

Negative energy balance and Ketosis: Consequences and monitoring in transition cows

Daryl Nydam, DVM, PhD; Paula Ospina, DVM, MPH,
Tracy Stokol BVSc, PhD; Tom Overton, PhD

Cornell University
College of Veterinary Medicine
College of Agriculture and Life Sciences

Negative Energy Balance and Ketosis: in TMR fed dairy herds

1. Background/Introduction

2. Recent research at Cornell

- Cow-Level implications
 - Disease
 - Reproduction
 - Milk production
- Herd-level alarms

3. Field Monitoring

NEB and SCK in transition dairy cows

- Introduction:
 - NEB (negative energy balance) and SCK (subclinical ketosis)
(e.g. Herdt et al; Duffield et al; Oetzel et al)
 - NEFA (non-esterified fatty acid)
 - BHB (beta- hydroxybutyrate)
- SCK the most costly disease in Ontario herds
(Lissemore et al '06)

NEB and SCK in transition dairy cows

Rapid change in cow's metabolic demands...



2x ↑Energy requirements

Energy (NE_L) Requirements 2 Days Before Versus 2 Days After Calving

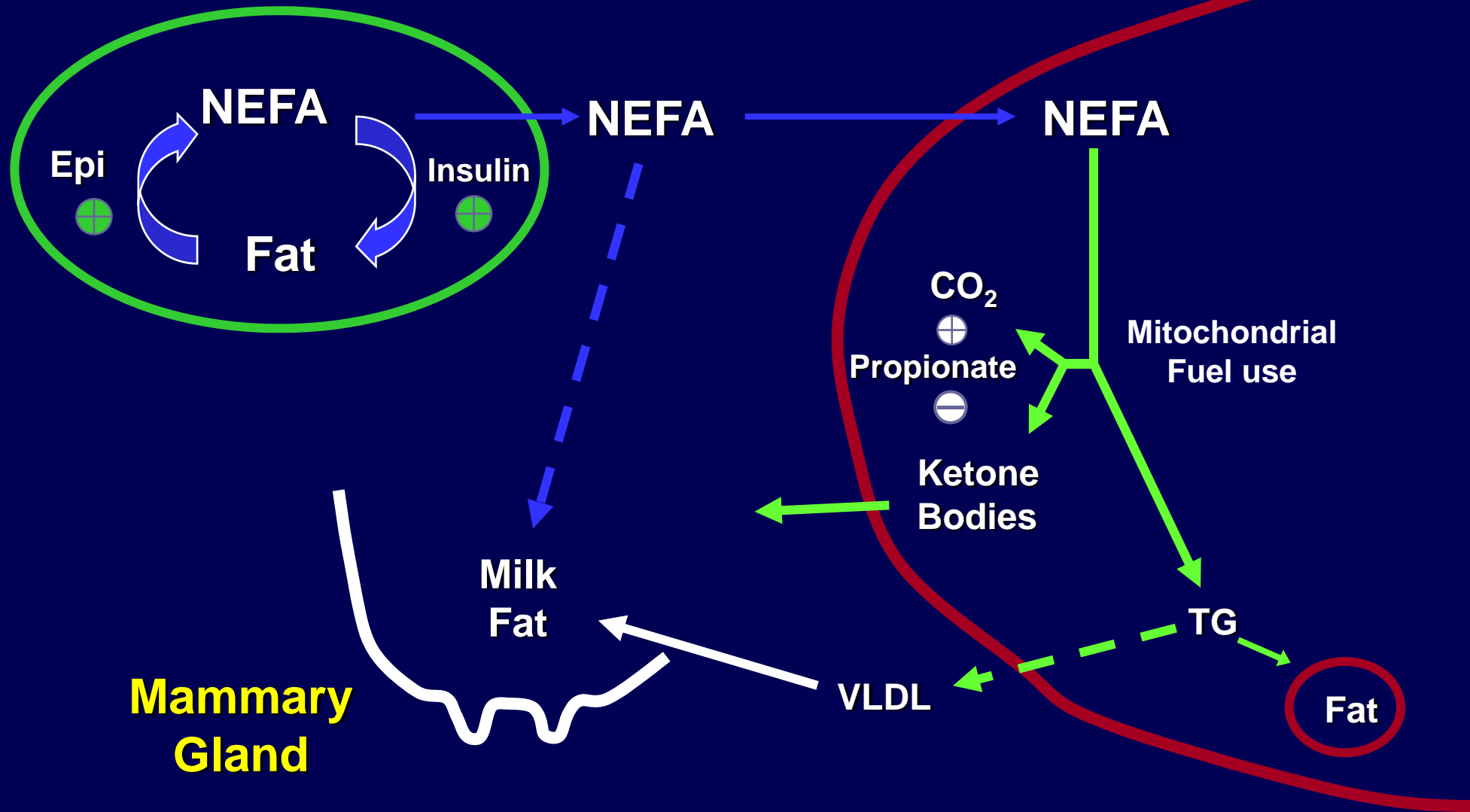
Function	725-kg Cow		575-kg Heifer	
	Pre	Post	Pre	Post
Maintenance	11.2	10.1	9.3	8.5
Pregnancy	3.3	---	2.8	---
Growth	---	---	1.9	1.7
Milk production	---	18.7	---	14.9
Total (Mcal)	14.5	28.8	14.0	25.1

Calculated from NRC (2001). Assumes milk production of 25 kg/d for cow and 20 kg/d for heifer, each containing 4% fat.

Courtesy of J. K. Drackley

Body fat

Liver



Non-esterified Fatty Acids (NEFA)

- In blood, come from adipose tissue
 - Mostly from mobilization of triglyceride
 - A little from dietary fats
- Measure of Negative Energy Balance
- Related to post-partum disease?

Subclinical Ketosis (SCK)

- Ketosis is the elevation of ketone bodies:
 - acetoacetic acid
 - Acetone (not metabolized, expelled in lungs)
 - β -hydroxybutyric acid (BHB)
 - Most stable in blood
- Results from negative energy balance (NEB) in early lactation or "insulin resistance" starting in dry period...
- May be secondary to diseases causing decreased feed intake

Punch line #1

- Transition dairy cows have a huge challenge
- All cows "visit" NEB in this period
- Can measure extent of it with NEFA and BHB

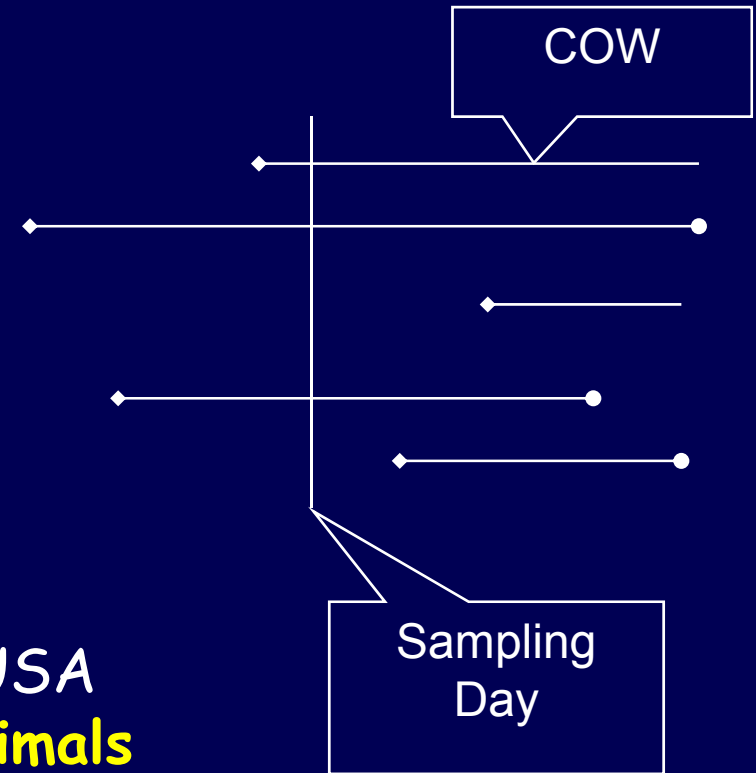
Objectives: Cow-level

1. Evaluate metabolites and their association with clinical disease, reproduction, and milk production in larger herds in the northeastern USA
2. Establish cow-level critical thresholds for NEFA and BHB



Materials and methods

- Prospective cohort study
 - **Cross-sectional sampling**
 - 1 visit
 - **Convenience sample of herds**
 - > 250 milking cows
 - Free-stall
 - TMR-fed
 - DHIA/ DC 305 herds in the NE USA
 - **Convenience sample of transition animals**
 - Healthy
 - 14-2 days pre-partum
 - 3-14 days post-partum
 - 1/3 heifers 2/3 cows
 - 15 cows/herd



NEB and SCK in transition dairy cows

- Data Collection
 - Predictors:
 - NEFA, BHBA (other metabolites)
 - Herd, BCS, DCC, DIM, LACT, season, diet, stocking density, pen moves, time of sampling, etc
 - Outcomes:
 - Clinical disease
 - Metritis
 - Retained Fetal Membranes (RP)
 - Ketosis (CK)
 - Displaced Abomasum (DA)
 - Reproduction
 - ME 305 milk production

NEB and SCK in transition dairy cows

Materials and Methods

- DC 305 → DairyOne → database
- Lab Analysis
 - Colorimetric Wet Chemistry (Hitachi 917, Roche)
- Data Analysis (SAS V 9.1)
 - Univariate Analysis
 - PROC Univariate, PROC Freq. PROC Lifetest
 - Multivariable Analysis and ROC curves
 - PROC Logistic, PROC Genmod, PROC Phreg, PROC mixed, MedCalc

Results

- 100 herds
- Mean= 838 cows
(range: 265 - 2770)
- 2758 animals
- Heifers: 991
- Cows: 1767



Disease Outcomes

Lactational Incidence (%)	<u>DA</u>	<u>Ketosis</u>	<u>RP/Metritis</u>	<u>Any of 3</u>
	3.3	7.0	12.1	19.6
Median DIM at Diagnosis				
	10	7	2/5	

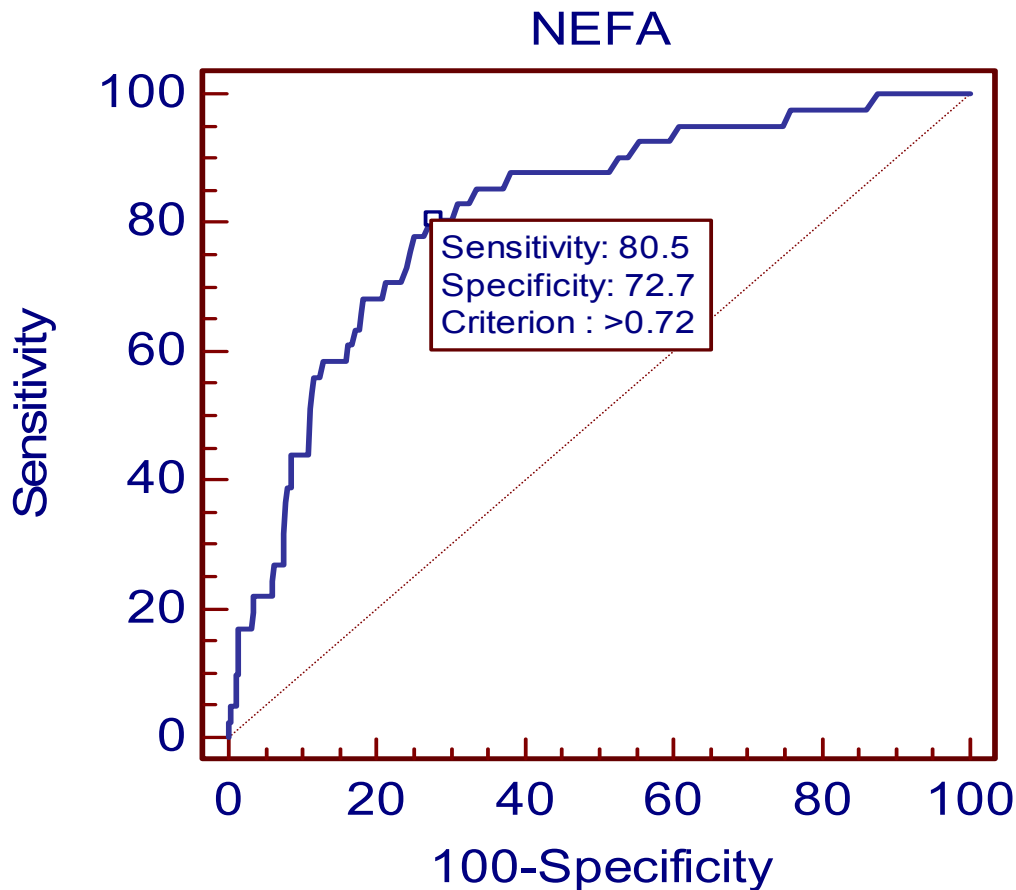
How to figure out what NEFA/BHB values are important?

- Receiver Operator Characteristic (ROC) curve
 - Plots True Positive Rate v. False Positive Rate
 - for all observed values in the data set against outcome of choice
 - Point with greatest combo of Sensitivity and Specificity is most useful
 - Area Under the Curve (AUC) tells how predictive the variable is of the outcome; the larger the better
 - .5 = coin flip
 - .5 to .7 = accurate test
 - .71 to .9 = very accurate test
 - >.91 = highly accurate and rare

ROC Curve

POST-partum NEFA: 0.72 (mEq/L)

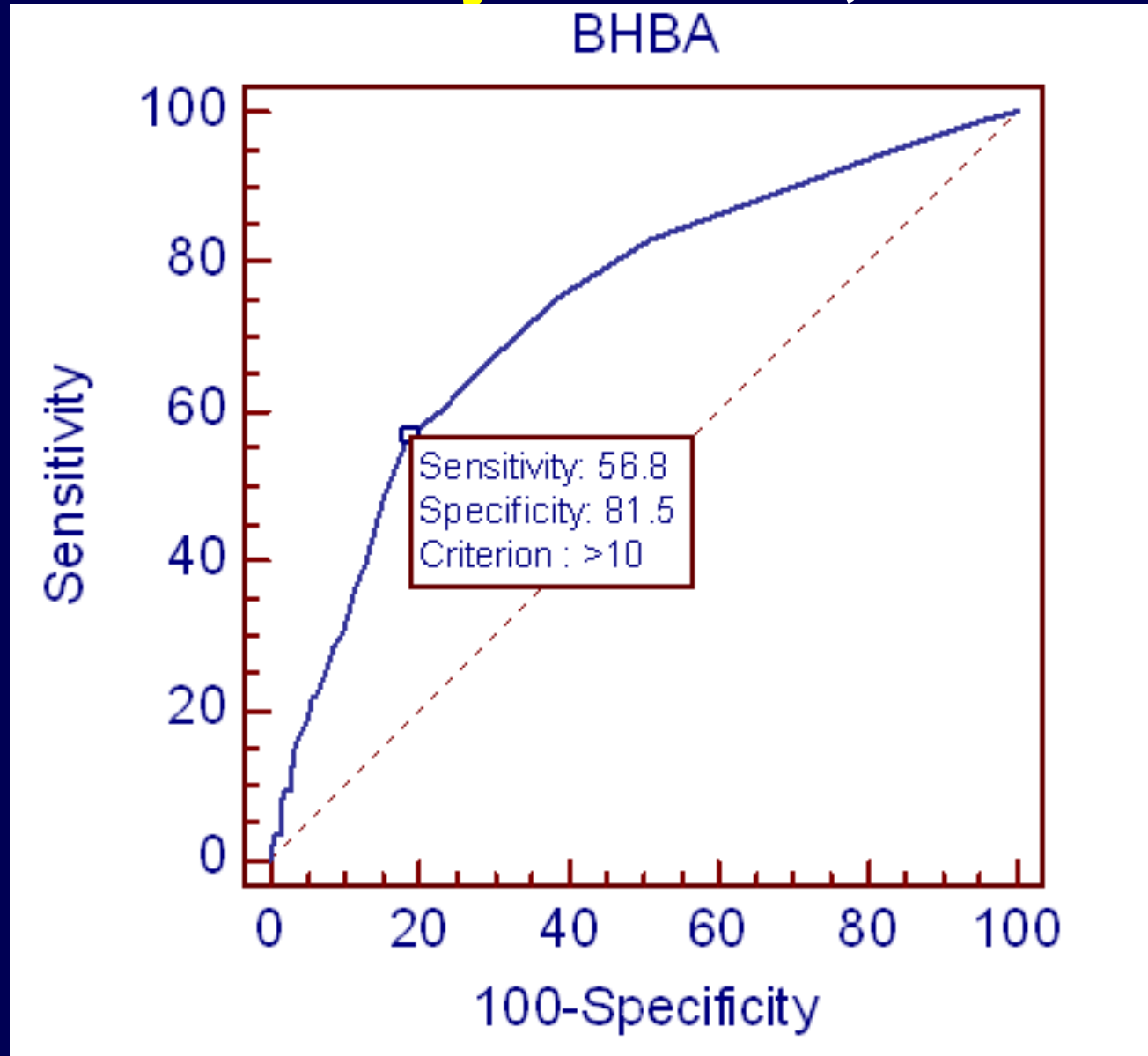
DA



AUC:0.81

Highly
accurate

Milk animals - **any Disease**; AUC: 0.74



Impact on disease outcome?

NEFA > 0.3 mEq/L in animals pre-partum

<u><i>Disease</i></u>	<u><i>Relative Risk</i></u>	<u><i>P-value</i></u>
DA	2.0	0.003
CK	2.0	0.0001
RP/Met	2.2	<0.0001
Any of 3	2.0	<0.0001

Impact on disease outcome?

BHB > 10 mg/dl in animals post-partum

<u><i>Disease</i></u>	<u><i>Relative Risk</i></u>	<u><i>P-value</i></u>
DA	6.9	<0.0001
CK	4.9	<0.0001
Met	2.0	0.02
Any of 3	4.4	<0.0001

Impact on disease outcome?

NEFA > 0.6 mEq/L in animals post-partum

<u><i>Disease</i></u>	<u><i>Relative Risk</i></u>	<u><i>P-value</i></u>
DA	9.7	<0.0001
CK	5.0	<0.0001
RP/Met	17	<0.008
Any of 3	4.4	<0.0001

Conclusion: Clinical Disease

Predicting any Dz: Sample \geq 12 animals

Risk Factor	Threshold	Risk Ratio	AUC
Pre-partum NEFA (mEq/L)	0.3	2.0	0.6 Accurate
Post-partum BHB (mg/dL)	10	4.4	0.74 Highly accurate
Post-partum NEFA (mEq/L)	0.6	4.4	0.81 Highly accurate

Punch line #2

- Cut-point for Northeast TMR fed freestall herds
 - NEFA pre-partum: 0.3 mEq/L
 - BHB post-partum: 10mg/dl
 - NEFA post-partum: 0.6 mEq/L
- High impact on disease, *esp. NEFA post-partum*
 - 2.0 - 9 X's more likely to develop diseases

Reproduction?

- Conception within 70 days post-voluntary waiting period
 - 95% of herds using ovulation synchronization protocol
 - Equal time at risk for all herds
 - Optimal calving interval
- **Covariates:** BCS, herd, season, parity, milk production

NEB effect on Reproduction

Table 1. Cox proportional hazard model of the effect of NEFA (≥ 0.27 mEq/L), covariates, and herd as a random effect on days to conception after voluntary waiting period for animals sampled pre-partum (n=1318)

Variable	Parameter estimate	Standard error	P-value	Hazard Ratio
NEFA ¹	-0.20	0.08	0.01	0.82
BCS ²	0.02	0.10	0.82	1.02
Season ³	-0.03	0.09	0.78	0.98
Parity ⁴	-.31	0.09	0.0005	0.73
ME 305 ⁵	-.03	0.09	0.73	0.97

¹NEFA: dichotomized \leq or ≥ 0.27 mEq/L.

²BCS: dichotomized at \leq or ≥ 3.75 .

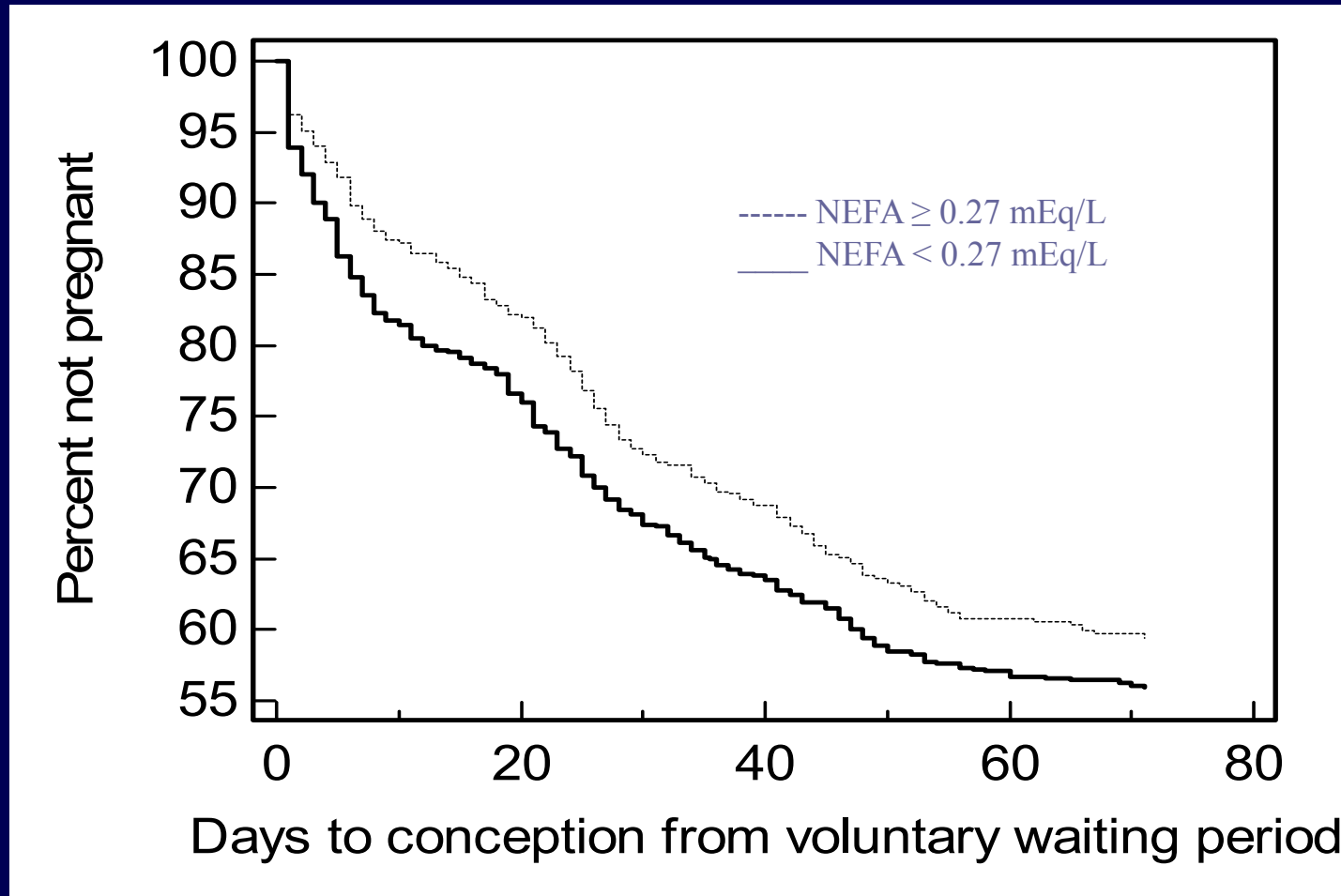
³Season: dichotomized into warm (May, June, July, August, September) versus cool months (October, November, December, January, February, March, April).

⁴Parity: dichotomized into ≤ 2 or ≥ 2 .

⁵ME 305: dichotomized into \leq or \geq median for animals sampled pre-partum.

Reproduction:

PRE-partum NEFA- time to pregnancy



Kaplan-Meier curves of time to pregnancy of 1318 cows and heifers with NEFA ≥ 0.27 mEq/L or < 0.27 mEq/L measured in serum 14-2 days pre-partum.

Results- Reproduction

Animals sampled **POST**-partum

- Cox proportional hazard models

Variable	P-value	Hazard Ratio
Post-partum NEFA ≥ 0.72 mEq/L	0.05	0.84
Parity > 1	0.01	0.81

Covariates: BCS, season, ME 305, BHB: p-values > 0.2

Results- Reproduction

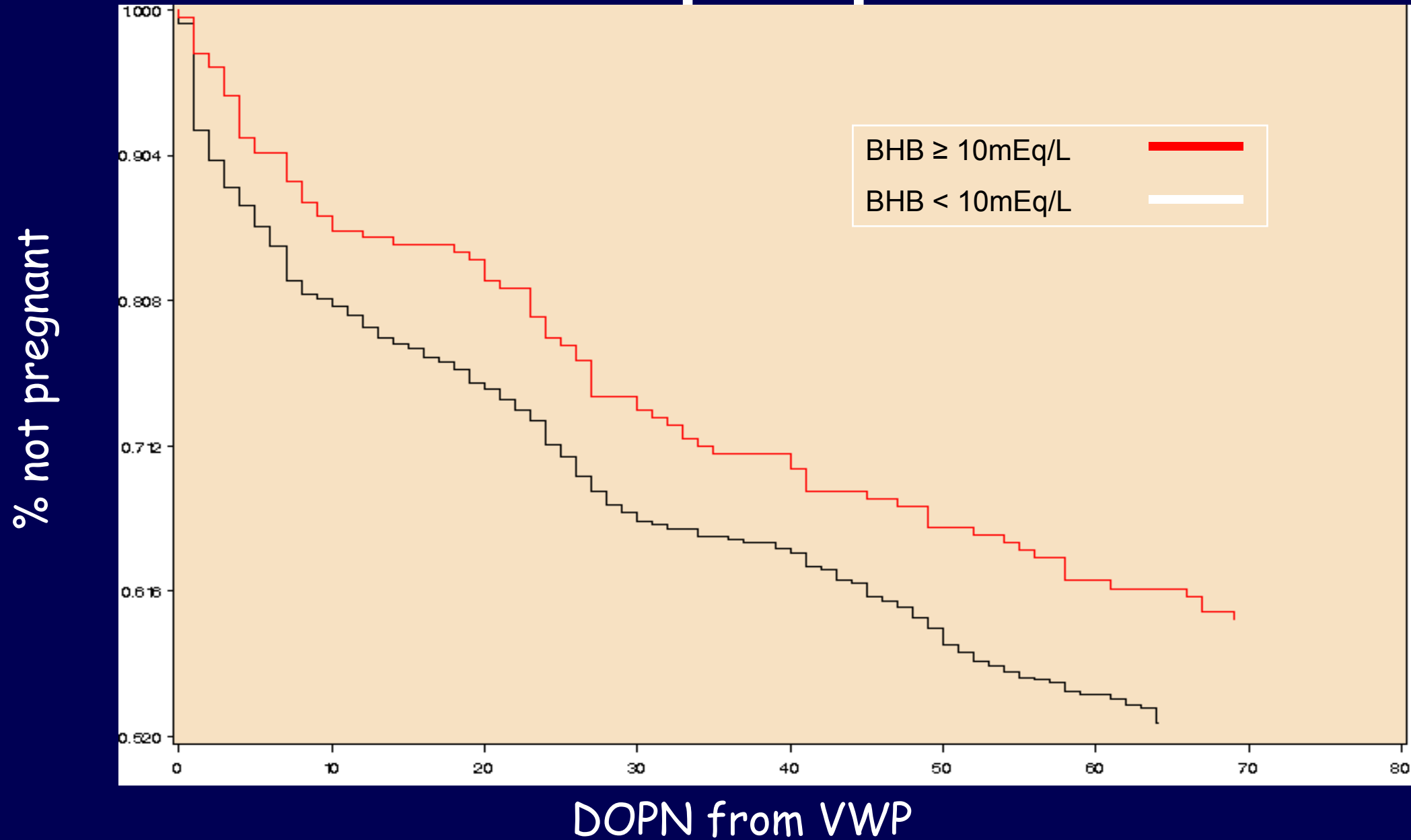
Animals sampled **POST**-partum

- Cox proportional hazard models

Variable	P-value	Hazard Ratio
BHB \geq 10mg/dL	0.1	0.86
Parity > 1	0.01	0.80

Covariates: BCS, season, ME 305: p-values > 0.2

BHB Cows post-partum



Milk production?

- ME 305 evaluated at 120 DIM
 - ME 305 milk can be compared between heifers and cows
 - 120 DIM ME 305 most accurate measure of milk production in our study
- **Covariates:** BCS, season, herd, parity, and interaction between parity and metabolite

NEB effect on Milk Production

Table 4. Mixed model for the effect of NEFA ($\geq 0.33\text{mEq/L}$), covariates, and herd as random effect on milk production measured by 120 DIM ME 305 (kg) for animals sampled pre-partum (n=1318)

Variable	Difference in ME milk yield (kg) for animals at or above category	Standard Error	P-value
NEFA ¹	-616	182	0.0008
Season ²	167	222	0.5
Parity ³	-526	226	0.002
BCS ⁴	-35	130	0.8
Parity*NEFA ⁵	225	270	0.4

¹ NEFA: dichotomized at $<$ or $\geq 0.33\text{mEq/L}$.

² Season: dichotomized into two seasons; warm (May, June, July, August, September) versus cool (October, November, December, January, February, March, April).

³ Parity: dichotomized at $<$ or ≥ 2 .

⁴ BCS: dichotomized at $<$ or ≥ 3.75 .

⁵ Interaction between parity and NEFA dichotomized at 0.33mEq/L .

Results- Production

Animals sampled **PRE**-Partum;

- Mixed effects models

Variable	P-value	Difference in ME milk yield (kg)
Pre-partum NEFA $\geq 0.33\text{mEq/L}$	<0.001	-616
Parity > 1	0.002	-526

Covariates: BCS, season, interaction: p-values > 0.4

Results- Production

HEIFERS sampled **POST**-Partum

- Mixed effects models

Variable	P-value	Difference in ME milk yield (kg)
Post-partum NEFA ≥ 0.57 mEq/L	0.02	+482

Covariates: BCS, season, BHB: p-values > 0.2

Results- Production

HEIFERS sampled **POST**-Partum

- Mixed effects models

Variable	P-value	Difference in ME milk yield (kg)
BHB \geq 10mg/dL	0.05	+381

Covariates: BCS, season: p-values > 0.3

Results- Production

COWS sampled **POST**-Partum

- Mixed effects models

Variable	P-value	Difference in ME milk yield (kg)
BHB \geq 10mg/dL	0.05	-328

Covariates: BCS, season: p-values > 0.2

Results- Production

COWS sampled **POST**-Partum

- Mixed effects models

Variable	P-value	Difference in ME milk yield (kg)
Post-partum NEFA ≥ 0.72 mEq/L	0.001	-662

Covariates: BCS, season, BHB: p-values > 0.3

Punch line #3

- Relatively small amount of NEB or SCK largely impacts reproductive performance and milk production
 - Current work suggests this may be due to immune compromise and subsequent endometritis, as well as ovarian function
- ~14-18% less likely to get pregnant
- ~1000 lbs less ME305 milk
 - Except heifers.....

NEB and SCK in transition dairy cows

How much opportunity is there to better manage a costly condition?



NEB and SCK in transition animals

- 1472 pre-partum animals
 - 45% (245/540) heifers NEFA \geq 0.3 mEq/L
 - 26% (246/932) cows NEFA \geq 0.3 mEq/L

NEB and SCK in transition animals

- 1315 post-partum animals
 - 25% (131/517) heifers NEFA \geq 0.7 mEq/L
 - 33% (267/798) cows NEFA \geq 0.7 mEq/L
 - 15% (77/517) heifers BHB \geq 10 mg/dL
 - 27% (214/798) cows BHB \geq 10 mg/dL

NEB and SCK in transition dairy cows

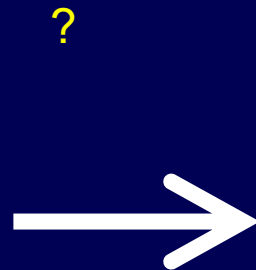
Results

- Unrecognized Clinical ketosis at ≥ 30 mg/dl:
 - 3 % (95% CI: 2.2 - 3.6%)

Herd alarm levels

- Herd alarm levels have not been well defined
 - Oetzel 2004

Proportion of
sampled animals
with elevated
metabolite levels



Herd level effects
on health,
reproduction
and production

Objectives: Herd-level

1. Determine the herd alarm level for the proportion of sampled animals with elevated metabolites resulting in herd-level effects
2. Describe the herd-level prevalence of elevated metabolite concentrations.

Materials and Methods

1. Diseases: percent of cases of DA, CK (within 30 DIM) in animals sampled
2. Reproduction: herd pregnancy rate calculated over 2, 21-day periods post-herd-voluntary waiting period
3. Production: Average ME 305 milk (evaluated at 120 DIM) from animals sampled
 - Heifers and cows were grouped in final analysis if outcomes between groups were similar

Animals sampled PRE-partum

If > 15% of animals sampled had NEFA \geq 0.27mEq/L		P-value
Herd pregnancy rate	- 1.2	0.005
DA and CK incidence in animals sampled	+ 1.4	0.01
Average ME 305 milk in animals sampled	-240kg	0.01

*15% of 15 = 2-3 animals

Animals sampled POST-partum NEFA

If > 15% of animals sampled had NEFA \geq 0.60 - 0.70 mEq/L	P-value	
Herd pregnancy rate (NEFA \geq 0.60)	- 1.3	0.007
DA and CK incidence in animals sampled (NEFA \geq 0.70)	+ 1.3	0.001

Animals sampled POST-partum BHB

If > 15% of animals sampled had BHB \geq 12 mg/dL	P-value	
Herd pregnancy rate	- 0.7	0.05
DA and CK incidence in animals sampled	+ 1.2	0.001

HEIFERS sampled **POST**-partum NEFA & BHB

If > 15% of heifers sampled had NEFA \geq 0.70 mEq/L		P-value
ME 305 milk	+300 kg	0.03
If > 20% of heifers sampled had BHB \geq 12mg/dL		P-value
ME 305 milk	-535 kg	<0.0001

COWS sampled **POST**-partum NEFA & BHB

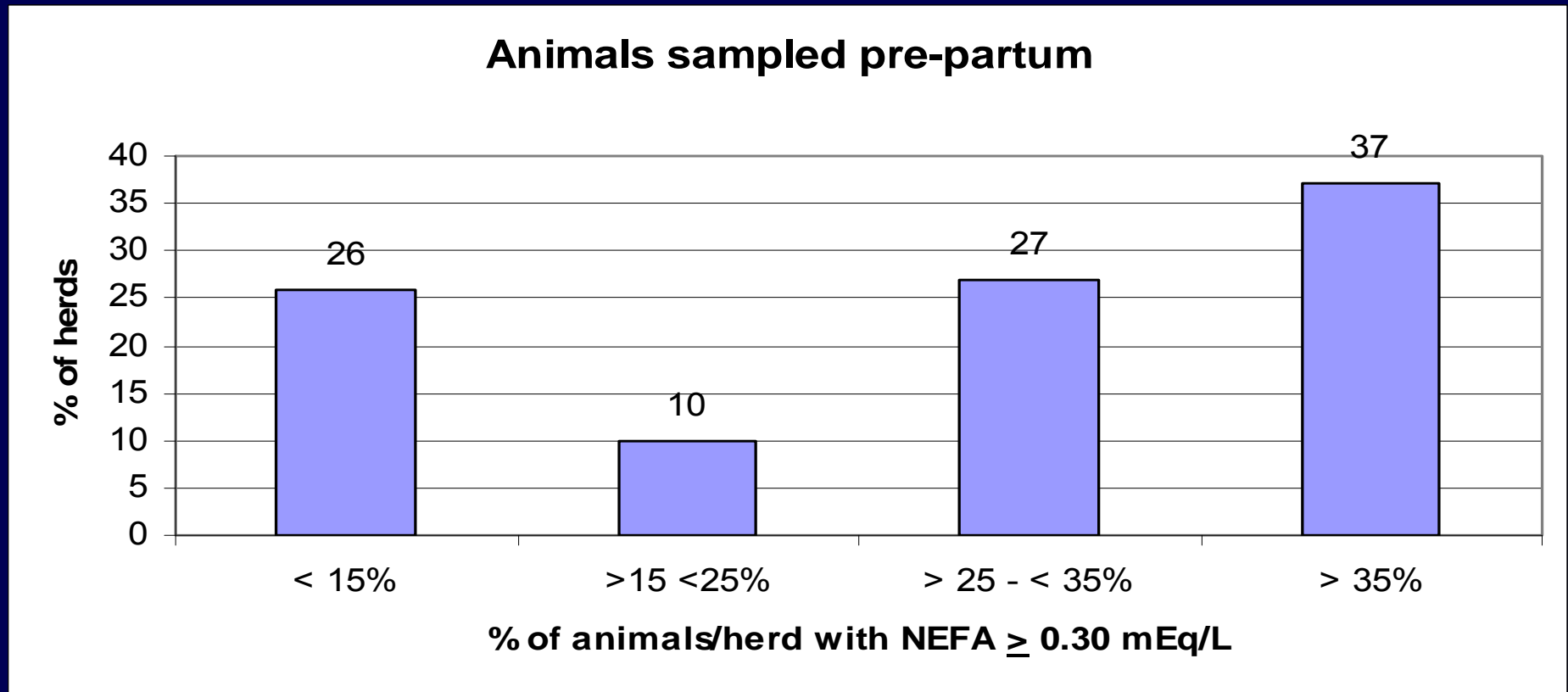
If > 15% of cows sampled had NEFA \geq 0.70 mEq/L		P-value
ME 305 milk	-584 kg	<0.0001
If > 15% of cows sampled had BHB \geq 10mg/dL		P-value
ME 305 milk	-349 kg	0.0004

Impact / Punch Line

Metabolite level	Herd Alarm	Effect
PRE -Partum NEFA \geq 0.3 mEq/L	15%	-1.2% PR +1.4% DZ -240 kg ME305 milk
POST -Partum NEFA \geq 0.6 ^a - 0.7 ^b mEq/L	15%	-1.3% PR ^a +1.3% DZ ^b Heifers:+300kg, Cows:-584kg
BHB \geq 10 ^a -12 ^{b*} mg/dL	15% *20%	-0.7% PR ^b +1.2% DZ ^b Heifers: -535kg*, Cows:-349kg ^a

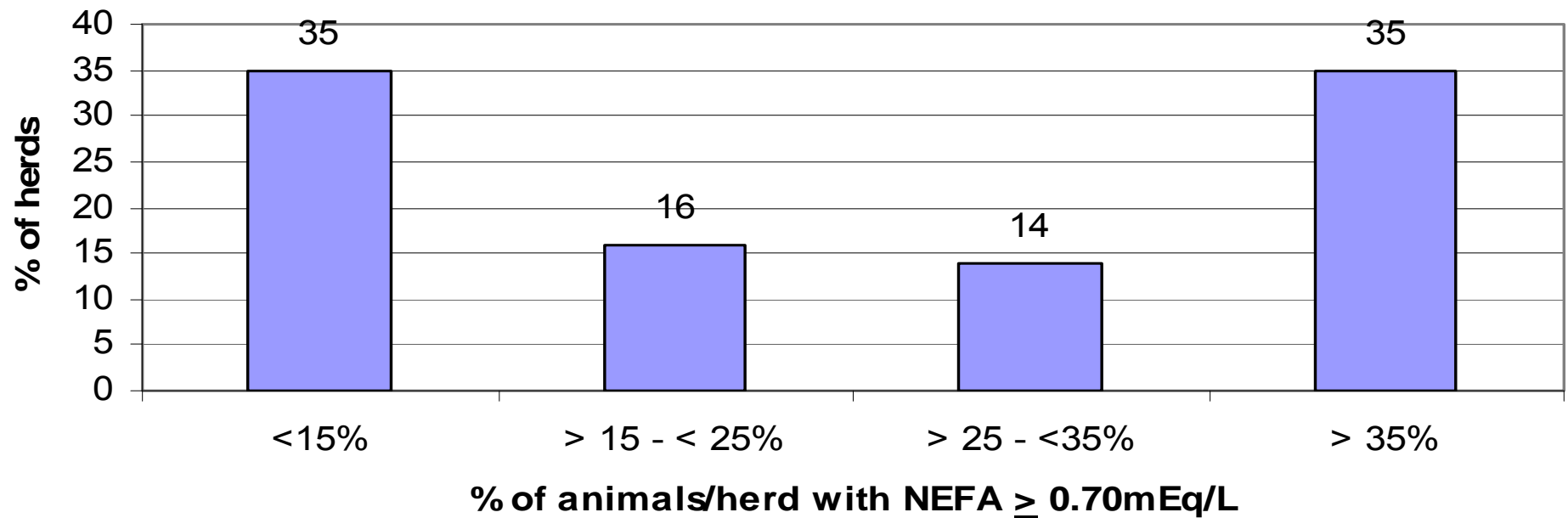
***15% of 15 = 2-3 animals**

74% of herds had >15% of animals sampled PRE-partum with NEFA \geq 0.30 mEq/L



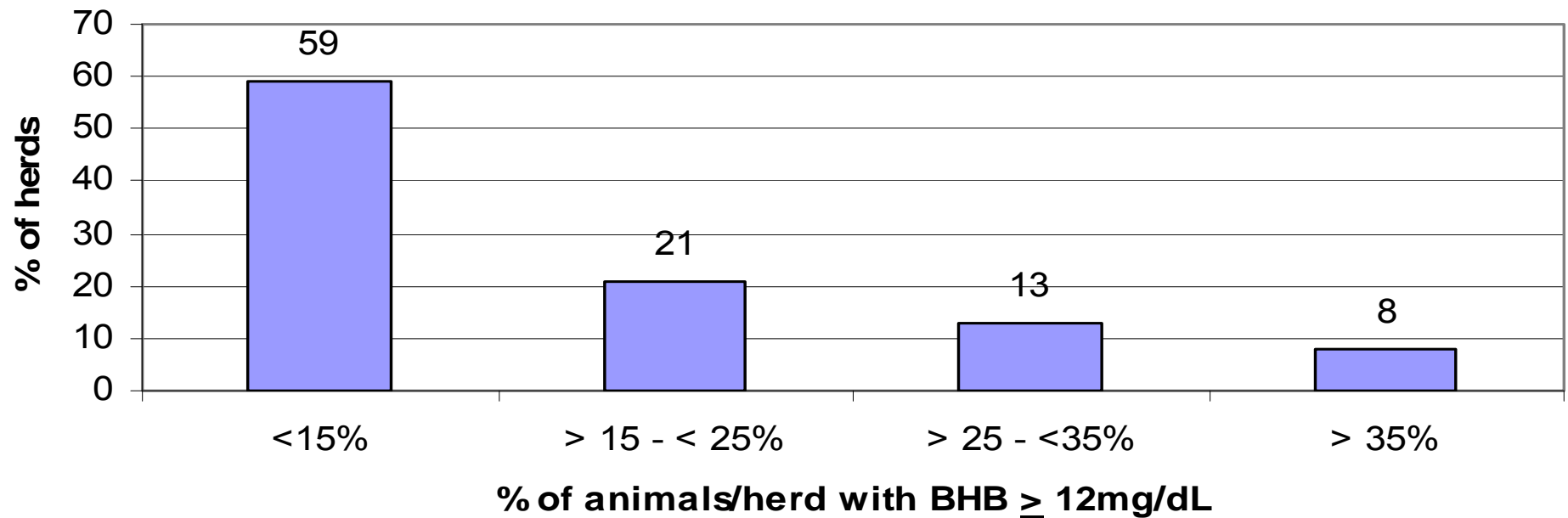
65% of herds had $> 15\%$ of animals sampled POST-partum with NEFA ≥ 0.70 mEq/L

Animals sampled post-partum



42% of herds had $> 15\%$ of animals sampled POST-partum with **BHB** ≥ 12 mg/dL

Animals sampled post-partum



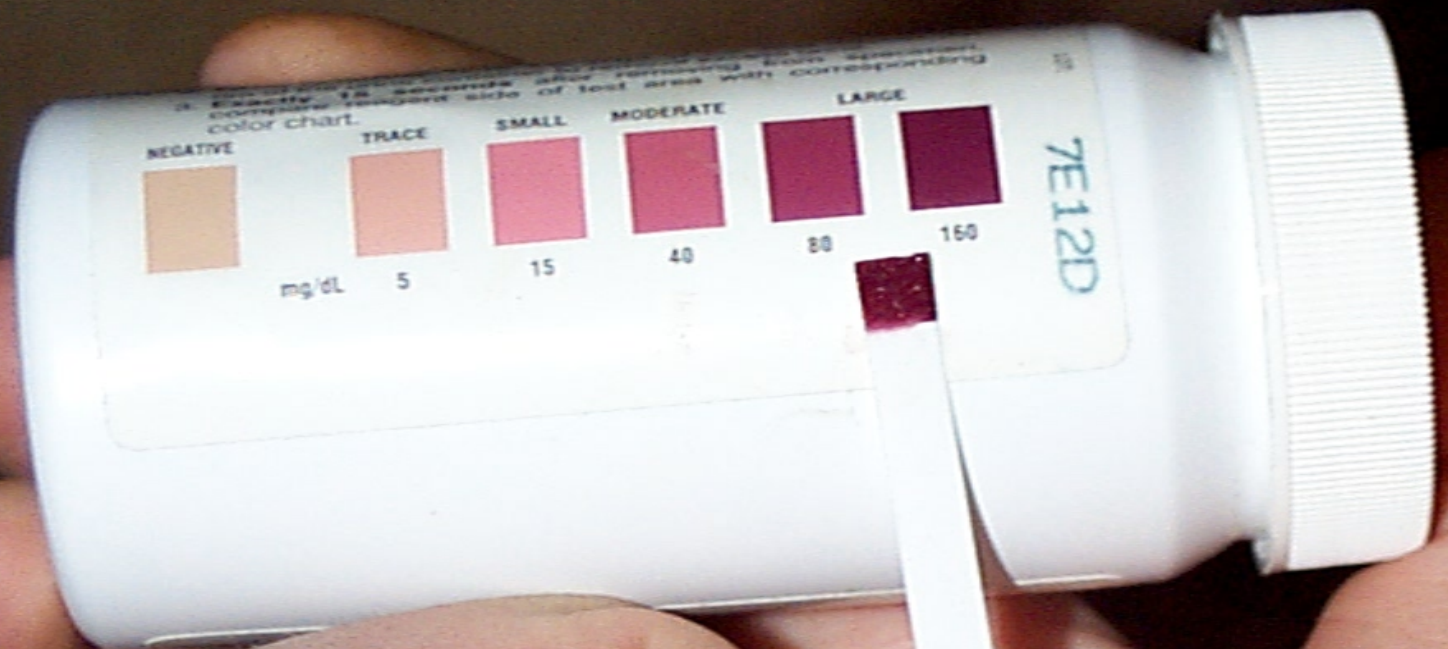
Punch Line #4

- Lots of opportunity for better management!!
- Herds and cows not selected because of problems
 - Unrecognized clinical disease
 - Cost of DA, metritis/RP, ketosis etc.
 - Reproductive Performance suffers

On-going monitoring in your herds:

- Goal:
 - Intervene early and proactively
- Ability to change things is harder though...

- Why:
 - Unfortunately lack of evidence for individual cow treatments...
 - But, Quantify herd problems, direct investigation, and motivate management changes for prevention



NEGATIVE



mg/dL

TRACE



5

SMALL



15

MODERATE



40

LARGE



80



160

7E12D



Bayer
Bayer

Cow-side tests for SCK

(compared to 14 mg/dl serum BHB)

Milk

- Keto-test
 - 100 mmol/L
 - Sen: 83%
 - Spec: 82%
 - 200 mmol/L
 - Sen: 54%
 - Spec: 94%
- Powder
 - Lacks good sensitivity

Urine

- Ketostix
 - Read in 5 seconds
 - Sen: 75%
 - Spec: 96%
- Acetest tablet
 - Lacks good specificity



Precision XTRA

- 1 drop of blood for meter
- Compared to 14 mg/dl serum BHB
 - Sensitivity: 91 - 100%
 - Specificity: 97 - 100%
 - Heuweiser JDS '09; Oetzel AABP '08; Burke MSU '07
 - ~ \$3.00/test and ~ \$40.00 for meter

Laboratory Analysis

- Use a lab with validated tests
- Red tops tubes preferred for both BHB and NEFA
- Keep sample chilled and separate serum ASAP
- Arrive at lab with 1-2 days
 - Can freeze serum for up to 1 month
 - Can get away with separating serum <24 hours and then sample in fridge for 3 days
- At Cornell, NEFA and BHB are \$7.50/test
- Pooling is not acceptable

Sample: A partial selection of animals from a larger group (herd) from which blood, tissues, etc are collected for testing. A judgment will be made about the larger group based on the findings from the sample.

Statistical Sampling:

- Tells us how lazy and cheap we can be:
 - Not enough time to see all the cows
 - Not enough money to "test" all the cows
- BUT, we're trying to make money by being correct at least some of the time...
- So we learn how to sample!

How confident do you need to be to make management decisions?

- 99% ?
 - 95% ?
 - 90% ?
 - 75% ?
-
- The more confident you want to be the more cows you have to sample!

What proportion of my cows have Y?

- Need to know:
 - Desired confidence about your fraction
 - The error you are willing to accept
 - Prevalence above which you care
 - Population Size

BHBA and NEFA suggestions

- 75% confident that sample proportion represents true population proportion
- If >15% of cows are in Negative Energy Balance, then it's a herd problem
- Want to estimate true proportion +/- 10%
- Target population has some effect, we'll say 35 cows at risk for NEB

2. Sampling to estimate a proportion

- 1.15 Z-value for desired confidence (2.58 for 99%, 1.96 for 95%, 1.64 for 90%, 1.15 for 75%)
- 0.15 Suspected prevalence (choose 50% if not sure)
- 0.1 Desired bound on estimate (absolute decimal value of desired precision around proportion)
- 35 Size of herd/group

====>
12 Sample size

If you want to be 95% confident, the sample size becomes 22 cows

Impact: Herd-level

Metabolite level	Herd Alarm	Effect
PRE -Partum NEFA \geq 0.3 mEq/L	15%	-1.2% PR +1.4% DZ -240 kg ME305 milk
POST -Partum NEFA \geq 0.6 ^a - 0.7 ^b mEq/L	15%	-1.3% PR ^a +1.3% DZ ^b Heifers: +300kg, Cows: -584kg
BHB \geq 10 ^a -12 ^{b*} mg/dL	15% *20%	-0.7% PR ^b +1.2% DZ ^b Heifers: -535kg*, Cows: -349kg ^a

***15% of 15 = 2-3 animals**

Impact: Cow-level

Metabolite level	Effect
PRE -Partum: NEFA \geq 0.3 mEq/L	Risk of disease \rightarrow 2 x's higher Reproduction \rightarrow 14-18% less likely Milk \rightarrow 616 kg less/lactation
POST -Partum: NEFA \geq 0.6 - 0.7 mEq/L	Risk of disease \rightarrow 4.4 x's higher Reproduction \rightarrow 14-18% less likely Milk \rightarrow Heifers +482 kg, Cows -662 kg
BHB \geq 9 - 10 mg/dL	Risk of disease \rightarrow 4.4 x's higher Reproduction \rightarrow 14-18% less likely Milk \rightarrow Heifers +381 kg, Cows -328 kg

Punch line #5

- **Sample size:**
 - ≥ 12 cows at risk
- **Cows at risk to sample**
 - Pre-partum: 14 to 2 days before calving
 - Post-partum: 3 to 14 DIM; NEFA a better predictor, BHB cheaper and easier to handle
- **Sample to take**
 - Serum (red top tubes)
 - Don't shake, keep cool
- **What to do with sample?**
 - BHB: Precision Extra
 - NEFA: Lab
- **What to do with results**
 - Interpret % above cut-point
 - Currently suggesting herd-level problem if $> \sim 15-20\%$ above cut-point for NEFA and $\sim 20\%$ for BHB

NEB and SCK in transition dairy cows

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NEB and SCK in transition dairy cows

Thanks for your attention!
Any Questions?

