Development of an *in vitro* intestinal digestibility assay for ruminant feeds

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Outline

- Introduction
- History of intestinal digestion assay
- Concerns with assays
- Updated assay
- Application to CNCPS
- Future
- Questions

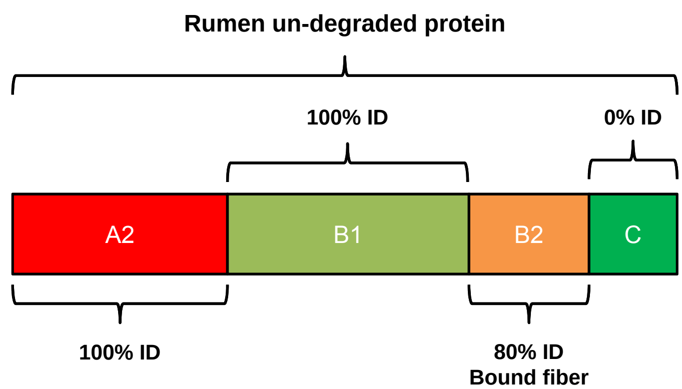
Introduction

- Intestinal digestion (ID) of metabolizable protein (MP) based on library values

  CNCPS feed library has static values for ID

- Intestinal digestion in CNCPS

INTESTINAL DIGESTIBILITY



Introduction

- Unavailable protein =
  1. 100% of C fraction (Acid detergent insoluble protein; ADIP)
  2. 20% of B2 fraction (Neutral detergent insoluble protein) - ADIP

History of ID assays for ruminants

- Tilley and Terry (1963) - two-step *in vitro* procedure
  - *in vitro* fermentation
  - pepsin digestion
- Calsamiglia and Stern (1995) –3-step procedure
  - *in situ* bag fermentation
  - centrifuge tubes -> abomasal and intestinal digestion
  - measure trichloroacetic acid (TCA)-soluble N

History, con't

- Gargallo et al. (2006) – modified 3-step
  - *in situ* bag fermentation
  - *in vitro* bag for abomasal and intestinal digestion
- Boucher et al. (2009a,b,c)
  - modified 3-step
  - precision-fed cecectomized rooster assay
  - immobilized digestive enzyme assay

ID of RUP from Alfalfa by various methods

Feed	ID, % of RUP	Ruminal Incubation	Method	Animal	Reference
Alfalfa hay	66.7	Yes	Mobile bag	lactating cow	de Boer et al., 1987
Alfalfa hay	66.0	Yes	Mobile bag	lactating cow	Erasmus et. al., 1994
Alfalfa hay	29.5	Yes	Mobile bag	Angus steers	Kononoff et al., 2007
Alfalfa hay	36.5	Yes	Mobile bag	Angus steers	Kononoff et al., 2007
Alfalfa haylage	17.2	Yes	Mobile bag	Angus steers	Kononoff et al., 2007
Alfalfa haylage	13.8	Yes	Mobile bag	Angus steers	Kononoff et al., 2007
Alfalfa meal	79.3	No	Mobile bag	Holstein bulls	Todorov et al 1991
Alfalfa meal	80.7	No	Mobile bag	Holstein bulls	Todorov and Girginov, 1991

RUP ID of Blood Meal by 3 Step or Modified 3 Step (Howie et al., 1996)

Feed	ID, % of RUP	Ruminal Incubation	Method	
Blood meal, ring-dried	82.4	Yes	3 step	In vitro
Blood meal, ring-dried	72.1	Yes	3 step	In vitro
Blood meal, ring-dried	72.0	Yes	3 step	In vitro
Blood meal, ring-dried	82.5	Yes	3 step	In vitro
Blood meal, ring-dried	77.2	Yes	3 step	In vitro
Blood meal, ring-dried	80.9	Yes	3 step	In vitro
Blood meal, ring-dried	90.3	Yes	3 step	In vitro

RUP ID of Blood Meal by 3 Step or Modified 3 Step (Boucher, 2008)

Feed	ID, % of RUP	Ruminal Incubation	Method
Blood meal, ring-dried bovine	68.7	Yes	Modified 3 step
Blood meal, ring-dried bovine	70.0	Yes	Modified 3 step
Blood meal, ring-dried porcine	88.7	Yes	Modified 3 step
Blood meal, ring-dried bovine	73.2	Yes	Original 3 step
Blood meal, ring-dried bovine	60.1	Yes	Original 3 step
Blood meal, ring-dried porcine	70.6	Yes	Original 3 step
Blood meal, ring-dried bovine	79.3	No	Modified 3 step
Blood meal, ring-dried bovine	76.8	No	Modified 3 step
Blood meal, ring-dried porcine	89.0	No	Modified 3 step

Concerns

- Use of bags
  - microbial barrier for digestion ⇒ ⇑ lag
  - sample loss
- Enzymes: Pepsin & Pancreatin
  - Profiles and activities undefined
  - Digestion process of ruminant a continuous process

Concerns, con't

Stern et al. (1997): stated

"Evident that an *in-vitro* technique to estimate protein digestion should include enzymes with activity and specificity similar to those found in digestive tract" and "be correlated with intestinal digestion and not total tract digestion".

Summary

- Intestinal digestion of feedstuffs are library values based on bound fiber and acid detergent fiber.

- Historically assays used bags for convenience and pancreatin as the intestinal enzyme.

ID assay ISSUES

- Bag loss
- Variability
- Enzymes

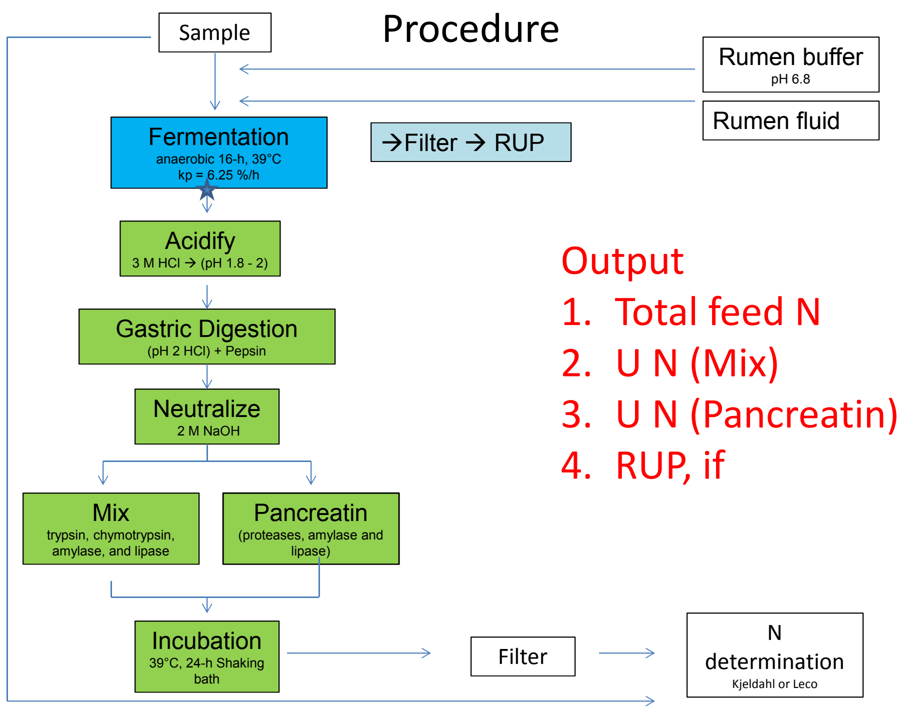
New *In Vitro* ID assay

- Modification of existing methods to better estimate N unavailable fraction
  - flasks instead of bags (sample loss, lag time)
  - physiological enzyme mix
    - reduce proteolytic activity variation
  - filtering residue on 1.5 µm, 90 mm glass instead of TCA precipitation

Inputs

- Feeds
  - 0.5 g / flask
  - 2 replicates / sample / enzyme treatment
- Blanks
- Positive control -- BM
- Negative control – heat damaged BM
- Corn silage ND residue (cell wall)
  - fermentation control ★
  - with rumen fluid
  - without rumen fluid

Procedure



Output

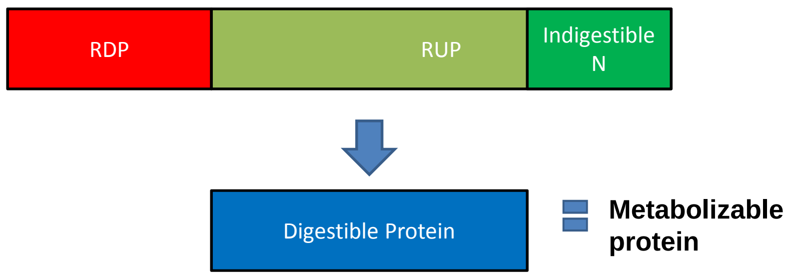
1. Total feed N
2. U N (Mix)
3. U N (Pancreatin)
4. RUP, if

Assay steps continued

- Use isolated corn silage aNDF as the fermentation control to ensure the in vitro step was viable
- Corn silage NDF is low in nitrogen – ↑ N recovery has been verified as microbial protein
- Thus, the corn silage NDF also serves as substrate to correct for microbial contamination and is NDF specific e.g. blood meal has no NDF, thus no correction.
- Must know sample solubility and particle size so RUP is retained on filter, IF..
- Standardize enzymes if ….
- Adaptable for commercial lab

How does this apply to the CNCPS?

IN RUMINANTS INTESTINAL DIGESTIBILITY IS A CALCULATION



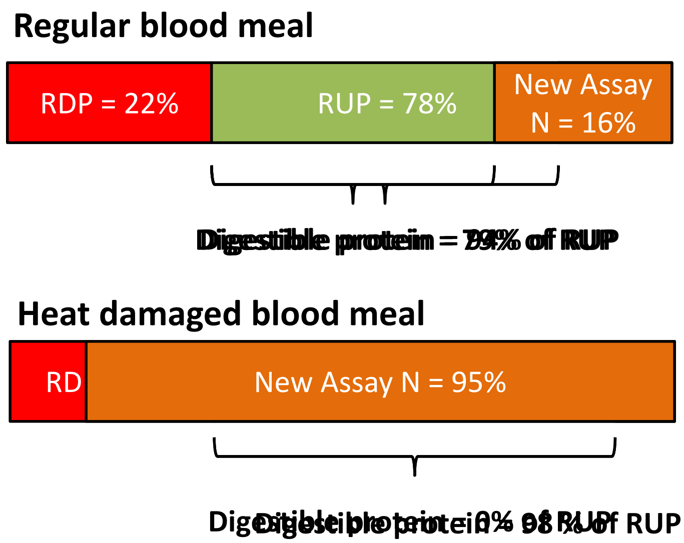
Intestinal digestibility = 1 – [indigestible N/ rumen un-degraded protein]

Comparison of ADIN and Ross in-vitro indigestible N

	Feed N (% DM)	ADIN (%N)	Ross In-vitro indigestible N (% N)
Regular blood meal	16.2	4.7	16
Heat damaged blood meal	16.1	1.8	93
Soybean meal solvent extracted	7.6	6.7	8
Soybean meal heat treated	7.3	7.9	11

Source: Ross, 2013

BLOOD MEAL EXAMPLE OF INTESTINAL DIGESTIBILITY



Calculation of RUP digestibility

	% RUP (CNCPS prediction)	ID (detergent system)	ID (In-vitro system)
Regular blood meal	77	94	79
Heat damaged blood meal	77	98	0
Soybean meal solvent extracted	48	86	83
Soybean meal heat treated	73	82	85

CNCPS example – 85 lbs of milk balanced for ME and MP

Animal Description	
Milk production (lb)	85
Milk fat (%)	3.7
Milk true protein (%)	3.1
Milk lactose (%)	4.78
Condition score	3
Target BCS	3
Age of first calving (d)	24
Calving interval (months)	13
Mature weight (kg)	750
Current weight (kg)	700
Age (months)	60

Diet description	lb DM
Corn Silage Processed 40 DM 45 NDF Medium	22.5
Corn Grain Ground Fine	11.0
Alfalfa Silage 20 CP 37 NDF 17 LNDF	8.8
Energy Booster	0.5
Soybean Meal 47.5 Solvent	3.3
Blood Meal	1.5
Soybean Hulls Ground	4.4
MinVit	2.2
Diet	**54.2**

Dietary composition and CNCPS predictions

Diet composition (% DM unless stated)	
CP	**15.6**
SolP (% CP)	40.9
Ammonia (% SP)	7.6
ADIP (% CP)	6.5
NDIP (% CP)	13.6
Sugars	2.0
Starch	27.5
Sol. Fiber	5.5
ADF	21.0
NDF	32.6
peNDF	56.6
Lignin (% NDF)	9.4
EE	4.2
Ash	8.2
Forage	57.7

CNCPS predictions	
ME (Mcals/d)	60.3
MP v6.1 (g/d)	2228
Inputted milk (lb/d)	85.0
ME Milk (lb/d)	85.0
MP Milk (lb/d)	85.0

Comparison of model predicted MP milk (lb/d) using the current vs new system to estimate ID

- Regular and heat damage blood meal was exchanged on a 1:1 basis.
- All other ingredients remained constant.
- ME allowable milk didn't change

	MP allowable milk (lbs) predicted by the CNCPS	
	Current System	In-vitro System
Regular Blood Meal	85.0	81.3
Heat Damaged Blood Meal	**85.8**	**62.2**

Summarizing

New *in-vitro* assay

- Utilizes half gram samples in flasks with filtration termination
- Porcine pepsin added at 60 %
- Intestinal digestion accomplished with physiological enzymes (trypsin & chymotrypsin).
- Residues are captured making further analysis (amino acids) possible.
- Adaptable for commercial lab

CNCPS vs *in-vitro*

- Detergent system in CNCPS overestimated MP of regular bloodmeal by 5 % and that of heat damaged bloodmeal by 27 %.

Comparing modified 3-step procedure vs in-vitro system

Feed	Modified TSP*		Cornell		
	Rumen	Pancreatin	Rumen	Enz. Mix	Pancreatin
	----% N Undigested----		-------% N Undigested-------------		
BM1	39.6	20.7	93.6	41.9	21.6
BM3	60.7	32.4	93.7	45.1	36.1
BM4	80.1	11.0	95.6	35.1	20.1
Menh1	86.1	8.1	86.3	20.8	16.5
Catfish	66.8	20.2	84.1	36.0	30.1
Menh2	91.0	13.7	89.7	22.7	21.6
DDGS1	100.0	9.6	97.5	76.4	63.3
DDGS2	100.0	5.0	92.7	34.5	30.7
DDGS3	100.0	8.6	83.9	27.6	23.8
DDGS4	100.0	9.7	81.5	24.2	16.8
SBM1	100.0	2.5	92.9	24.7	5.9
SP1	100.0	10.3	92.7	31.1	10.1
SP2	100.0	6.1	84.1	12.2	5.1

*Boucher, et al. (2009 a,b)

Feed	Modified TSP* Rumen	Modified TSP* Pancreatin	Cornell Rumen	Cornell Enz. Mix	Cornell Pancreatin
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DDGS1	100.0	Bag? 9.6	97.5	76.4	63.3
DDGS2	100.0	inhibit 5.0	92.7	34.5	30.7
DDGS3	100.0	bug 8.6	83.9	27.6	23.8
DDGS4	100.0	removal 9.7	81.5	24.2	16.8
SBM1	100.0	2.5	92.9	24.7	5.9
SP1	100.0	10.3	92.7	31.1	10.1
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Catfish	66.8	20.2		84.1	36.0	30.1
Menh2	91.0	13.7		89.7	22.7	21.6
DDGS1	100.0	9.6	Bag	97.5	76.4	63.3
DDGS2	100.0	5.0	Loss?	92.7	34.5	30.7
DDGS3	100.0	8.6	or	83.9	27.6	23.8
DDGS4	100.0	9.7	Enzyme	81.5	24.2	16.8
SBM1	100.0	2.5		92.9	24.7	5.9
SP1	100.0	10.3		92.7	31.1	10.1
SP2	100.0	6.1		84.1	12.2	5.1

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Feed	Modified TSP*		Cornell		
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	----% N Undigested----		-------% N Undigested-------------		
BM1	39.6	20.7	93.6	41.9	≈ 21.6
BM3	60.7	32.4	93.7	45.1	*in vivo* 36.1
BM4	80.1	11.0	95.6	35.1	20.1
Menh1	86.1	8.1	86.3	20.8	16.5
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*Boucher, et al. (2009 a,b) †correlation: r=0.801; P < 0.001

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Summarizing assay comparison

Modified 3-step

- ➢ Bag issues
  - Loss - degrading more RDP & ID
  - Microbial barrier – inhibit removal
- ➢ Enzyme

in-vitro system

- ➢ Appears microbial correction works—feed specific?
- ➢ Undigested N higher with Mix than Pancreatin – more physiological?

Comparing digestion calculated using the detergent system vs in-vitro system

ADIN vs. Undigested feed N using Enzyme Mix or Pancreatin

Feed	Feed N % DM	ADIN % N	% Undigested Feed N	
			Enz. Mix	Pancreatin
Anchovy	11.50	1.3	25.5	20.1
Alfalfa sil	3.80	6.1	23.2	21.9
Bakery waste	1.80	3.3	20.6	23.6
BM0	16.20	4.7	22.9[a]	8.0[b]
BM285	16.89	1.1	0.0	-
BM300	16.20	7.5	4.6	-
BM350	15.13	0.9	23.6	-
BM800	16.50	1.8	2.8	-
Canola 1	6.50	6.3	16.2	12.5
Canola 2	6.60	0.0	14.0	14.0
Citrus	1.04	15.8	55.0	45.4
Corn Germ	4.27	11.2	18.5	9.4

ADIN vs. Undigested feed N using Enzyme Mix or Pancreatin

Feed	Feed N % DM	ADIN % N	% Undigested Feed N	
			Enz. Mix	Pancreatin
Anchovy	11.50	1.3	25.5	20.1
Alfalfa sil	3.80	6.1	23.2	21.9
Bakery waste	1.80	3.3	20.6	23.6
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BM285	16.89	1.1	0.0	-
BM300	16.20	7.5	4.6	-
BM350	15.13	0.9	23.6	-
BM800	16.50	1.8	2.8	-
Canola 1	6.50	6.3	16.2	12.5
Canola 2	6.60	0.0	14.0	14.0
Citrus	1.04	15.8	55.0	45.4
Corn Germ	4.27	11.2	18.5	9.4

ADIN vs. Undigested feed N, con't

Feed	Feed N % DM	ADIN % N	Undigested feed N Enz Mix†	Undigested feed N Pancreatin†
Corn Gluten	3.13	16.9	28.7	18.9
CGF	3.08	11.2	20.7	16.2
Corn Sil 1	1.40	9.2	30.0	25.9
Corn Sil 2	1.30	8.6	13.9	21.1
DDG1	4.90	13.1	11.7	9.5
DDG2	6.40	32.7	27.9[a]	13.6[b]
Hay sil	2.40	12.5	29.6	31.9
SBM	7.60	6.7	7.8	7.6
Soy1	7.70	6.5	9.0	4.3
Soy2	7.30	7.9	11.1[a]	6.6[b]
Wheat Midds	3.30	3.1	9.3	7.2
Heat dam BM	16.10	1.8	95.0	95.0

[ab]Means with different superscripts in same row differ ($P < 0.05$) using Duncans Multiple Range tests. Not all samples were statistically evaluated.

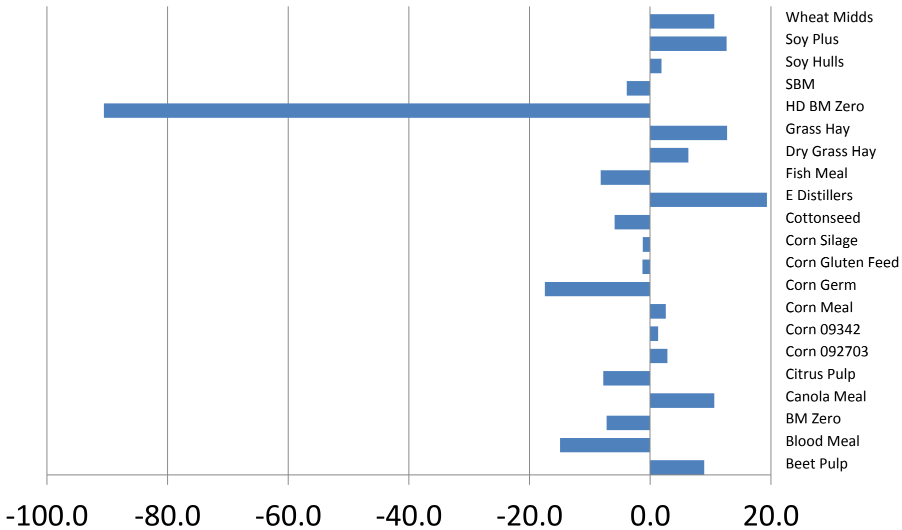
†correlation: r=0.881, $P < 0.0001$.

ADIN vs. Undigested feed N, con't

Feed	Feed N % DM	ADIN % N	Undigested feed N Enz Mix	Undigested feed N Pancreatin
Corn Gluten	3.13	16.9	28.7	18.9
CGF	3.08	11.2	20.7	16.2
Corn Sil 1	1.40	9.2	30.0	25.9
Corn Sil 2	1.30	8.6	13.9	21.1
DDG1	4.90	13.1	11.7	9.5
DDG2	6.40	32.7	27.9[a]	13.6[b]
Hay sil	2.40	12.5	29.6	31.9
SBM	7.60	6.7	7.8	7.6
Soy1	7.70	6.5	9.0	4.3
Soy2	7.30	7.9	11.1[a]	6.6[b]
Wheat Midds	3.30	3.1	9.3	7.2
Heat dam BM	16.10	1.8	95.0	95.0

[ab]Means with different superscripts in same row differ ($P < 0.05$) using Duncans Multiple Range tests. Not all samples were statistically evaluated.

Difference in estimated indigestibility between current model library inputs and assay data –> positive means more available protein than currently predicted by the current inputs
Average of the differences is -3.3 units (-HD BM)



Summary

- Assay appears to provide reasonable and repeatable results
- Sample loss is minimized using the in vitro system and the updated filtration procedures
- Enzymes are standardized and re-evaluated if required, especially when new enzymes are purchased
- RUP estimation was not in our objectives but appears to be necessary for commercial acceptance – use of corn silage ND residue for bacterial contamination

Summary

- Report undigested N as % of feed N

- Assay is compatible with commercial laboratory procedures – lab will need to know basic chemistry/makeup of the sample

- The procedure departs from the detergent system used to frame the feed library 30 years ago thus blurring the line between NDIP and ADIP but supplies a more biologically correct undigestible fraction

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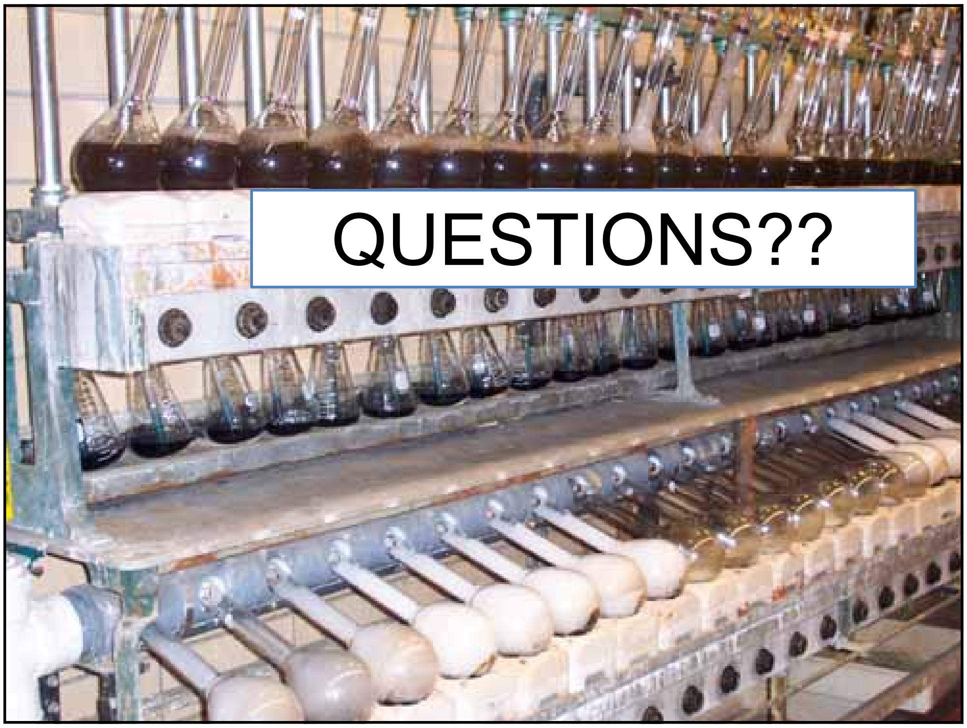
QUESTIONS??