their normal body function should be able to compensate over time to improved health. Reduced cellular inflammation would be particulaly important in restoring normal cellular functions in deer. By allowing normal cell processes to work properly would bolster the deer's immune system to help in the reduction of bacterial infections.

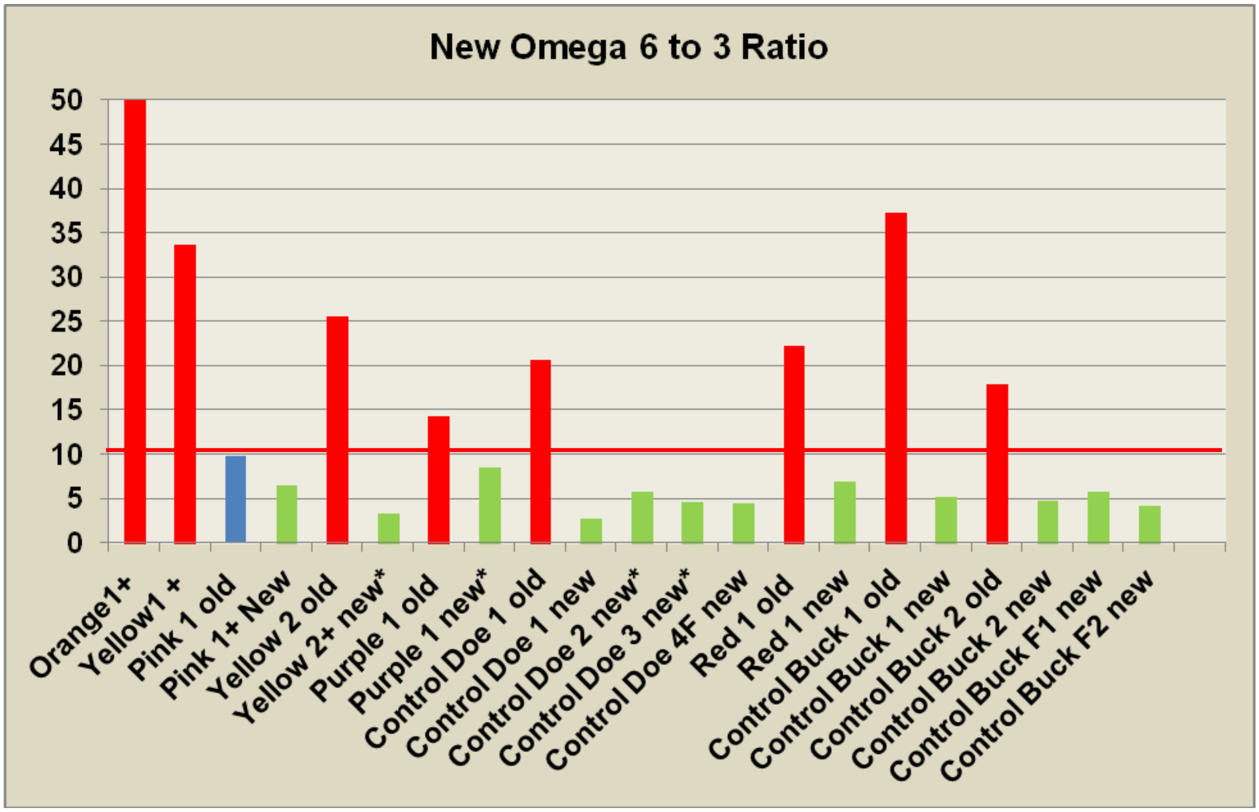


Table 13. Review of past study deer 6 to 3 ratios (red bars) showng pro-inflamation markers on different rations as compared with their coresponding new 6 to 3 ratio (green bars) being non-inflamatory with study deer on the same new ration. Does noted with an * are pregnant.

This year blood sampling shows lowered 6 to 3 ratios of blood values of the same deer in this study along with other farmed deer in different geographical areas of the state not in the initial study. There were also some other adult Does included in this study in the interests of nutrition for reproductive performanes due to these Does had not fawned the last 2 years (control Doe 2,3). Each of these 2 Does had twin fawns this spring. These fawns were also included in this review (Doe fawn 4 , Buck fawns F1,F2) from these other farms fed the test ration to continue the review of any positive or negative effects relating to fetal programming and the support of immunity in deer.

By reducing the pro-inflamatory process we will be able to follow all health aspects on all 3 farms under the same ration support where as the deer biological signiture closer together though they are geographicaly separated. This in turn will provide a basis for comparable bloods provided from wild harvested deer in comparrison of like mechinisms in review of disease process'.

Fecals

The fecal review this year (Table 14) shows more similarities of the fecals on all deer from all 3 farms. Though there were multiple organisms that were present in a nearby farm well water source very little orgaisms from the quarantined farm well water resinated in the deer fecals. The fact that other wells show a higher load of organisms that appear in all deer supports the the assertion that a water sanitation program on the deer farm would be warranted. Staying ahead of any negative possibilities that could have a negative affect on your deer would support your bottom line.

Another area of interest is the presence of some common organisms (Phase 2.5 Table 4) shared from the fecal tesing of Yellow1 upon her death. What is different is that the akkermansia bacterium in the current deer testing is less than the 18% of Yellow1. Akkermansia is an organism normally residing in the intestinal tract that, when elevated, can be detrimental and negatively impact the intestinal lining allowing for more opportunistic organsims and infection to occur.

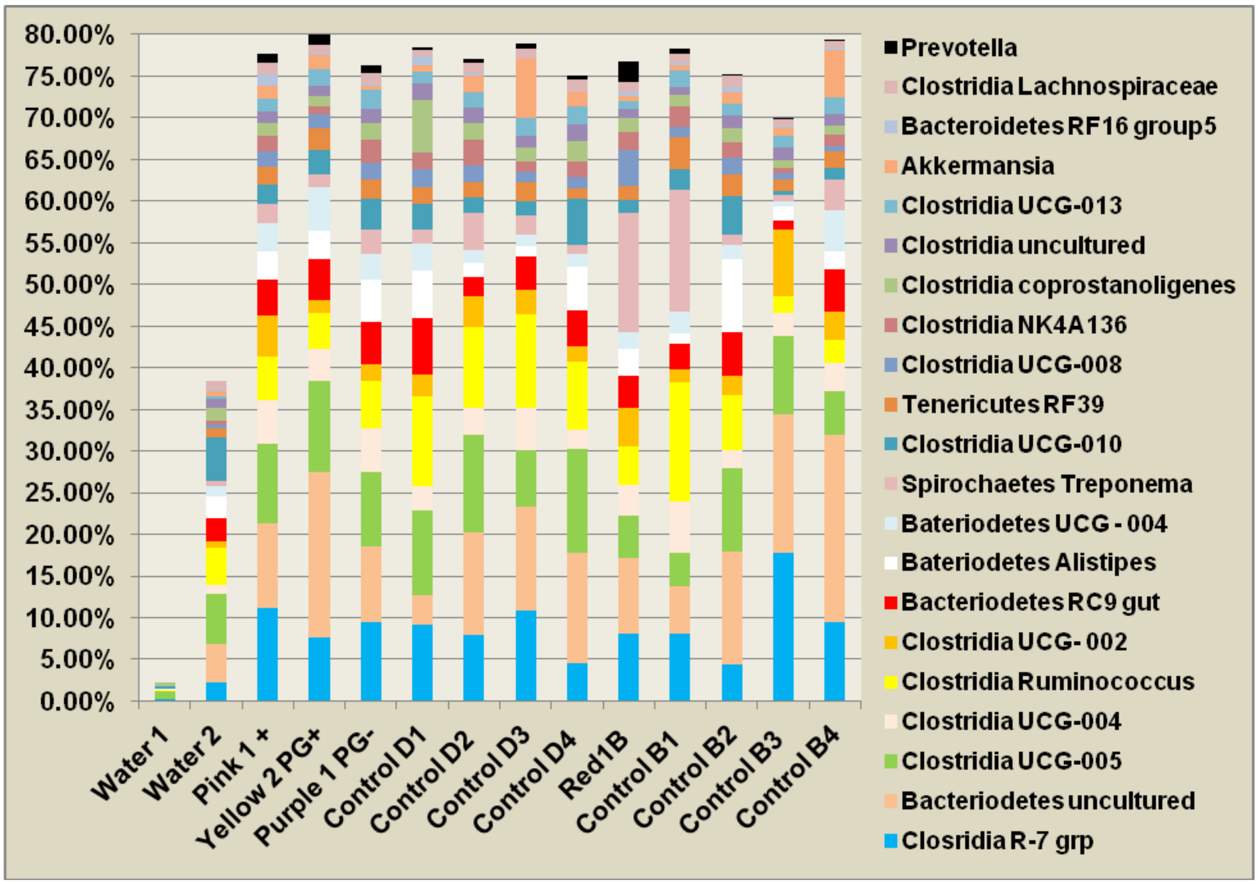


Table 14. Deer on a common ration pellet signiture closer than deer on past different rations though they are still geographically apart on different water / hay / forage sources.

Left too Right: July 15th shows Purple 1 and Yellow 2 still carrying fawns to be born soon. Pink 1 was not determined to be pregnant but shows good body conformation.

Urine

Samples for urine were collected from the deer on the quarantined farm (Table 15). This was to provide a comparrison of the urine sample by Yellow 1 upon her death in December, 2018. None of the negative organisms present in the current deer urine, collected this spring, were present when Yellow 1 died last December (Phase 2.5, Table 5).

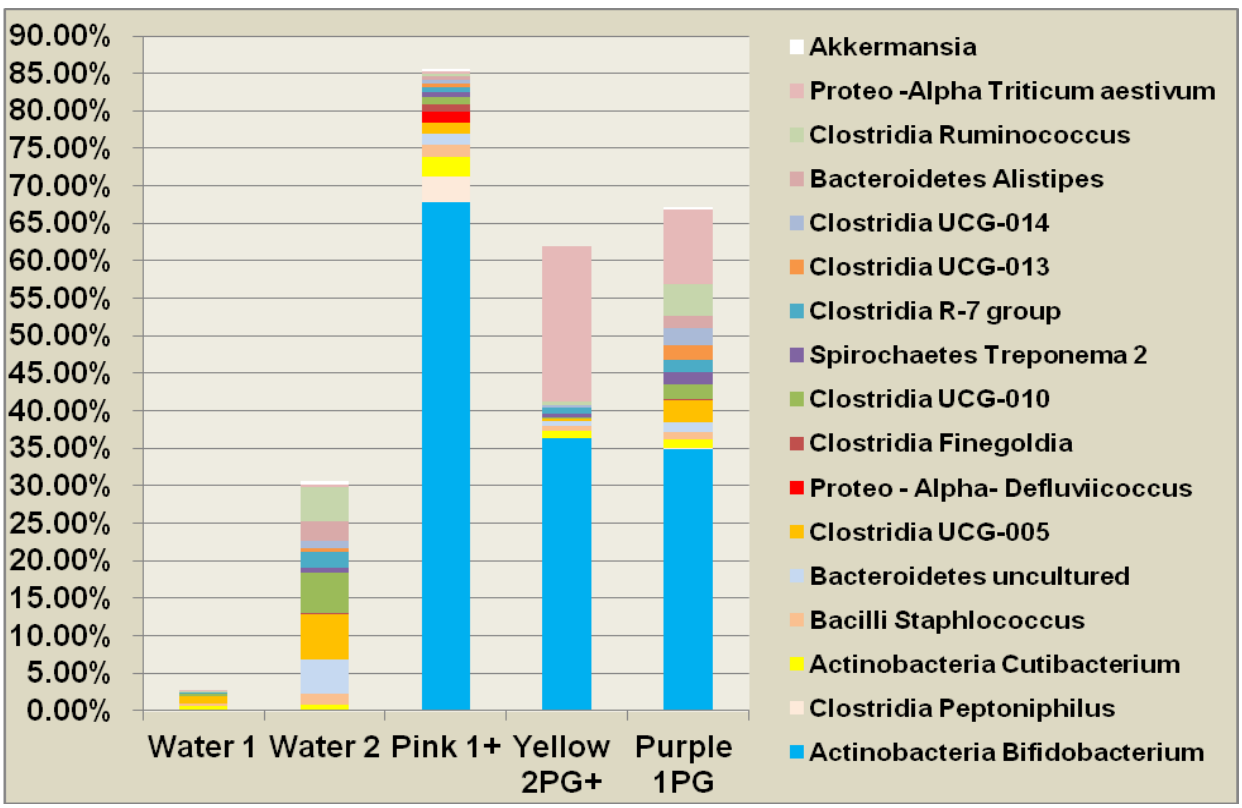


Table 15. Urine collected from the 3 Does on the quarentine farm as compared to 2 different farm water supplies.

Summary of findings

In summary, this spring's samples collected from the deer involved in this study were taken from the drinking water source and feed in addition to nasal, oral, blood, fecal and urine of deer. The current bacterial organisms identified were compared to the same deer profiles from past years of testing. This years bactrial profile shows a more positive health promotion this year as compared to past negative associated bacterial organisms in the deer. These positive organisms were also different than the negative associated organisms that were found in the two deer that died with CWD as described in Phase 2.5. Review of additional blood markers pertaining to the metabolic health status provided a basis of the deers for overall stability. Other blood markers reviewed from the deer in this study showed that deer from all 3 farms geographically separated had a reduction of an omega 6 to 3 ratios that would be supportive of anti inflamatory process'.
Though two Doe were found to be rectally positive for CWD by IHC methods, one deer was pregnant while the other deer was not pregnant. There was one Doe deer that was found to be a non- detect rectally for CWD and is pregnant at this time along with a buck deer with an unknown CWD detect status due to insufficient rectal tissue follicals present in the submitted sample. This coming falls' seasonal changes with the impending demand of the deer to produce a winter hair coat as well as body fat accumulation in preparation for the winter season will be something to monitor.

Discussion

In review of the overal study updates, we started with 3 unknown farm health assessments for deer in a effort to determine what is a normal assessment of the control farm deer as compared to differences to a farmed deer quarantined for CWD.

As we learned, the health status of each deer in the study it became apparent that the farmed deer under quarantine for CWD had a higher negative organism composition than the deer of the control farms. It was also noted that the 2 control farms also differed in their respective health status though not negatively associated. These health differences identified from the quarentined farm were from sources such as water (e-coli, excess iron), feed (psudomonus) and hay/forage (ecoli, psudomonus, excess minerals) products provided or fed to these deer. It is important when sourcing feeds for nutritional inputs of deer to test and quantify their healthier attributes before providing to deer. This would provide the deer the opportunity to perform to a healthier production status.
By changing the feed inputs on the 3 farms as same provided a healthier profile to the deer for the opportunity to measure and follow identified healthier markers in the deer from the improvement of the nutritional inputs. These improvements showed a reduction in negative associated organisms identified in past deer samples. This change from negative to a more positive associated organism expression should provide support to the deers immune system. This in turnt would minimize the opportunity of negative environmental organisms to create negative conditions in your deer.

Taken collectively, this provides the research the unique opportunities for the continuing health assessment of these remaining deer through this years pending fawning timepoints and the pending falls seasonal changes. This provides the availability to also monitor these deer for the past conditions in the disease role in loss of body condition (body fats) or slowness of winter hair coat changes. This information will continue to help the industry in the continuing effort to find answers for the remediation of or elimination of CWD as we know it.

By developing and implimenting a sound feeding and biosecurity program for your farm will support your farming activities while minimizing the risk factors we have delineated in this research review to date.

Information provided to date would also be applicable for wildlife agencies or groups in the interests and understanding of disease process' of free ranging wildlife for develpoing effective best managemnet practices. By understanding areas of opportunities to reduce potentials for the spread for gram negative bacterial organisms or other potential pathogens that wildlife species carry would reduce the potential of any cross over contamination to livestock species (2. Shelli Dubay).

I appreciate the opportunity to provide this information to the industry and look forward to the pending updates from this Phase 3 study installment in a follow up of these deer in a Phase 4 proposed continuence hopefully without the potential need of a Phase 3.5.

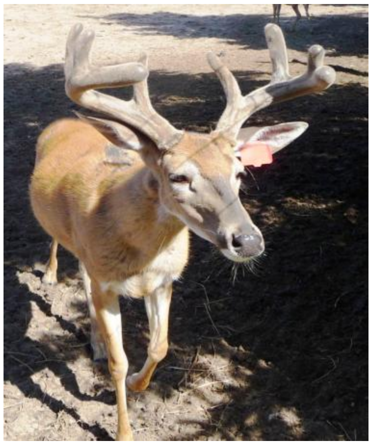
You the member, hold the key in supporting this continued research so your input is highly valued.

In PPE from left to right: Jerome Donohoe, Dr. Amy Robinson, Joel Espe, Ray Hanson and Brad Heath. Thank you goes out for those who participated with special thanks to Joel Espe (all photos) Nu Dart (darts) and Zoo Pharm (BAM kit) kind donations supporting CWD research.

Contact your Industry Leaders to have a conversation supporting CWD research.

WOW/WCFF whitetailsofwisconsin.com , NADeFA schafer@nadefa.org , or DBC tim@dbcdeer.com

Submitted: Jerome Donohoe, ag_o3@earthlink.net



Buck Red1 keeps tabs on Purple 1 (still pregnant) while Pink 1 looks on as Yellow 2 feeds to nourish' her first fawn (Yellow 3) born 7-24-19.

Disclosure Statement: Though there are Non - disclosure agreements with the farms in this study to protect confidentiality and any perceived research Bias , A.O.S. declares there are no conflicts of interest generated with or between the 3 farms and or the WOW / WCFF Foundation as designed and funded.

Reference 1: Metabolic changes and induction of hepatic lipidosis during feed restriction in llamas.

Am J Vet Res. 2001 Jul; 62(7):1081-7. Tornquist SJ[1], Cebra CK, Van Saun RJ, Smith BB, Mattoon JS.

Objectives: To determine whether feed restriction induces hepatic lipidosis (HL) in llamas and to evaluate the metabolic changes that develop during feed restriction.

Conclusions and Clinical Relevance:

HL in llamas may be induced by severe feed restriction, particularly in the face of increased energy demand. Llamas with weight loss attributable to inadequate dietary intake may develop biochemical evidence of hepatopathy and HL. Increases in serum concentration of bile acids and activities of GGT, AST, and SDH may indicate the development of HL in llamas and identify affected animals for aggressive therapeutic intervention.

Reference 2 : Environmental Factors Influencing White-tailed Deer (Odocoileus virginianus) Exposure to Livestock Pathogens in Wisconsin. Authors: Shelli Dubay. Christopher Jacques , Nigel Golden, Bryant Kern, Kathleen Mahoney, Andrew Norton, Devi Patnayak, Timothy Van Deelen PLoS ONE 10(6): e0128827. doi:10.1371/ journal.pone.0128827.