

Proteases: A tool to improve soybean meal quality

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SBM quality is variable

- A result of variations in environmental conditions, varieties, agricultural practices and processing conditions
- High quality SBM is characterized by 2 parameters (that are largely controllable by heat processing):
 - low trypsin inhibitor content
 - high protein solubility
- Trypsin inhibitors may lead to poor litter quality, poor feed conversion, low body weight and thus economic loss

	Recommended range for good quality SBM ¹	Reported values for commercial samples ^{2,3}
Trypsin inhibitor activity (mg/g)	1.55-2.3	1.8-6.5
Protein solubility in KOH (%)	78-85	68-88
Urease (Δ pH)	0-0.05	0-0.05

¹Ruiz & de Belalcázar, 2004, Ruiz, 2014, personal comm ,

²Thakur and Hurburgh (2007) J Am Oil Chem Soc 84 & de Coca-Sinova et al. (2008) Poult Sci 87

³Own work (analysis performed at Universidad Politécnica de Madrid)

Effect of RONOZYME ProAct on SBM of different quality

9 commercial SBM samples

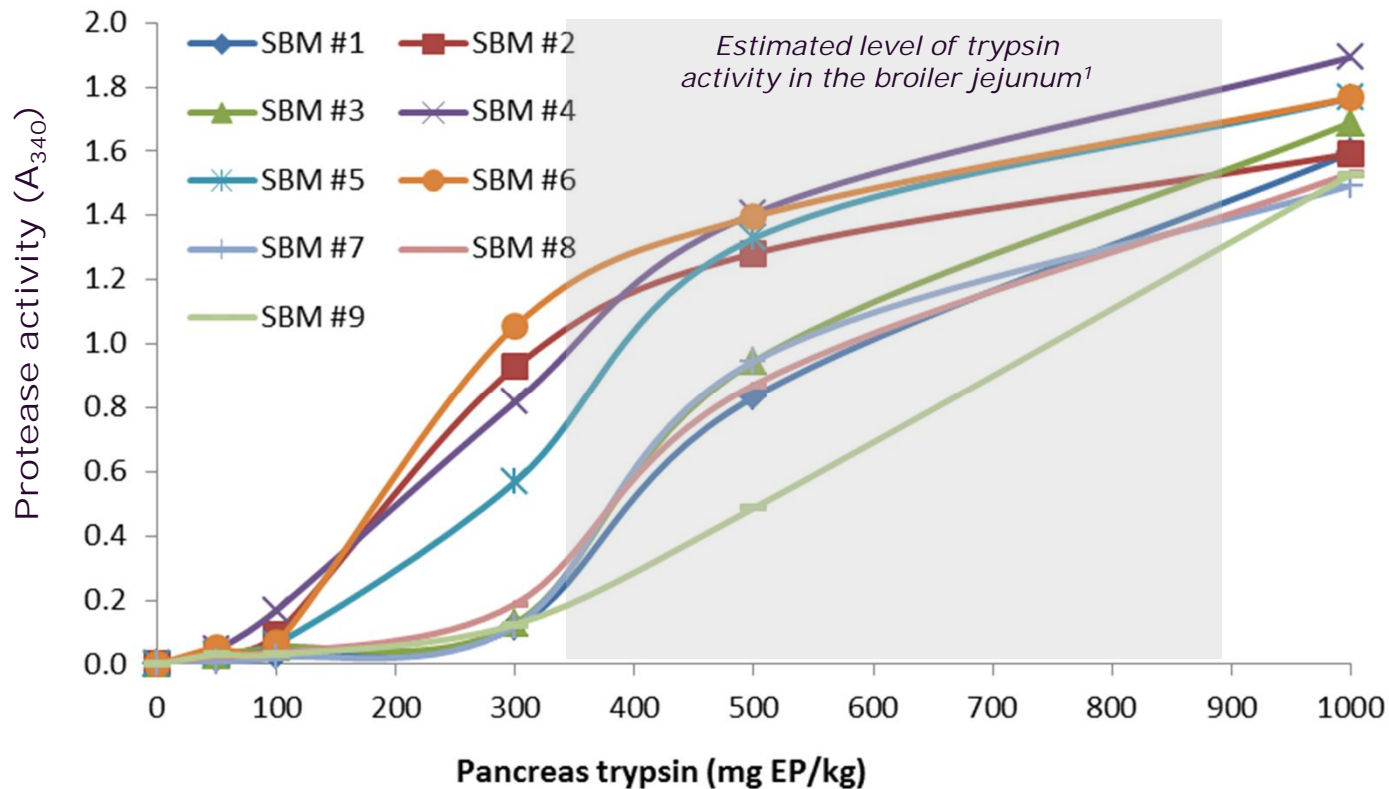
Protease treatment:
3 hours @ pH 7,
40°C, 450 rpm

Colorimetric analysis of
cleaved peptide bonds



Pancreatic trypsin is affected by variation in SBM quality

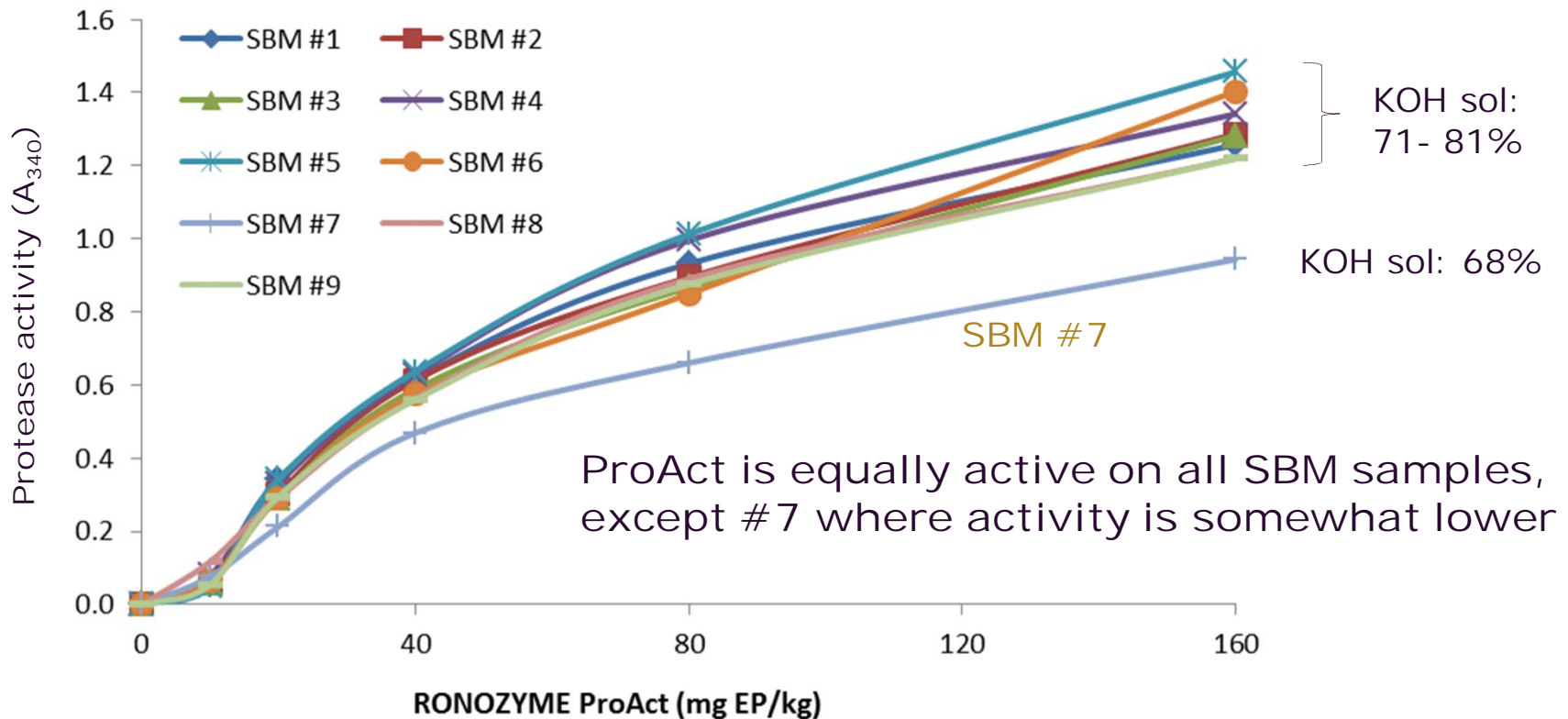
9 commercial SBM samples from Brazil, Argentina and US



- Activity of pancreatic trypsin vary largely between SBM samples
- No one chemical parameter can explain the variable respons

RONOZYME® ProAct is not affected by variation in SBM quality

9 commercial SBM samples from Brazil, Argentina and US



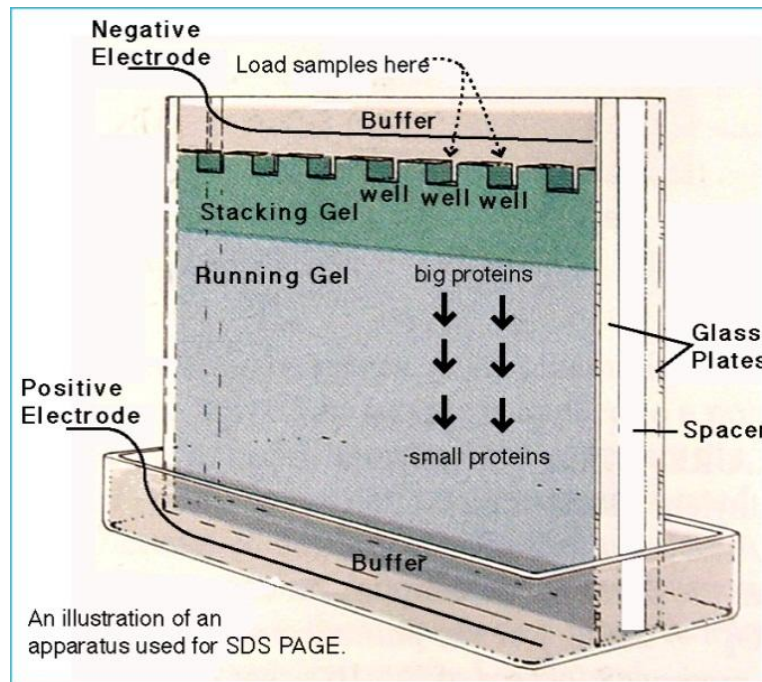
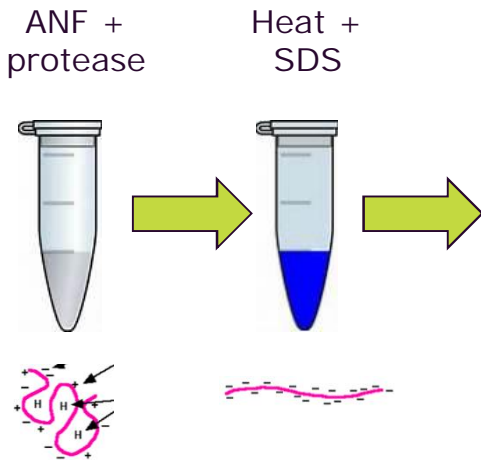
ProAct is equally active on all SBM samples, except #7 where activity is somewhat lower

Degradation of purified antinutritional factors (ANF)

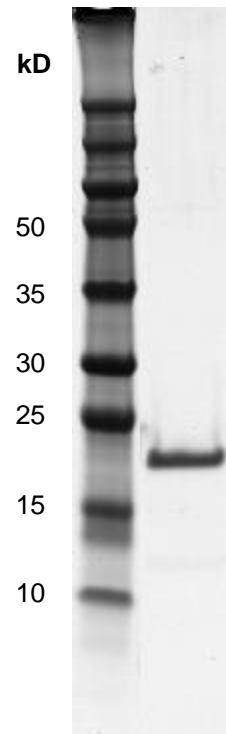
Protease treatment: 2h, 37°C, pH 7 → Protein visualized by SDS-page

Ratio between purified ANF and purified protease = 10:1

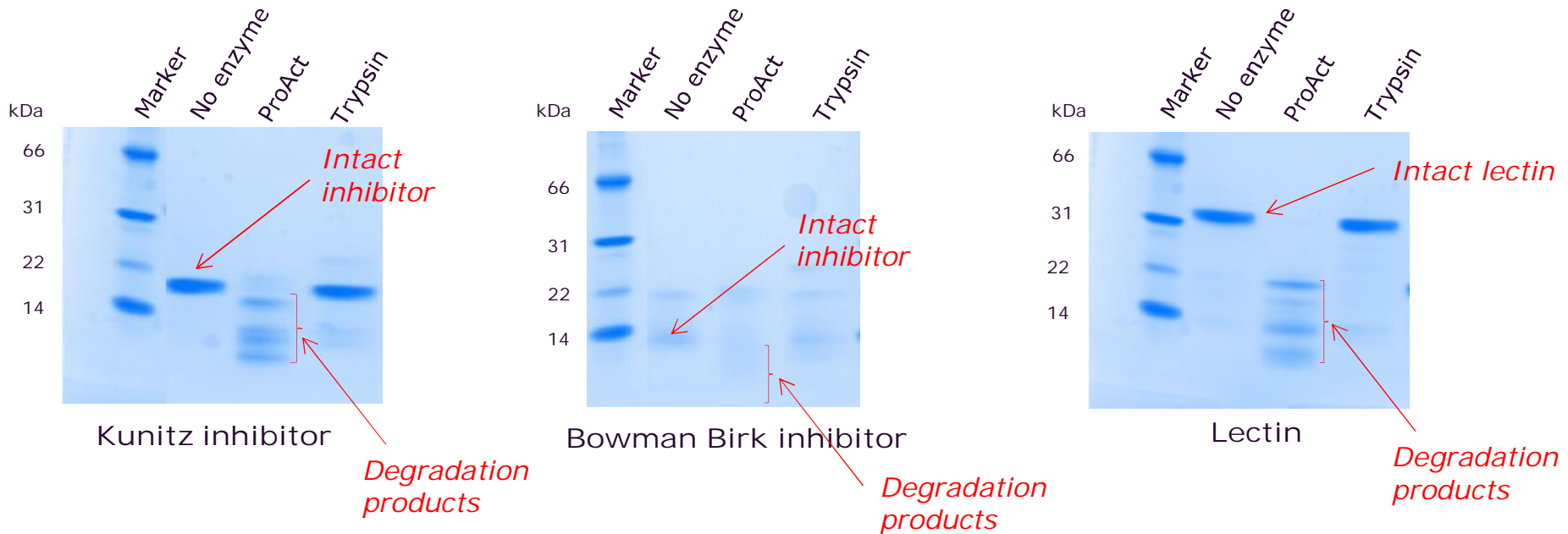
SDS-page:



Visualization e.g. by staining



Degradation of purified antinutritional factors (ANF)




Degradation efficiency (%)

	Kunitz inhibitor	Bowman Birk inhibitor	Lectin
ProAct	96	64	98
Trypsin	4	29	11

Potential role of ProAct in relation to SBM quality

Under-processed SBM
(high trypsin inhibitor level; high protein solubility)

- 
- The endogenous protease system impaired
=> reduced growth performance
 - ProAct degrade trypsin inhibitors
 - ProAct complements the endogenous protease system in the degradation of feed protein

- The endogenous protease system is functional
- Protein solubility limit protein utilization
- ProAct increase protein solubility
- ProAct complements the endogenous protease system in the degradation of feed protein

Over-processed SBM
(low trypsin inhibitor level; low protein solubility)

Conclusions and implications

- Low SBM quality as a result of high trypsin inhibitor content can lead to poor litter quality, impaired FCR, low bodyweight and economic loss
- Batch-to-batch variation in SBM samples affected pancreatic trypsin to a large extent in contrast to RONOZYME® ProAct, which was largely unaffected
- Purified trypsin inhibitors are degraded *in vitro* by RONOZYME® ProAct
- Addition of an effective microbial protease to feed has the potential to assist the pancreatic proteases in hydrolyzing protein in diets containing SBM despite trypsin inhibitor level – and in this way act to level out differences in soy quality

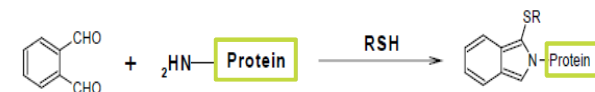
TIME FOR DISCUSSION

Effect of ProAct and trypsin on various soy substrates

Different soy substrates

Protease treatment:
3 hours @ pH 7,
40°C, 450 rpm

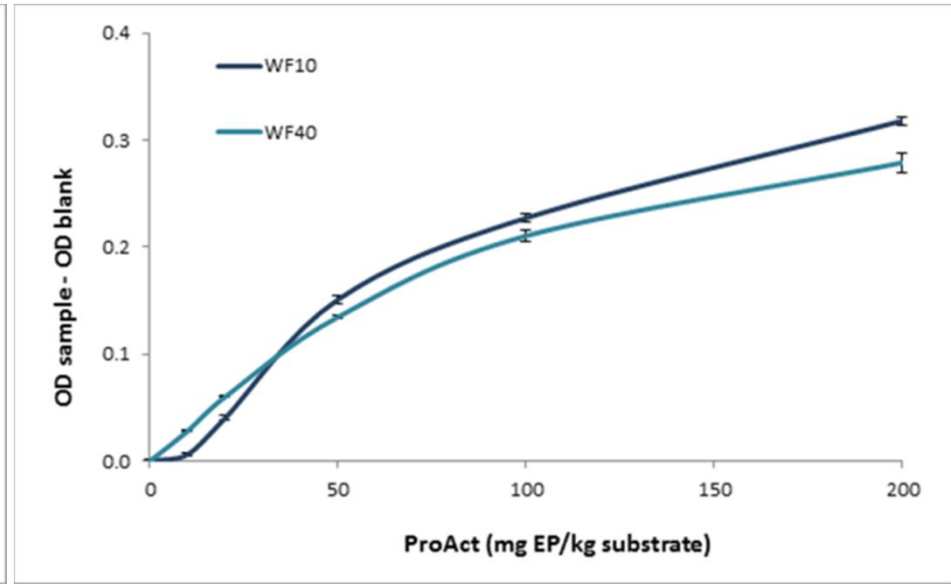
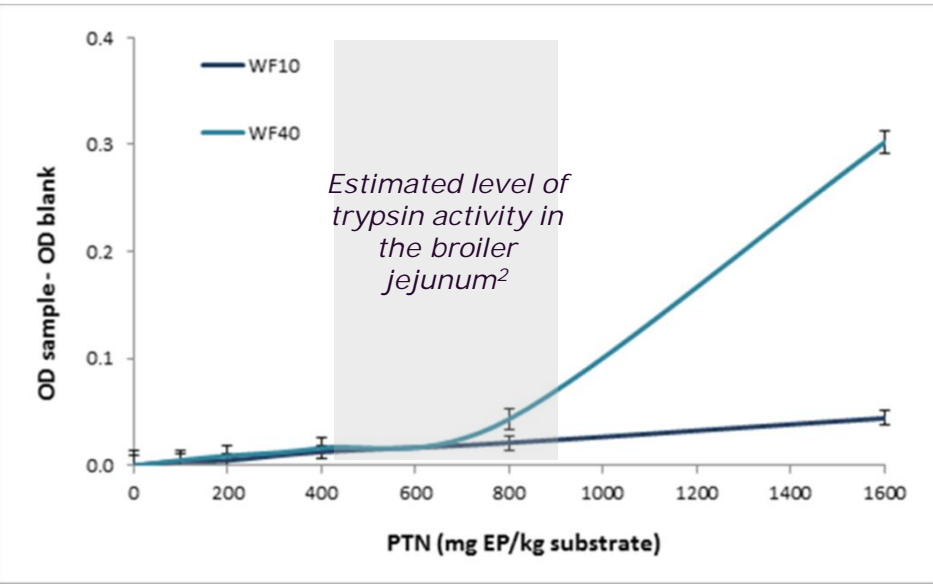
Colorimetric analysis of
cleaved peptide bonds



Effect on autoclaved soy whiteflakes

Oil extracted soy whiteflakes (non toasted) were autoclaved @ 125°C for 10 or 40 minutes to simulate under- and over-toasting

	Urease , ΔpH	TIU/mg ¹	KOH protein solubility, %
Whiteflakes autoclaved 10 min (WF10)	0.28	25.4	80
Whiteflakes autoclaved 40 min (WF40)	0.01	4.7	65



Under-toasting (high inhibitor level) reduced the activity of trypsin (PTN)

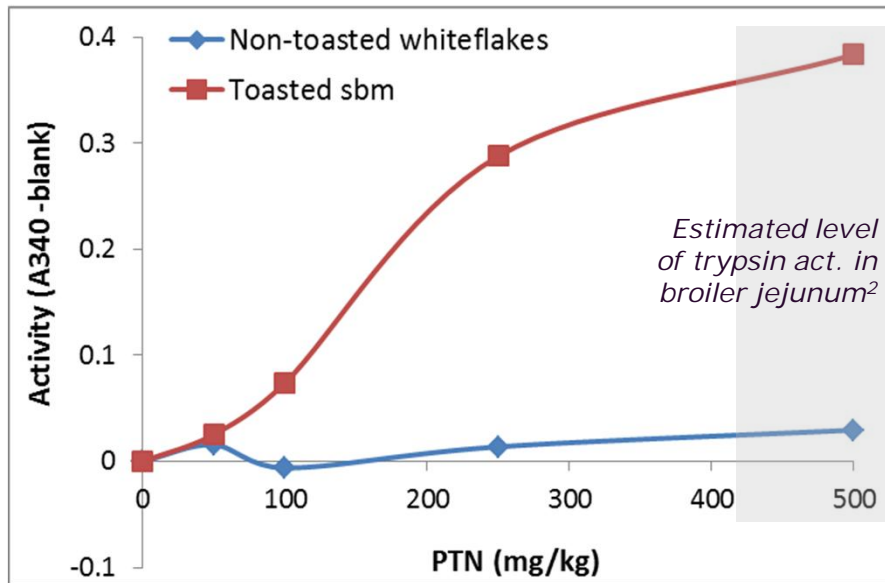
ProAct worked equally well on under- and over toasted SBM

¹ AOCs method; ² Ren et. al. (2012) Poul. Sci. 91

Effect on non-toasted and toasted SBM

Oil extracted soy whiteflakes (non-toasted) and the same whiteflakes after industrial toasting (SBM) were used as substrate

	Urease, Δ pH	TIU/mg ¹
Whiteflakes (WF)	2.04	14
Toasted sbm (SBM)	0.02-0.03	-



Trypsin (PTN) had almost no activity on non-toasted SBM (whiteflakes)

Effect of protease products on SBM of different quality



Products dosed 10x rec. dose for broiler diets

Statistical significance within each dataset was tested using All-pairs Tukey-kramer HSD, $P < 0.05$

Background

SBMs are highly variable with regards to crude protein content, protein solubility and trypsin inhibitor activity

Table 5 Comparison of soybean meal quality study results (12% moisture basis)^a

	Origin	N	Protein (%)	KOH sol (%)
2004	USA	40	47.5	87.7
	Argentina	44	45.9	84.3
	Brazil	22	48.8	83.3
	India	18	46.6	86.1
	Asia	15	47.4	86.2
	China	5	46.3	88.3
	Other	9	47.8	86.2
1995-- 1999	USA	311	47.2	85.0
	Argentina	70	45.5	79.9
	Brazil	136	46.7	80.4
	India	143	47.5	76.3
Baize 1999	USA	16	47.7	83.4
	Argentina	10	45.0	76.2
	Brazil	14	46.9	78.7
	India	17	46.8	80.6

Table 1. Determined composition and protein quality of the soybean meal tested (% , as-fed basis unless stated otherwise)¹

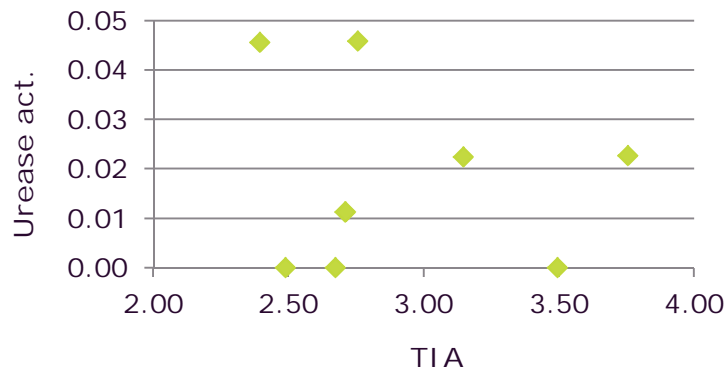
Component	Soybean meal ²					
	Rosario	Ilheus	Paranaguá	Santos	Spain	United States
DM	88.9	88.2	88.4	88.5	89.4	90.2
CP	46.1	45.5	47.2	45.2	50.6	48.6
TIA, ⁵ mg/g	6.5	5.1	4.1	5.1	2.4	1.8
Urease activity, mg of N/g per min 30°C	0.03	0.01	0.02	0.04	0.00	0.00
PDI ⁶	12	14	12	15	11	10
KOH solubility	80.9	80.5	84.2	81.6	85.2	84.3

modified from de Coca-Sinova et al. (2008) *Poult Sci* 87

Analytical data on 9 SBM samples (Prof. Mateos)

	#1	#2	#3	#4	#5	#6	#7	#8	#9
	USA	USA	USA	ARG	ARG	ARG	BRA	BRA	BRA
Crude protein (%DM)	53.8	52.4	52.9	53.6	52.9	52.8	53.7	54.2	53.2
PDI (%DM)	25.6	24.8	9.7	10.4	11.7	10.1	7.0	15.2	12.5
KOH solubility (%DM)	80.2	79.7	78.6	75.4	77.3	71.4	67.9	77.2	81.4
Urease activity (Δ pH)	0.02	0.00	0.02	0.01	0.00	0.00	0.05	0.05	0.01
Trypsin inhibitor activity (mg/g DM)	3.75	3.49	3.15	2.71	2.67	2.49	2.39	2.76	2.71
Heat Damage Index (Aminored – Evonik)	5.00	2.00	8.00	11.00	12.00	15.00	25.00	14.00	16.00

Urease act. vs TIA



KOH sol. vs TIA



SYNERGY WITH PANCREATIC PROTEASES

Substrate: Commercially toasted SBM

Incubation: 3 hours, pH 7, 40°C

Enzymes: Pancreatic Trypsin Novo (PTN), RONOZYME® ProAct

