Peck Farm Research Report

Title: Identifying Nutritional Characteristics' of a Cervid Farm under CWD Quarantine

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The Wisconsin Cervid Farmers Foundation (WCFF), North American Deer and Elk Farmers Association (NADeFA) and Deer Breeders Corporation (DBC) have collectively funded the following review for the continued CWD research investigation of 6 Whitetail deer on a Wisconsin farm located in Iowa County, Wisconsin. The purpose of this collective funded research Phase 1 proposal will review and monitor the deer's health / wellbeing, reproductive status of these quarantined deer. Once established over the next 4 years Phase 2 if approved will provide a more detailed understanding of the CWD disease process while in captivity if any deer advance in showing any clinical signs of the disease process.

This captive deer herd is located in Iowa County Wisconsin where back in 2002 was the first location of CWD detected in the wild deer population in the State of Wisconsin and is still considered today the highest CWD endemic area in the State. With this farm being of single fence will provide a good model for wild to captive interaction.

The information within this report is to provide any Cervid Farmers the opportunity to review the inputs and outputs of your own farm operations. This information is to help improve your own Bio Security plans and or on farm sanitary practices as deemed necessary. As we collectively learn how this disease continues to be transmitted in the wild and or subsequently spreads to the farm hopefully will provide insight on how to keep the disease outside of your fenced operation.

In the Beginning

This farm had been considered CWD negative since 2002 until one deer (1.5 year male) died from a goring incident in early January of 2016 from another male deer (10 year old) in the same pen. Upon submitting the deceased deer's tissues for CWD testing results noted that this deer was positive for CWD via IHC test results for the lymph nodes and brain. This was a first for the farm since the beginning of CWD testing in the wild deer population around our farm in 2002.

Since the farm is a single fenced farm, along with other livestock as a petting zoo, the CWD is speculated to have come from the wild deer in the surrounding area for which is considered the highest risk endemic area of the State (Iowa County) for CWD positive wild deer.

We decided we wanted to offer the opportunity to Whitetails of Wisconsin (WOW) through their Wisconsin Cervid Farmers Foundation (WCFF) as an effort to study the deer on this farm to find answers to the CWD disease process instead of depopulation of our remaining deer. A 4 year agreement was approved to move forward.

In the initial stage of review of the deer on the farm was conducted by Dr. Tracy Nichols of the USDA Wildlife services for the collection of certain tissues (laryngeal, rectal, blood, fecal, etc…) to ascertain the remaining live deer CWD status through potentially developing an opportunity for anti- mortem testing methods and procedures. The funds covering the USDA portion of this project (personnel, travel, lodging and equipment…) were covered by the USDA.

Initial Report of Findings

After 2 negative suspect rectal tissue samplings of the deer over the last 2-8 months it was determined to wait a year for the next sampling. To date, the residing deer on this farm have all tested as non-detects for the CWD prion through their second rectal biopsy testing procedure. Two of the original deer (10 year old buck and adult doe) in this herd died a week after the second testing procedure for rectal tissue harvest for unknown reasons as no necropsies were preformed. Both deceased deer heads were collected and sent in for lymph node testing for CWD and as consistent with the rectal biopsies both deer tested negative for CWD. This left an interesting question as why

older deer on the farm were negative for CWD but a yearling deer tested positive for the CWD prion in the lymph node as well as in the brain noting to be a more advance state of the disease process.

A buck fawn (1 month old) born late into the herd had died a month later for unknown reasons and no tissues were submitted for CWD testing. A liver sample was collected and submitted for mineral analysis. The autopsy did not show any gross legions though the carcass but lacked any appreciable body fat. The liver panel results showed a mineral deficiency.

A subsequent study design was created by Jerome Donohoe from the WCFF with the multi organization funding support provided by Wisconsin Cervid Farmers Foundation, North America Deer and Elk Farmers Association and Deer Breeders Corporation (WCFF, NADeFA, and DBC). This initial support will provide a comprehensive review in phase one as to the general nutritional and reproduction status of the remaining deer on the farm. This base understanding is needed to understand the onset of a disease process in varying ages of deer that continues to have a negative impact in the Cervid industry.

Moving Forward

Upon my interview and subsequent review of the quarantined farm with the farm owner for the future care of these deer (next 4 years) it was noted that there were some challenges with housing conditions that warranted further review.

One of these challenges was the deer looked to be under nourished.

Note: Doe, quarantined farm (left) shows signs of nutritional insufficiencies vs. healthy Doe from control farm.

The farm operation was set up to have a petting zoo for the public to come and see the deer with their children. The deer were kept somewhat hungry so they would readily eat the shell corn bought by the public to feed the deer. The corn was sold in quarter dispensers as a farm income source supporting the deer.

Upon my review of the feed and water system I noted that these animals might have less than optimal nutrition available to them on a daily basis.

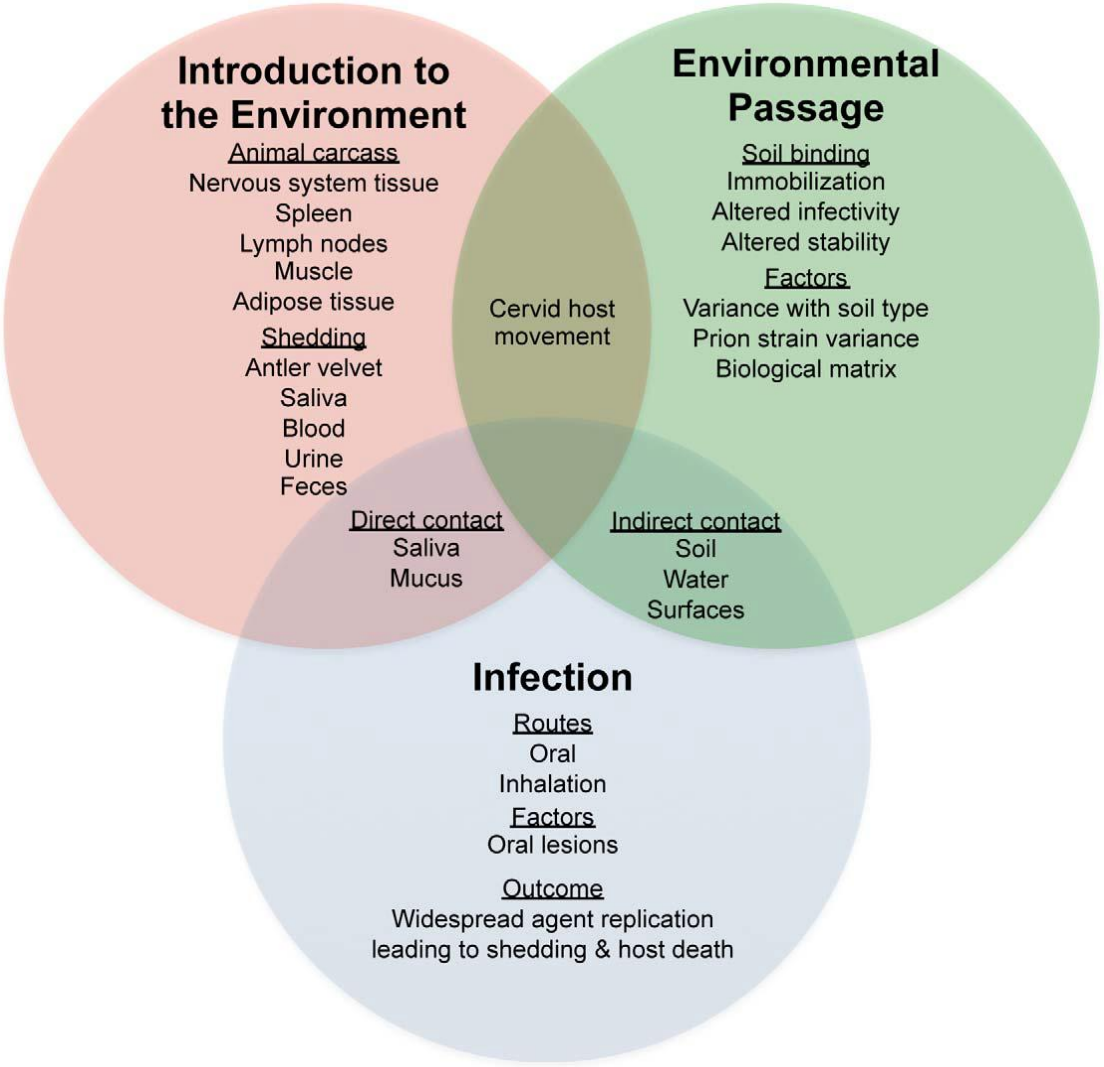
Note: Deer's water supply from deep well filling old cheese vat for Koi fish. Vat open for many other animal species to use. Wood feeder protected by rain / snow but is open to other vermin to use.

Over time, continued nutritional deficiencies in any animals physiological system would leave open the opportunity for reduced immunity against disease processes including the possibility of contracting CWD from the environment.

My past work experience comes from the Laboratory Animal Research field (32 years) at the Medical College of Wisconsin. I provided housing and dietary research supports for developing research animal model systems for the study of human / animal disease process' or medical complications. These developed animal systems varied from the use of various rodents to various livestock species and primates. These supportive research activities directly impacted the development of key nutritional findings supporting the research program for both the animal and human health. Since retiring from the Lab Animal Research field I refocused my research to help grow a USDA Agricultural Food Program for continuing animal and human health supports through nutrition. The current program titled N-3 polyunsaturated fatty acids and human health and disease utilizes animal model systems for feed / food development for livestock and human alike. I have spent the past 8 years developing improved animal and human healthier food platforms for both animal and human consumption through the use of research rodent and livestock models systems. My past work is the basis for this current research proposal hypothesis.

Research Hypothesis:

I believe Cervids, supported with optimized feed, forage and water through nutrition along with genetics can stave off the debilitating disease onset of CWD.



Factors influencing horizontal transmission of prion disease in the environment.
doi:10.1371/journal.ppat.1003113.g001

Feed and nutrition is not considered part of the horizontal transmission process.

Research rationale:

To effectively review the nutritional status of deer on the farm currently under CWD quarantine we will need to find at least 2 comparable deer farms outside of the CWD endemic area of Southern Wisconsin to act as controls.
This is important as to eliminate the potential of feed and or water contaminations that might be present within the endemic area. Though the farm had a CWD negative status since 2002 until 2016 it will also be necessary to utilize 2 control farms that have a current CWD negative status' since 2002 from outside of any CWD affected areas of the State.

The control farms we have identified in this study continue to use lymph node tests their deer for CWD through the Federal CWD Surveillance Program. These farms both have a negative CWD status since year 2002. Since these farms are negative status we will only use collected rectal biopsies from the deer on the farm that is under quarantine for CWD diagnosis potential.

All farms (1 quarantined and 2 control) that are to be used for this study are currently under a NON-Disclosure clause as to maintain their privacy and to remove any research bias of this study. Upon certain research findings it may become necessary to include more farms in from other geographical locations to support certain test materials of anticipated findings supporting the hypothesis of this study (phase 2).

The reason for using 2 control farms in this study is that the cervid industry has gained much knowledge to date regarding certain areas of research and the speculations surrounding the CWD process or its mode of contamination. There has been little research conducted on the overall nutritional compentcies of the cervid deer to stave off the onset of the CWD disease process neither on a farm nor in the wild deer population. The only exception of nutritional review was with trace mineral compositions of copper, zinc and magnesium in genetically altered rodent model systems designed for early onset of CWD after prion exposure. Most other research is to develop testing mechanism for detection of the CWD prion.

In Iowa County (endemic area) surrounding the farm the deer population is considered by many to exceed the carrying capacity of the natural vegetation. One would also think that the high deer population, in relation to available feed sources on the landscape, increases likelihood for improper nutrition. In reality, the reproduction status of these deer seems to be unaffected and the deer population in this CWD endemic area continues to grow each year.
Though the available natural vegetation or agricultural crops supports reproduction little is known to a potential reduced immulogical deficiency effect through lack of proper nutritional quality in this geographical area of the State.

Organizations reviewing the wild cervid population or the farmed cervid industry have spent a lot of money over the years on CWD detection in the cervids to only a research “hindsight approach” just to eliminate them from the landscape once determined to be positive for the CWD prion.

To break this cycle of “hindsight approach” we need to look at the beginning causes of how the protein got that way. Several vendors support nutrition for the Cervid industry. However, there are still many that utilize their own custom mixes of deer feed, forages or water because they feel it is what works best on the farm or it’s the “cheaper too” idea. The question then becomes, without measuring (testing) to know what is needed in your feed, forages, water or other things provided the deer for supporting healthy animals, how do we manage the farm?

My research approach Phase one is to review the health status of the Farmed Cervid Industry through the eyes of these 3 farms in a proactive manner, to help educate, and get industry farmers thinking to the endpoint of potential elimination of health risks to our farmed cervids re: overall health

and nutrition status. This ongoing process over the next 4 years will include areas of geographical location, farm CWD status, feedstuffs, water, parasites (endo / ecto), gender and genetics.

Feed stuffs (grain / hay / other) and water sources can be carriers of many different organisms that could harbor contaminating properties including CWD prions. These areas of feed, water and their sources for use on the farm will be initially explored in this review.

To best represent this study from a control perspective to the quarantined deer farm two control deer farms were selected to best represent the farmed cervid industry.

Farm #1 being a larger breeding farm with a current CWD negative status since FY2002 feeds a commercially prepared pelleted feed provided from outside of the CWD endemic area.

Farm #2 being a smaller breeding farm also has a current CWD negative status since FY2002 but feeds a ground custom textured feed ration based on corn.
Farm #2 was selected for this custom mixed ration as being close in ingredients to the ration provided in quarantined farm 3 but ingredients are from outside of the CWD endemic area.

Farm 3 is our farm currently quarantined for a CWD positive animal in early 2016 but had a CWD free status since 2002. This farm feeds a ground custom textured feed ration based mostly of corn grown in the CWD endemic area.

Due to the original body condition of the deer on this farm was in question due to lack of reproductive status the nutritional status of this farms feed was upgraded to a custom pelleted ration made from ingredients from outside of the endemic area.
By providing proper nutritional supports for the deer during this study will insure a reproductive status for the longevity of the study. The owner of the farm in his original agreement was to maintain the deer and feed the deer during the 4 year timeframe. The farm owner agreed to pay for this feed upgrade and is not considered part of the requested funds in support in this study.
An approval letter was secured from the Department of Agriculture Trade and Consumer Protection (DATCP) for the approval to remove samples for testing from the quarantined deer farm. This approval works through the farm owner and the farm’s herd veterinarian to ensure animal health compliance during this study.

The following detailed farm matrix

of the 3 farms is provided to help other Cervid Farmers to glean from this research effort to help self evaluate what works best for farms and what might work best on your farm. The results of these findings are also a look into what can be used as best practices in support of the hypothesis - Can the Cervid specie supported with optimized feed, forage and water through nutrition along with its genetics stave off the debilitating disease onset of CWD?

Methods and Materials

- **Farm Matrix Descriptions**

Farm 1 - Control Farm – CWD Negative 16 Years (2002)

Total acres fenced: 40 – 380 deer = 9.5 gross deer per acre density

Double fenced: 10' on inside and 8'on the outside
Gate type, closures and fencing
Padlock on main gate
Lockable 2-way gate latches along with chain link closures
Fencing material - high tensile deer fence
Wood Posts are spaced 20 feet apart
Steel fence posts are 10 feet apart
2 gates per pen

Handling facility:

- 16 - 8x8 inside holding stalls
- 3 foot wide tunnel system with sliding gates / drop chute for immobilizing animals.
- After release from drop chute deer can be sent to:
  a. 8x8 holding stalls
  b. one of 2 outside pens or
  c. back thru the tunnel system

Forage type for pens:

- Half alfalfa and half grass , grass only pens, dirt, trees

Feed and Delivery system:

- Feed is a pelleted feed commercial with 18% protein fed year round
- Wood gravity feeder - hold 800 pounds
- Smaller covered trough feeders - hold 200 pounds
  Other commercially available feeders

Hay / Treats:

- Hay is 100% alfalfa grown on farm, feed 3rd and 4th cut
- Peanuts , apple dobbins
- Fly control: cattle rubs with flypes saturated with Diesel fuel
  boss Ultra pesticide mixture.

Water / Delivery systems:

- Fresh Water supplied 365 with Jug waters in all large pens
- Plastic horse water buckets

- Metal tank type waters.
- 100 gal tanks

Vaccinations and time of year:

- Wormer – Long Range in the fall, summer fecal tests / no parasites
- Does are vaccinated with Covexen 8
- Bucks are vaccinated with Covexan 8 and Fusoguard.
- Fawns are given probiotics at Birth (Energizer)

Farm 2 - Control Farm – CWD Negative 16 Years (2002)

Total acres fenced: 7 – 40 deer = 5.7 deer per acre density

- Double fenced: yes
- Gate type: swing gates, chain closures w/padlocks
- Padlock on main gate
- Lockable 2-way gate latches along with chain link closures
- Fencing material -high tensile deer fence
- Wood Posts are 20 feet apart
- Some fence posts are 10 feet apart
- 1 to 2 gates per pen

Handling facility:

- 2 - 8x8 inside holding stalls
- Tunnel system with sliding gates / drop chute for immobilizing animals.

Forage type for pens:

- Mostly clover / grass / chicory, grass only pens, one pen / trees

Feed and Delivery system:

- Feed is a custom textured feed fed year round
- Barrel feeder used

Hay / Treats:

- Dry hay from local farm, baleage ,
- Peanuts

Fly control – none

Water / Delivery systems:

- Fresh Water supplied 365 with Jug waters in pens
- Plastic water buckets

Vaccinations and time of year:

- Wormer – Long Range in the fall, summer fecal tests / no parasites
- Does are vaccinated with Covexen 8
- Buck and Does vaccinated with Covexan 8 and Fusoguard
- Fawns are given probiotics at Birth (Energizer)

Farm 3 - Quarantined 2016, CWD Negative 14 years (2002)

Total acres fenced: 1 - 6 deer = 6 deer per acre density

- Single fenced: yes
- Gate type, spring type self closure
- Padlock on main gate
- Fencing material - high tensile deer fence
- Wood Posts are 6-8 ft apart
- 1 gate per pen

Handling facility: none

Forage type for pens: none, dirt

Feed and Delivery system:
textured feed mix, corn, oats, mineral, etc.

- Wood gravity feeder - hold 300 pounds

Hay / Treats:

- Hay is older type from other part of farm
- Shell corn treats (grown on farm) through $0.25 / pay dispensers

Fly control – none

Water / Delivery systems:

- Fresh Water supplied from artisanal well into stock tank w/fish

Vaccinations and time of year: none

Farm Water Profiles

- The water characteristic of farm 1 has two wells on the property whereas one is considered a shallow well (20ft.) and the other a deep well (180 ft.). The deer water source on this farm is supplied from the shallow water well via an industry supplied watering station (jug) that keeps

sun, dirt and other debris out of the drinking water and from freezing during sub zero temperatures in the winter. This farm is located in a heavily used agricultural area part of the state.

- The water characteristics of farm 2 has one well on property (325 ft.) that is the primary source of water for the deer on this farm. The water is supplied from the well via an industry supplied watering station (jug) that keeps sun, dirt and other debris out of the drinking water and from freezing during sub zero temperatures in the winter. This farm is located in a light agricultural / wooded area part of the state.

- The water characteristics of farm 3, is spring water fed from an artisanal well as a clean water source that runs 24 / 7 year round and is used for all animal species of livestock on the farm. This spring water is the primary water source for the deer on this farm and is delivered to the animals via a large stock tank that also holds Koi and other small fish species. The deer have access to this water source for not only drinking but for standing / lying in the stock tank on hot summer days. This farm is located in a heavily used agricultural part of the state and is the highest CWD endemic zone for positive wild deer on an annual basis.

Water supply testing on each farm will include results for:

a. Water Package – Total Coliform Bacteria (e-coli), Nitrate, nitrate- Nitrogen, pH, Alkalinity, Hardness, Chloride, Conductivity, Corrosively index, Fluoride

b. Water Metals Package – Arsenic, Calcium, Copper, Iron, Lead, Magnesium, Manganese,

   Potassium, Sodium, total Sulfur and Zinc

Farm Feed Profiles

- Farm 1 uses an industry supplied pelleted feed and a feed with probiotics added for which is supplemented with lush alfalfa, grass and clover mixes growing in the pens and available to all deer on a year round basis. During the winter months a high quality alfalfa is supplemented alongside the pelleted feed free choice.

- Farm 2 utilizes a custom feed mix from a home recipe with corn, roasted beans, oats, and trace minerals with yeast cell wall products fed as a textured feed. The animals are provided this feed on a limited basis of approximately 3-4lbs feed each day (seasonal) in an open bucket feeder where other feed intake is from the lush growth in all pens of grass, clover, chicory. During the winter months a supplemental alfalfa bailage or dry hay is provided.

- The feed used on farm 3 is a custom family recipe used on this farm for many generations. This custom mix is based on a general mix of corn, roasted beans, oats and trace minerals that is fed as a textured feed. An upgraded pellet feed was used for supporting health and

reproduction. Hay fed to the deer is grown in the general area, pens were absent of any kind of grass / clover cover and was considered to be a dirt lot.

Farm feed testing to be performed on each farm:

- Each farm will have its respective base feed tested as a Grain Mixes/Byproducts for: moisture, crude protein, phosphorus, ash, Neutral Detergent Fiber, calcium, magnesium, potassium and fat.

- Each base rations will also be tested for their total minerals such as phosphorus, calcium, potassium, magnesium, sodium, sulfur, iron, manganese, zinc and copper.

- Each base ration will also be submitted for a bacterial screen utilizing a next generation 16S rRNA genetic screen for detection of any pathogenic organisms that might be present in the base feed sources provided to the deer.

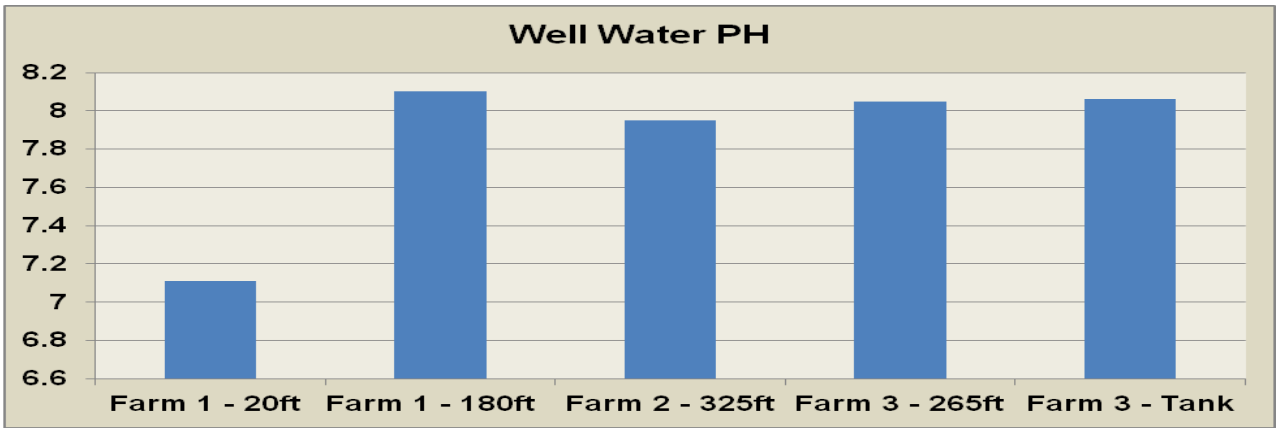
Fecal Characteristics

- Fresh fecal samples will be collected on farms 1, 2 and 3 as a random sampling from areas on each farm to best represent several animals on the farm. Fecal samples will be submitted for a bacterial screen utilizing a next generation 16S rRNA genetic screen for detection of any pathogenic organisms deposited by the animals on their respective farms.

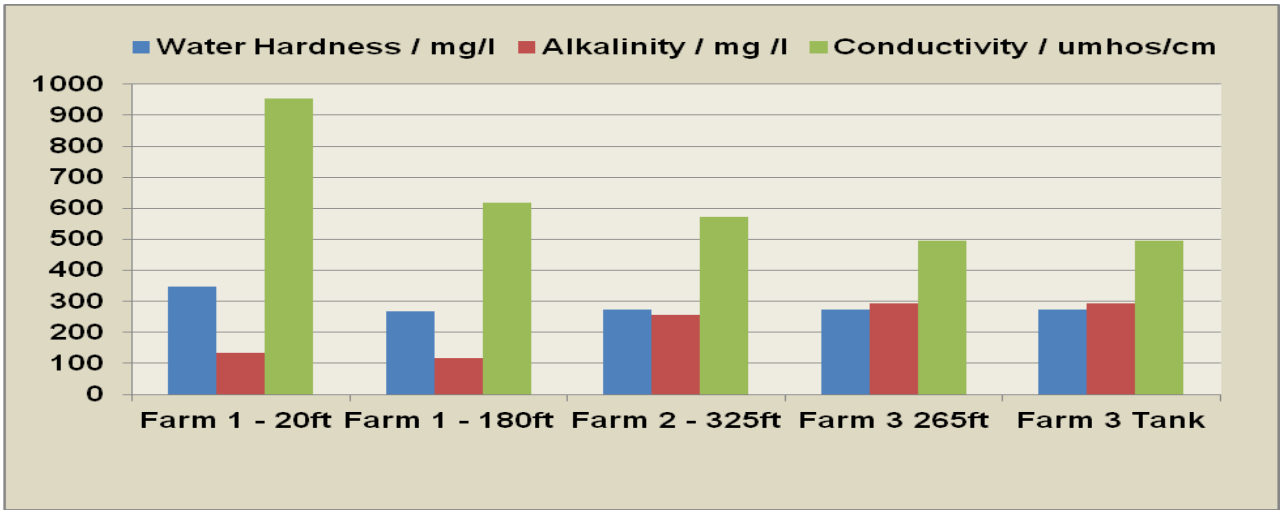
- Fecal samples will also be submitted for internal parasites and wormed accordingly.

Results:

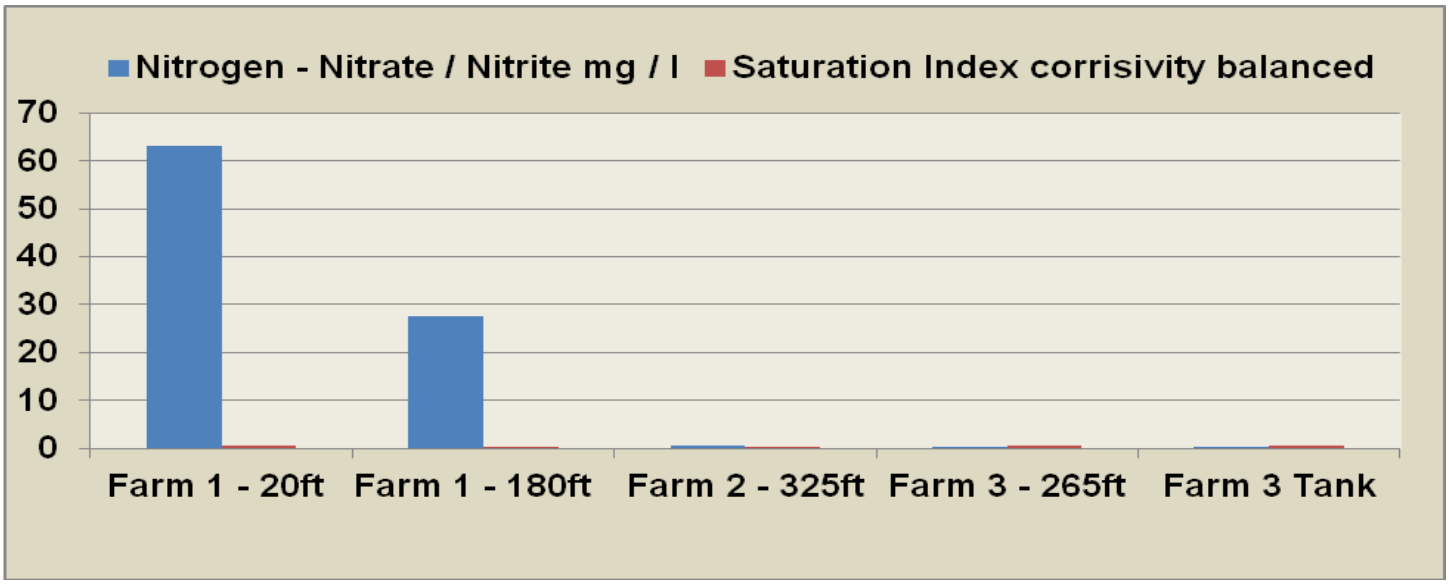
Water characteristics - University Stevens Point



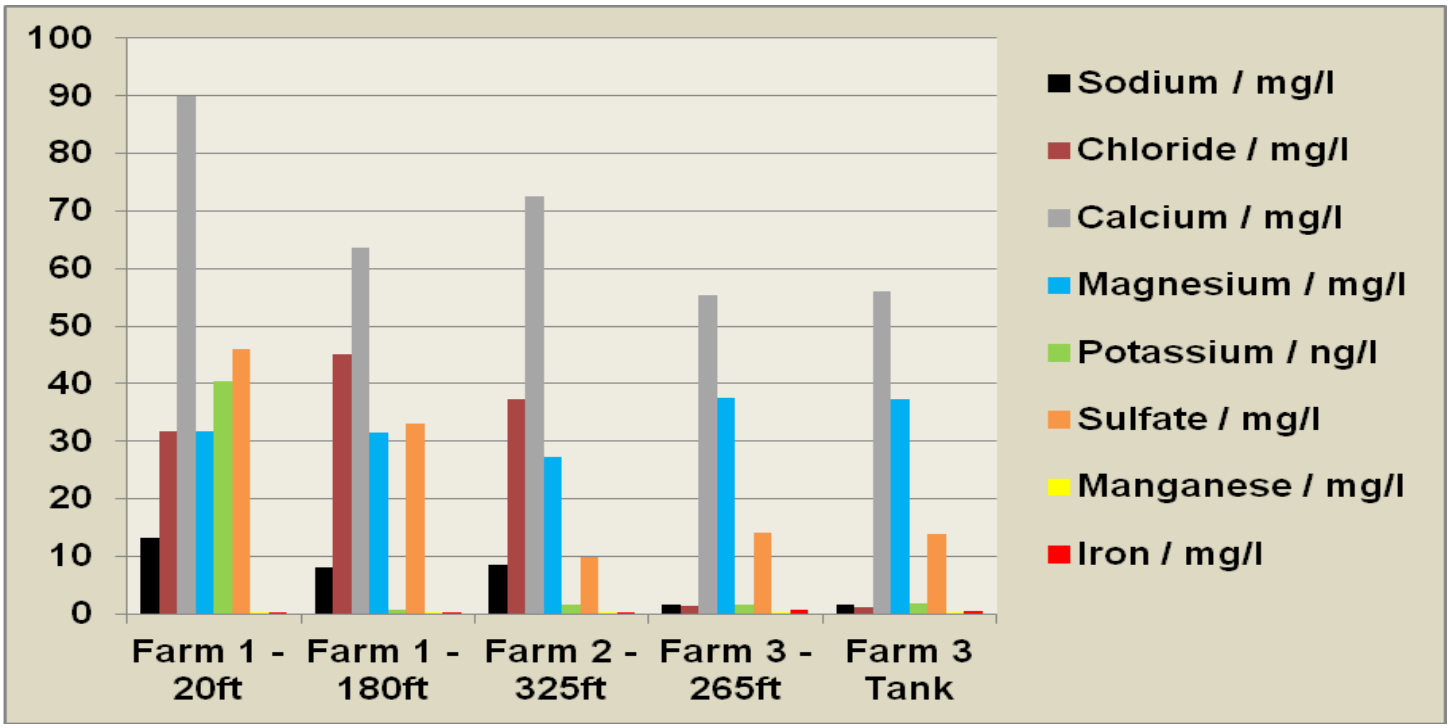
All well water pH tested above the neutral value of 7. Well water pH in an alkaline condition might stave off certain negative organisms.



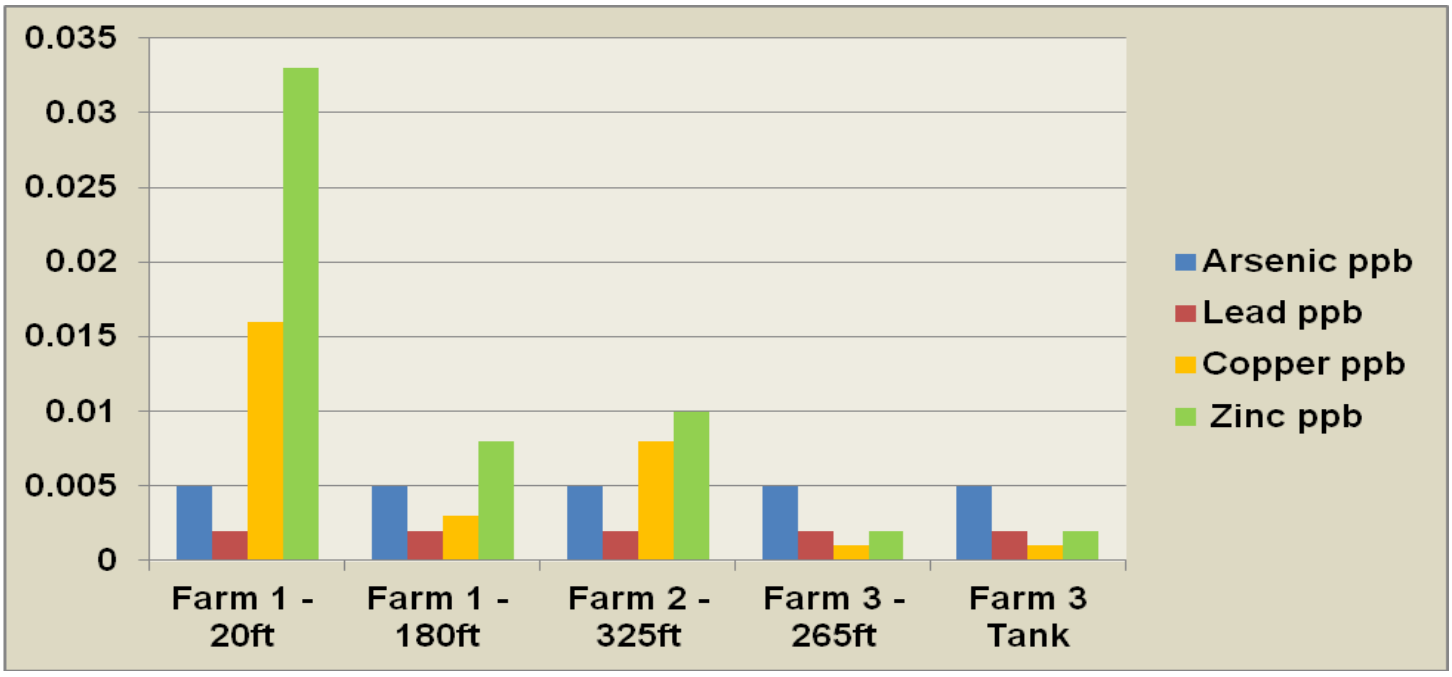
Water hardness was similar in all water samples with alkalinity / conductivity differing in Farm 1 vs. Farms 2 & 3.



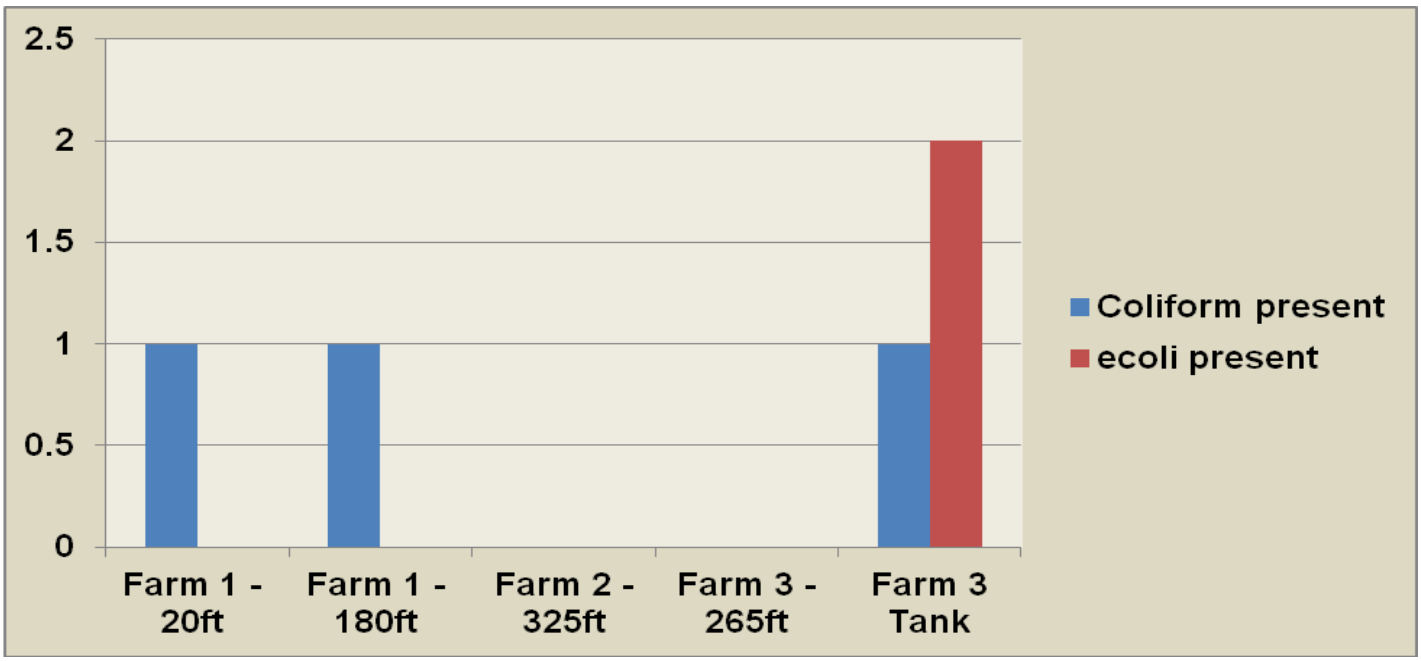
Water nitrate/nitrite levels in samples were only found in Farm 1 vs. Farm 2&3. Farm 1 is located in an agricultural production area of the state where these fertilizer types are used in crop production.



Water calcium, magnesium and sulfate were the only appreciatable minerals present in the waters of Farm 3 as compared to Farms 1 & 2.



Consistant low levels of arsenic and lead were present in all water samples. Levels of copper and zinc were elevated in Farms 1 & 2 vs Farm 3 though below the national threshold of 1ppm (copper) and 5ppm (zinc).



Well waters on Farm 1 & 3 shows coliform contamination with only Farm 3 showing E.coli in the stock tank used for the deer's drinking water. Though well water supply in farm 2&3 shows the watering receptacles' hygiene can make a difference re contaminants'.

Concentrations of Potentially Toxic Nutrients and Contaminants in Drinking Water Generally Considered Safe for Cattle [a]

Element	Upper-limit Guideline (mg/L or ppm)
Aluminum	0.5
Arsenic	0.05
Boron	5.0
Cadmium	0.005
Chromium	0.1
Cobalt	1.0
Copper	1.0
Fluorine	2.0
Lead	0.015
Manganese	0.05
Mercury	0.01
Nickel	0.25
Selenium	0.05
Vanadium	0.1
Zinc	5.0

[a] Reprinted with permission from the National Academies Press, copyright 2001, National Academy of Sciences.

Water quality goes hand in hand with the animals feed on a consistent basis. This means that if the farm water supply is not presented in a clean potable manner there is the likelihood that water quality will be diminished. One case in point; a farm decided to use a water softener to help mitigate the effect of high iron and muddy looking water every time it rained. This worked great until it was asked if the water was ever tested. Upon review of the raw well water source and the soften water provided for the household and the deer it was found to contain 5 times the sodium content of the unfiltered well water. In review, it was determined that the unit was undersized to handle the volume of water to properly deliver the amount of softened water on the whole farm. A larger unit was put in place for which took care of the problem or so was thought. Later in the fall the unit lost water pressure at the holding tank. This meant that the water supply to the deer was compromised again, this time no water. After a long and frustrating deconstruction of the softener it was determined that the backflow self cleaning part of the watering system was clogged with the iron deposits that grew and clogged the discharge port.

This brings up an important point that proper water and feed on the farm are critical to support healthy growth in animals on the farm on a year round basis. Checking the water in the dead of winter for frozen watering receptacles, especially in the northern USA, are paramount for access to fresh water for the deer. Without it they will go off feed furthering the opportunity to compromise their overall health, growth, and reproduction.

Feed characteristics – UW Forage Lab

Feed Name	Farm 1	Farm 2	Farm 3	Farm 3*
Dry Matter	87.12	86.38	87.01	90.1
Moisture %	12.88	13.62	12.99	9.9
Crude Protein %	19.89	14.68	15.59	22.44
NDF	30.52	18.79	16.22	26.97
Non Fiber Carb.	33.82	53	58.28	33.02
Total Fat	5.92	4.99	6.66	4.12
TDN	71.4	77.17	85.48	67.2
Net Energy LAC,3 X	0.74	0.8	0.9	0.69
Net Energy, Maint.	0.82	0.92	1.05	0.75
Net Energy, gain	0.53	0.62	0.73	0.47
Metabolizable Energy	1.24	1.35	1.52	1.15
Macro Minerals % DM				
Phosphorus P	0.87	0.51	0.38	0.73
Calcium Ca	1.31	1.07	0.09	1.58
Potassium K	1.26	0.79	0.62	1.45
Magnesium Mg	0.36	0.37	0.15	0.44
Sodium Na	0.27	0.35	0.04	0.42
Sulfur S	0.49	0.31	0.17	0.52
Micro Minerals ppm				
Iron Fe	367.4	277	43	663.7
Manganese Mn	347.41	142	21	334.76
Zinc Zn	332.9	192	26	308.47
Copper Cu	72.99	44	4	102.66
Ash % DM	9.85	8.54	3.25	13.45

Farm 1 feed is a commercially available pelleted feed vs. farm 2 being a custom textured feed made by the farmer that is lower in protein and trace minerals as compared to the commercial feed used on farm 1. Farm 3 original feed shows a low protein and lower mineral content in the custom textured feed. Farm 3* feed was upgraded to a pellet feed used in lieu of the farms nutrient deficient textured feed. This could explain no fawn reproduction this current year.

Fawn Liver	test	Norm Range ug/g
Arsenic	<0.05	<0.01
Cobalt	<0.05	<0.01
Copper	5.3	15-140
Iron	196	120-300
Lead	<0.05	<1.0
Manganese	2.7	3.0-8.4
Molybdenum	0.43	nd
Selenium	0.29	0.2-1.10
Zinc	34	30-110
Boron	<0.05	nd
Cadmium	<0.05	<0.001-6.0
Calcium	120	25-60
Chromium	0.15	nd
Magnesium	252	160-210
Nickel	<0.05	nd
Phosphorus	3080	850-1090
Potassium	4620	2080-3300
Sodium	2380	925-1024

Original feed used in lactation for farm 3 deer shows multiple deficiencies. In the deceased fawn, a liver panel shows low levels of the trace minerals copper and manganese. There was also excessive mineral pooling in the fawns liver from calcium, magnesium, phosphorus, potassium and sodium.

Novel ID tag		Genetics	D.O.B.
Orange 1	Doe	GG	6/7/2010
Pink 1	Doe	GG	5/20/2012
Yellow 1	Doe	GS	6/4/2012
Purple 1	Doe	GS	7/8/2012
Red 1	Buck	GS	6/4/2015
Yellow 2	Doe	GS	5/20/2016

Genetic ID's and birth dates of current deer on farm 3.

Fecal characteristics: Parasitic screen

Farm 1 – tested yearly, negative status, utilizes injectible vaccine wormer

Farm 2 – worms yearly, negative status but doesn't conduct fecal exams

Farm 3 – 1 / 5 positive stomach worms -17 worm count, follow up fecal check post worming = 2 worm count

Bacterial – 16S rRNA genetic screen for Feed and Fecal

Chart Key		
Control Feed Farm 1	**Pellet**	**Farm 1**
Control Feed Farm 1+ w / probiotic	**Pellet**	**Farm 1+**
Control Feed Farm 2	**Textured**	**Farm 2**
Quarantine Farm Feed 3 (Original)	**Textured**	**Farm 3**
Quarantine Farm Feed 3 (replacement) w / probiotic	**Pellet**	**Farm 3+**
Control Fecal Farm 1		**Farm 1**
Control Fecal Farm 1+		**Farm 1+**
Control Fecal Farm 2		**Farm 2**
Quarantine Fecal Farm 3		**Farm 3**
Quarantine Fecal Farm 3+ (30 day)		**Farm 3+**
Quarantine Fecal Farm 3++ (120 day)		**Farm 3++**
Control Fecal Wild Deer Northern Wisconsin non endemic zone		**WDNW**
Control Fecal Wild Rabbit Southern Wisconsin endemic zone		**WRSW**

The chart key above lists the pathway to review the following feed and fecal charts.

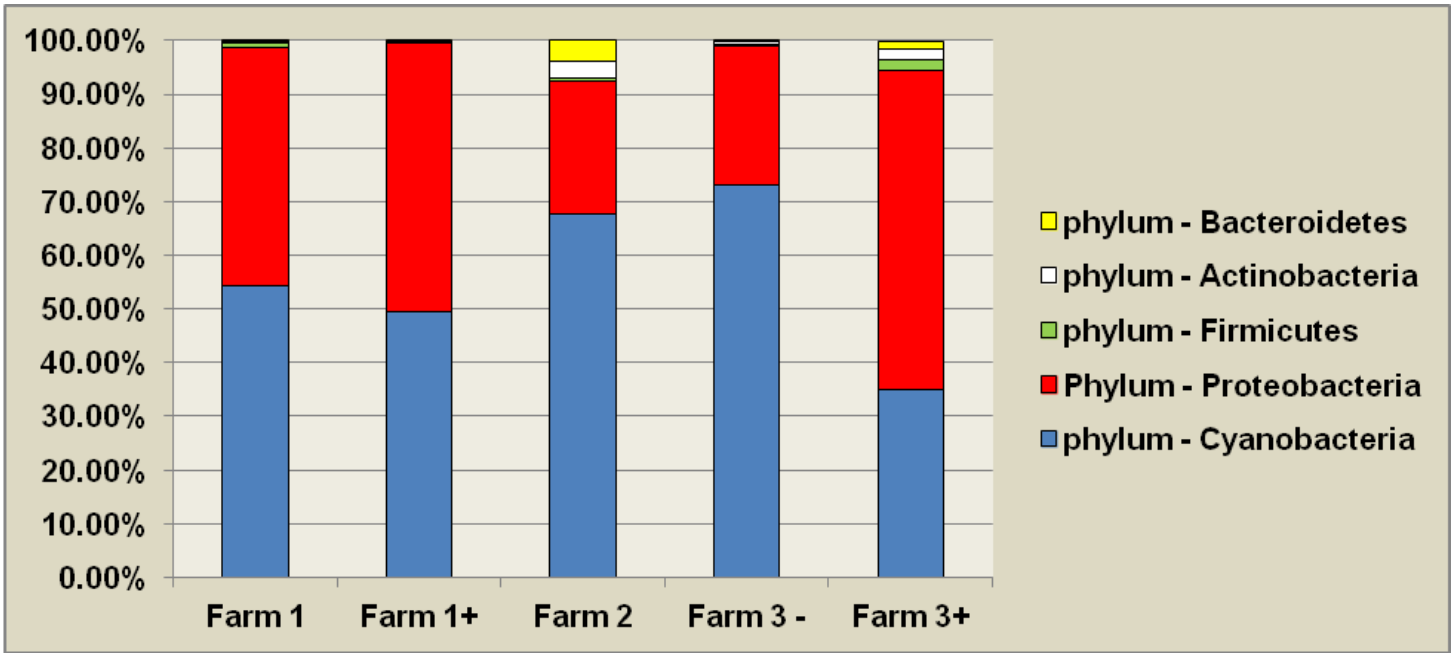
There are additional feed and fecal results added to this review to look into additional bacterial information supporting this research effort. This informarion was generated by farm 1+ diet , farm 3+ diet and farm 3+ / farm3++ fecals along with a wild rabbit fecal from the quarantined deer farm located in the endemic CWD area. Another fecal sample was collected from a wild whitetail deer from the northern forest of Wisconsin far from any farm crops and the quarentined deer farm as a comparable.

In understanding the bacterial world, one needs to review the nomenclature of how bacteria are identified to understand the data charts provided in this report.

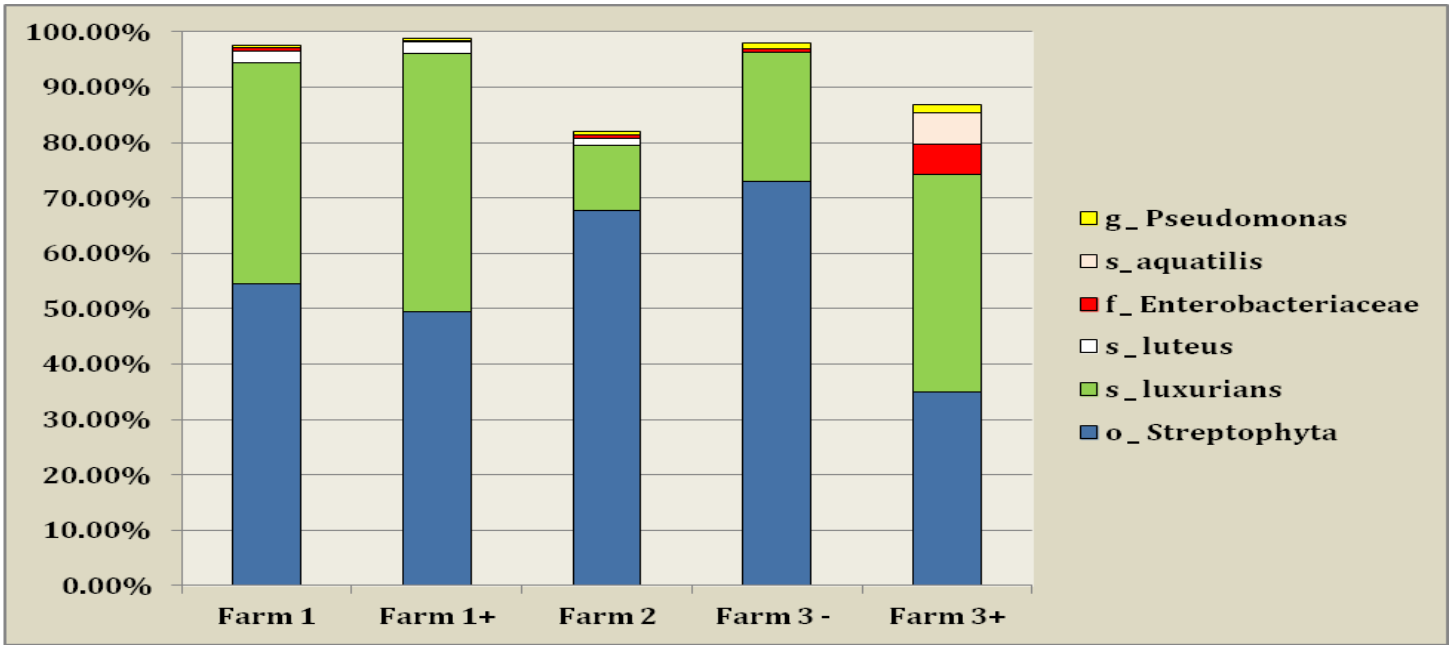
Kingdom = (b) bactria, (p) phylum, (c) class, (o) order, (f) family, (g) genus, (s) species

Not all results provided in this review drills down to the species level of the identified bacteria detected. This is due to the limitation of current 16S rRNA genetic technology available today in research for specie specifics. There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a milliliter of fresh water.

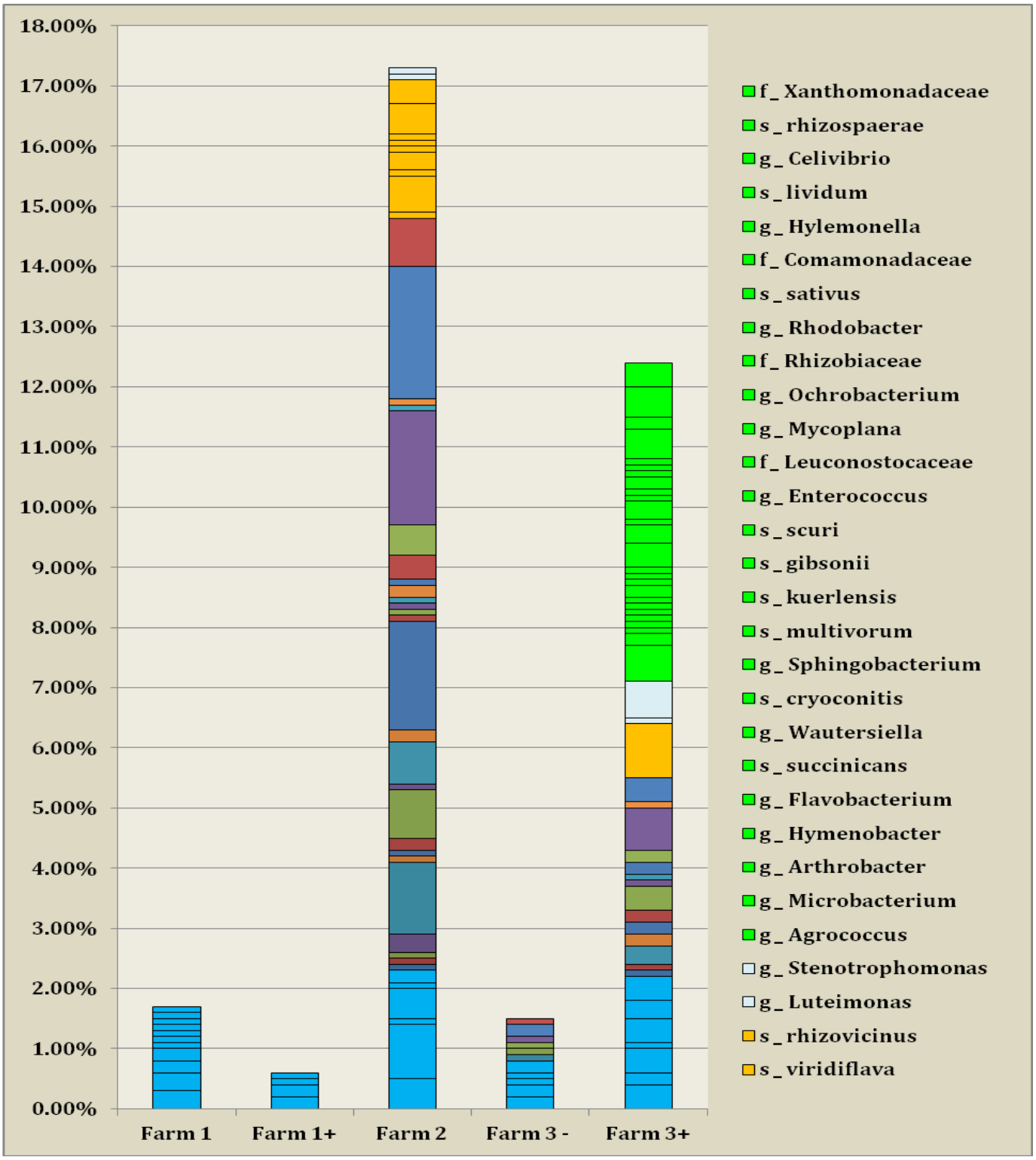
Feed Results



Generally feed samples should be low in bacterial counts. All feed samples reviewed for this report share up to 5 phylums of bacteria for which 2 phylums make up the majority of total bacteria (blue / red).



In the 5 phylums of feed bacteria, their contents makeup totals 73 different bacterial organisms. The top 6 organisms listed equalled up to 97.5% by volume above 1% in volume dominated by 2 (blue / green).
(o = order, f = family, g = genus and s = species).

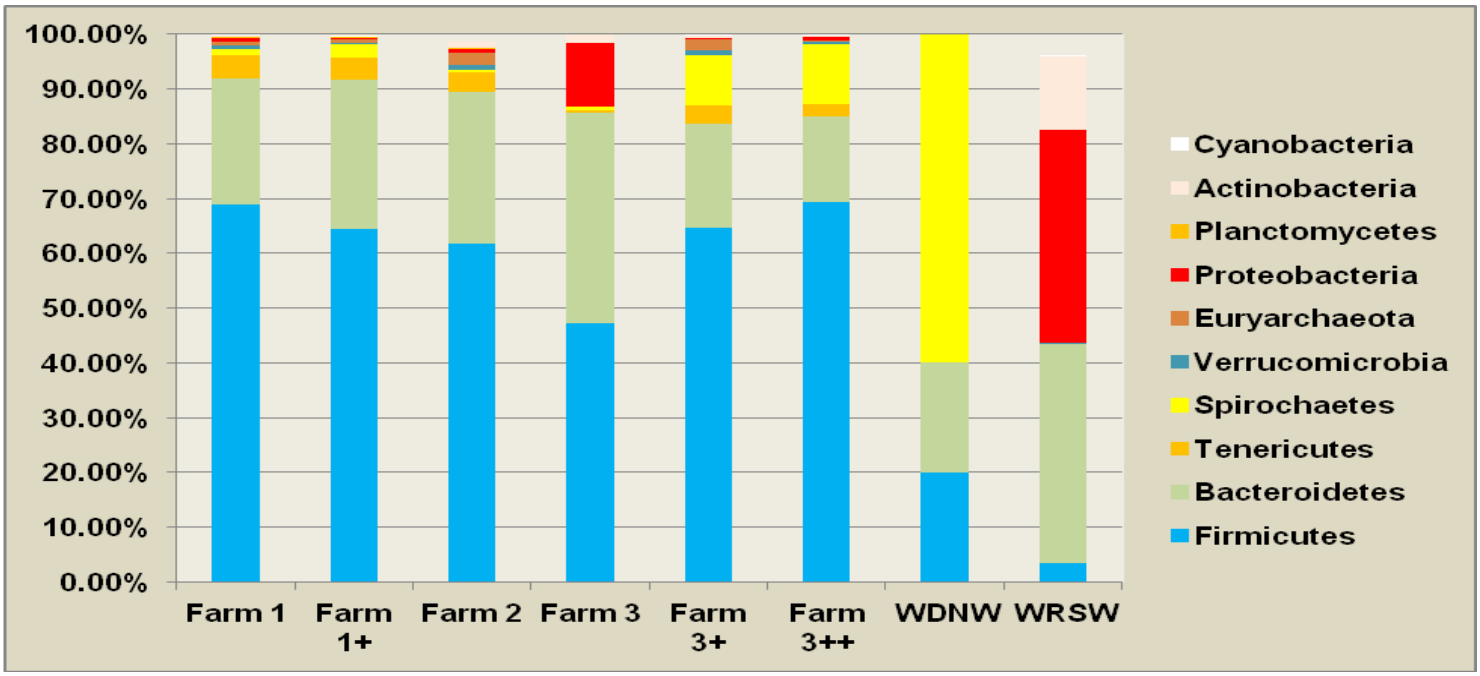


The remaining bacteria under 1% by volume, equals up to 67 organisms. Though these organisms are under 1% each one has the capacity to grow in the digestive tract given the right condition. Color coding shows the similarities and differences of feeds tested. Pellet feed shows lower amounts of organisms present due to possible pellet process temperatures.
O = order, f = family, g = genus and s = species

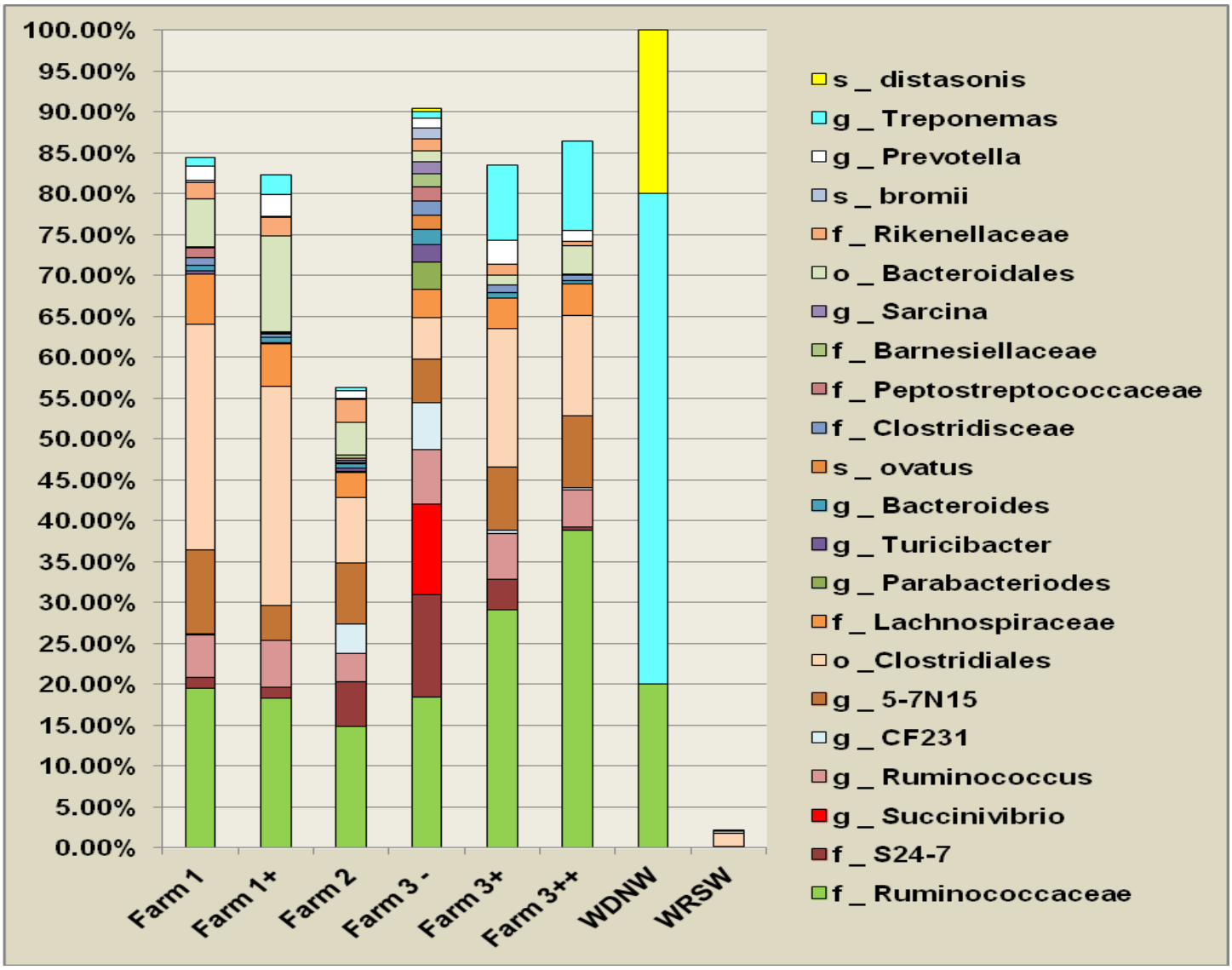
Fecal Results

Generally, bacterial organisms from a ruminant's fecal material will differ somewhat from feed bacteria since the rumen in deer is a digesting fermentor. That means there is a need for a mix of certain bacterial organisms to aid in proper digestion of feedstuffs and water consumed by the deer. Feed ingredients rely on soils, added fertilizers and water to grow. The rumen of the deer generally holds a diverse set of conserved organisms but some can be quite different depending on dietary intakes as well as the deer's geographic location. This is an important point for the farmer to pay attention to when one might buy and/or sell deer. Across the country, deer farmers feed different rations which can be impacted by geographic location. Geographic origin and transport can also affect how deer respond to changing food stuffs and how the rumen responds to those changes. If the rumen does not receive a consistent ration to maintain the current bacterial status it could cause a bacterial shift which could lead to an increased stress response that could include death of the deer. To help prevent this stress, one should review the feed from the selling or receiving farm and try to blend the 2 different feeds for the deer's consumption at least 2 weeks ahead of moving the deer to a new location. This will help reduce any dietary shock stress from the animal(s) movement to a new location since the movement itself is a stress on the deer.

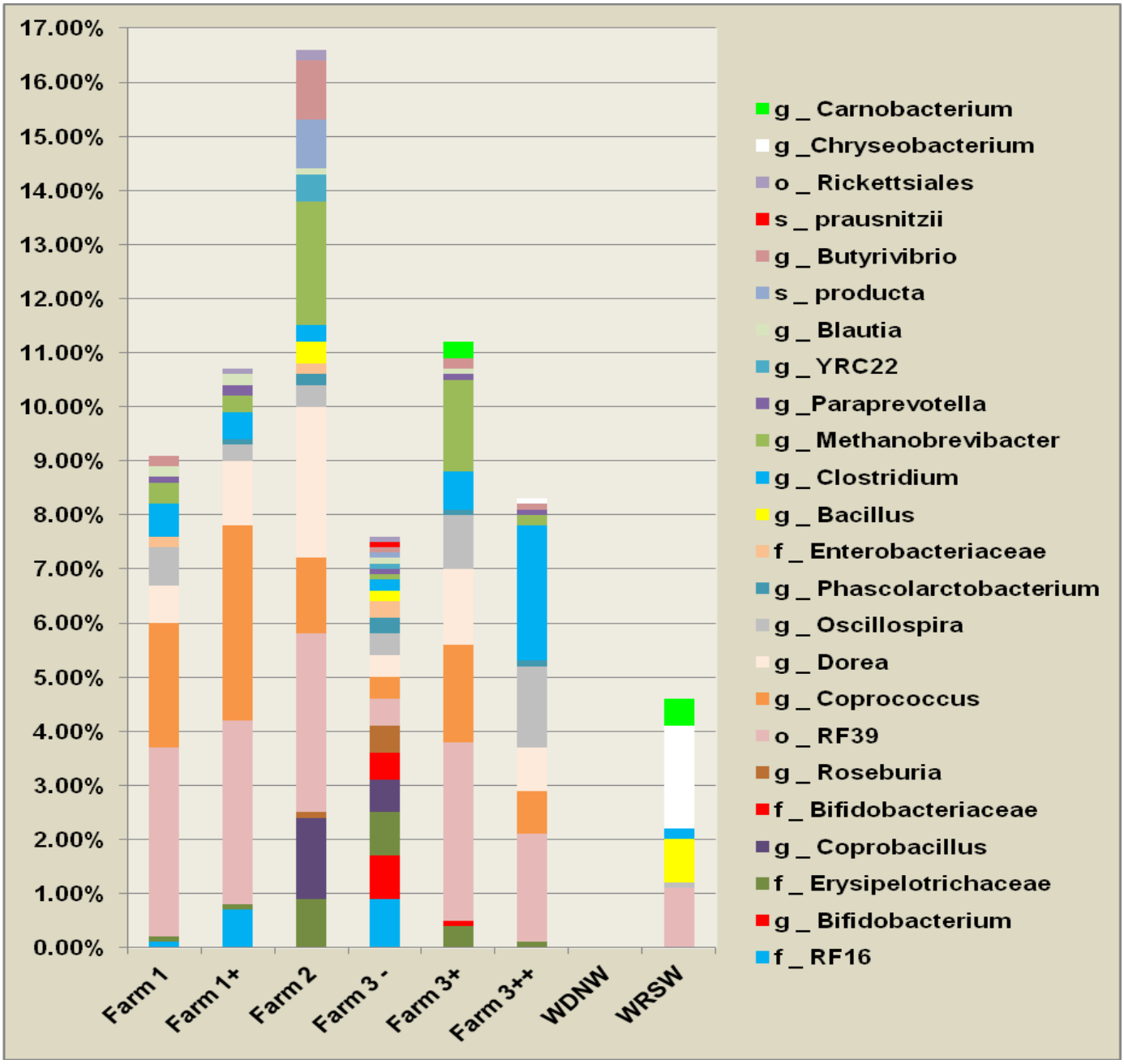
The following fecal charts are set up to review bacterial differences focused on farm 3 (quarantined herd) vs. other healthier non-quarantined farms.



The top 10 phylum organisms identified in fecal samples equalled up to 98.6% above 1% by volume.There were a total of 47 total bacterial organisms identified within the 10 phylums. There was a definite bacterial phylum shift in fecal samples provided from farm 3, to farm3+ and farm 3++. This shift was due in part to replacing the original deficient ration with a custom replacement ration supporting health and reproduction. Fecal's were collected for testing 30 and 120 days post feed change.

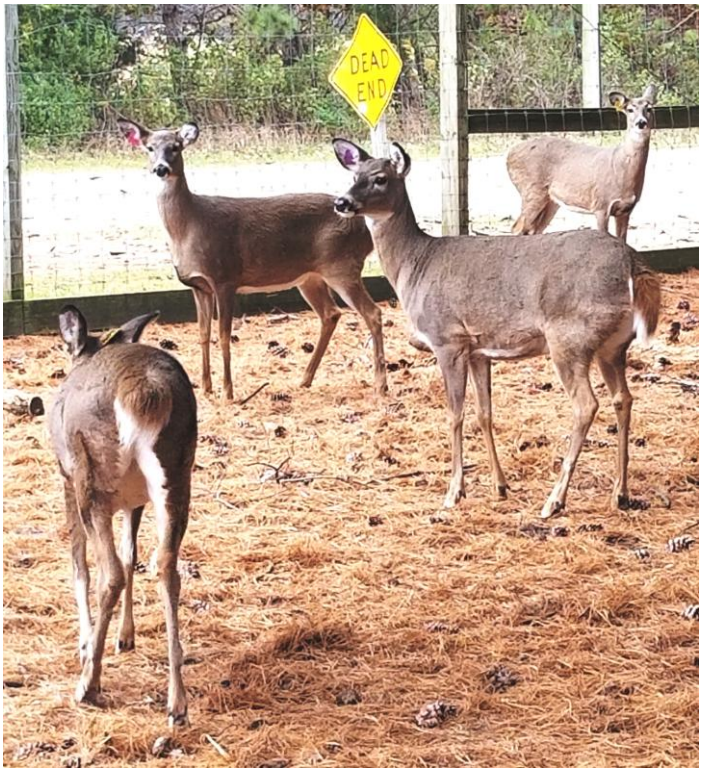


There were a total of 46 bacterial organisms identified across all deer feces tested. The top 22 organisms avgeraged 57% to 100% by volume of all baterial present above 1%. The only bacterium above 1% was genus_Succinivibrio (red) that was unique to farm 3 under the original ration. This organism did not show up upon subsequent testing farm3+ and farm3++. Bacterial species distasonis (20%) from the wild deer from northern Wisconsin was the only other unique bacteria associated with the farm 3 deer (0.40%). Color coding shows the balance of organisms that are similar or different in volumes across all deer fecals.
(o = order, f = family, g = genus and s = species).



There were a total of 46 bacterial organisms identified across all deer feces tested. The bottom 24 organisms equal up to 17.5% by volume of all baterial present below 1%. The Actinobacteria; genus –Bifidobacterium (red 0.8%) , Actinobacteria; family – Bifidobacteriaceae (red 0.5%) and the Firmicutes; specie – prausnitzii (red 0.1%) were unique to farm 3 deer. The Firmicutes; genus – Carnobacterium (lime green) was unique to Farm 3 deer fecal (0.3%) as well as the rabbit fecal (0.5%) from the endemic zone. The Bacteroidetes; genus – Chryseobacterium (white) was the only organism unique to the ferm 3 deer and the rabbit feces tested from the endemic zone location. Other color coding shows the balance of organisms that are similar or different in volumes across all deer fecals.
(o = order, f = family, g = genus and s = species).

Buck born 6-4-15 on farm 3 did not grow any antlers by September his yearling year 2016. Spring of 2017 no fawns were born showing lack of fertility in deer on the original farm 3 ration. After ration improvement in November 2016 the bucks antler growth in 2017 was an expected result of dietary improvement.



The above buck showed breeding interest in trailing the does in November, December 2017 and was seen mounting at least 1 doe January 11, 2018. With 1 - yearling, 3 - 5 year and 1- 7 year old doe in the mix for breeding this fall 2017 hopefully there will be fawns in the spring 2018? There is always one in a group that is camera shy.

Discussion

In the hypothesis of this review, we looked to provide information to the Cervid industry into what constitutes the health of a deer on a farm to stave off a disease process such as CWD. Usual onset of a disease process in a mammalian system has to do with many environmental stressor(s) of some sort whether related to age, nutrition, health status, genetics etc. In review of these normal farm operations in comparison to a quarantined farm operation reflects towards nutritional stressors we reviewed regarding the water source, feed source, parasitic control and fecal micro biome.

The farms water sources showed that deer farm 3 had a clean water source from the well, but had contamination of coliform and ecoli where as farm 1 had coliform counts but no ecoli from its well and farm 2 had neither contaminates of its well water supply. All farm water sources had a general neutral to alkaline ph with trace minerals showing the least amount in farm 3. Since on the farm operations revolve around being in rural environments filled with other wildlife species it is imperative that cleaning watering receptacles to ensure providing clean fresh water for the deer's daily consumption will minimize such contamination potentials from coliform and or E.coli sources from other species drinking from these watering sources.

Feeds sources used for the nutritional supports of the 3 farms were reviewed and was noted that farm 3 had a lower protein and major mineral deficiency as compared to feed used on farm 1 and the replacement feed used on farm 3+. The makeup of farm 3's original ration noted that the vitamin mineral package used was of a drier component that did not tactify to the base grains used in this feed formulation. This was noted after initial review by measuring feed fines from the main bulk feed storage bin. Though the feed fines equaled only 11.4% by weight, it accounted for 87% of the trace element ingredients for molasses, salt, vitamins and calcium in the total ration. Farm 2 also had a lower protein value but a higher trace mineral package but was still short on these elements as compared to the feed from farm 1 and farm 3+ rations that were of a pellet composition. There is nothing wrong with one mixing their own ration for their deer if one knows what they are attempting to accomplish in a finished feed for all nutrients needed for their deer. In the case of farm 3, the lack of proper ingredients led to a lack of minerals in the finished ration being delivered to the deer for proper consumption. As the feed fines (vitamins and minerals) sifted out onto the ground it was not being consumed by the deer. This lack of vital ration components was supported in the liver panel from the deceased fawn showing concentrated minerals and deficient mineral compositions within the liver. This could also explain why there were no fawns born this past year due to low nutrition up to the breeding season. There is a need though to understand the proper nutrition dynamics of what goes into nutritionally supporting the deer and their immune system year in and year out if we are to increase our knowledge of the disease process' such as the onset of CWD. Having proper nutrition onboard goes beyond disease suppression for the proper growth and production of the animal.

Pellet feed can hold most nutritional components designed in feeds with the proper pelleting and handling process without creating excessive feed fines. It is also important to maintain moisture in feed pellets as not to have excessive moisture over 10%. This is typically an industry standard that will help reduce any potential for mold or mycotoxin growth in any finished feed product supporting

growth for the deer. Mycotoxin testing for the feeds used in this study was not part of the phase 1 portion of this review.

Feed samples were reviewed using 16S rRNA sequencing to determine any pathogenic contamination of the feeds. There were no pathogenic organisms found in the feeds tested. Most organisms found in the feed were digested to a point in the rumen and did not show up on the fecal panel or were well below a 1% in value. This is a good sign knowing that feed tested to date in this review show little negative organisms that could be contributing to a disease process, but this was only a look at 5 total feed types on 3 different farms. Pellet feed type had the lowest overall bacteria levels of all feed types used.

Fecal samples and test results were reviewed and found that farms 1 & 2 practice their worming protocol with an injectable product as a customary farm practice. On farm 3, the first fecal samples noted only a stomach worms (17cnt) contamination. All deer on farm 3 were subsequently wormed through a medicated feed that is available commercially for use in the deer. Upon finishing the worming treatment a retest of fecal from the deer showed stomach worms were still present but at a lower count(2). A follow up during the winter months for review of the fecals on farm 3 will provide information as to the need to worm the deer again before last frost in the spring.

Fecal samples were also reviewed using 16S rRNA sequencing to determine any pathogenic contamination being shed by the deer on farms 1, 2 or 3. Other fecal materials were also added to the review process in this study from other sources. Fecals were generated from the upgraded feed used on farm 3 (3+ & 3++), along with adding a wild cottontail rabbit fecal from farm 3's location and a fecal sample from a wild whitetail deer from Northern Wisconsin.
No pathogenic organisms were found in any samples. There was a bacterial shift noticed in farm 3 from the original ration to the upgraded ration as noted in the fecal results collected on day 30 and day 120. On farm 3 the unique bacteria found in the base feed fecal result Succinivibrio, inhabits the rumen of cattle and sheep, playing an important role in the digestion process. They colonize their hosts soon after host organisms are born. They are sensitive to changes in diet, age, and environment. Some strains of Succinivibrio or Succinimonas have been shown to cause disease, but they are rarely pathogenic in humans. The shift in this organism was deemed to be for the good as it now resembles a more common bacterial environment found in farms 1&2.

Bacterial species distasonis (20%) from the wild deer from northern Wisconsin was the only other unique bacteria associated with the farm 3 deer (0.40%). This organism is a normally found in aiding digestion in the digestive tract.

Summary

This phase one research review of a whitetail deer farm held under quarantine for CWD was to help answer the hypothesis "Cervids, supported with optimized feed, forage and water through nutrition along with genetics can stave off the debilitating disease onset of CWD." This review found that the deer farm under quarantine was providing what would be considered a nutrient deficient ration to the deer on this farm by improper mixing of all ingredients to hold together.

Though the yearling deer that originally died from a goring incident from another deer, subsequently tested positive for CWD, by IHC method, both in the lymph nodes and brain area. Upon further rectal biopsy testing, the rest of the deer on this farm have presented no clinical signs of CWD to date. The current ages of the deer on this farm are from 1.5 to 7 years and are expected to convert to CWD positive status given the brain involvement of the CWD prion in the brain of the positive deer from this farm.

Review of the nutritional and bacterial status of the deer on this quarantined farm and the 2 other CWD negative farms provides a basis for future follow up of these farms. In the event that the deer residing on the quarantined deer farm successfully reproduce providing younger animals to include in the study or if current deer start to show clinical symptoms' of the disease process, a follow up can be initiated with the use of the baseline data for comparison.

The endemic area where the quarantined farm is located generally produces 5-6 deer which test positive for CWD in the wild population yearly. The quarantined farm, being a single fenced facility will provide future opportunities for nose to nose contact with the wild deer population of this CWD endemic area. Due to the quarantined animals improved body condition, another round of rectal biopsies are planned to update the status of determining an onset of CWD within this small herd.

Other areas of interest resulting from this review include deer farmers paying attention to farm supports for their deer regarding water quality, feedstuffs, animal transfers and sanitation practices by embracing Bio-Security program development.

Continued and ongoing review and monitoring of Phase 1 components is warranted as the onset of clinical signs of CWD could take multiple years. A Phase 2 scope will be constructed adding to the baseline information of work is warranted to ascertain any health status changes (good / bad) to the deer held under CWD quarantine on this farm. There are other farms in Wisconsin that have been determined to have CWD who continue to operate as hunt preserves. These farms could provide critical test materials from the positive side of the CWD disease process. This would provide pertinent information in comparison to current baseline information derived to date. This would speed up future research findings in understanding this disease process. To achieve this next step more research funding is needed for the continuance of this study.