Protein Digestion and Absorption: Limits

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Overview

• The gastrointestinal tract
• Movement of digesta
• Protein digestion and absorption
• Patterns of amino acid release
The GIT

- pH and rate of passage
GIT – pH at 22 d of age
(mean of 35 birds)(min/max) (Angel et al., unpublished data)

Crop
5.3 (4.3/6.5)

Proventriculus
1.9 (1.0/2.8)

Gizzard
2.5 (1.6/3.2)

Duodenum
6.0 (5.6/6.3)

Jejunum
6.5 (6.3/6.8)

Ileum 6.8 (6.4/7.0)

Ceca
6.4 (5.9/6.9)

Large intestine
6.7 (6.3/7.3)

Total residence time (n=12), h
*10 d age = 2:52 m (2:01 – 3:10 )
*22 d age = 3.31 h (2:49 – 4:02)
*30 d of age = 3:55 h (2:50 – 5:05)
Residence time of digesta by segment of the GIT at 22 d of age
(Angel et al., unpublished)
Mean feed particle size 0.822 mm (corn/SBM diet)

<table>
<thead>
<tr>
<th>Segment</th>
<th>Residence Time (days)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crop</td>
<td>5.3</td>
<td>12 m (&lt;5 – 60 m)</td>
</tr>
<tr>
<td>Proventriculus</td>
<td>1.9</td>
<td>P+G 37 m (20 – 72)</td>
</tr>
<tr>
<td>Gizzard</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>Jejunum</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>6.8</td>
<td>83 m (65 – 125)</td>
</tr>
<tr>
<td>Ceca</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>Large intestine</td>
<td>6.7</td>
<td></td>
</tr>
</tbody>
</table>

pH data n = 35
Residence time data n = 12
Angel et al., unpublished
Rate of passage through the small intestine and duodenum at different ages. Calculated by dividing the mean amount of isotope recovered from the segment by the daily intake of $^{141}\text{Ce}$. Uni et al 1995
Retrograde movement of digesta

- Proximal and distal
- Proventriculus and gizzard
- More know about proximal (back peristalsis from duodenum and jejunum into gizzard)
- Key in interpretation of passage rate and segment digestibility data
- Methodology to account for this?
Sklan et al., 1978

- Injected radioactive isotope into bile duct
- 40% of injected dose reached the gizzard (most within 2 min)
- After 2 min 70% of injected dose was in gizzard and duodenum
- After 20 min 50% of the dose seen at 2 min was still in the gizzard – slow clearing time
- If injected into upper jejunum, 20% of the dose present in gizzard at 2 min
Impact of water pH on GIT pH -
H₂O pH 5.8/8.1: diet pH 6.2 at 8 and 38 d of age (n=10)

Angel et al., 2010
Role of gastric acidity

• Denature (unfold) proteins to allow access for enzymes to hydrolyze
• Extent of protein hydrolysis in gizzard correlated to time of exposure to pepsin/HCl (Keller, 1968)
• Any inhibitions to this first step will inhibit subsequent steps
  – Type of protein
  – Acidity in gastric area
Impact of diet Ca gizzard pH

$r^2 = 0.33
P < 0.001$

Kim et al., 2013

Corn-SBM diet, no added inorganic phosphate
$tP = 0.44\%$, $PP = 0.32\%$, $nPP = 0.12\%$

Limestone added to increase Ca

Analyzed Ca 0.21 to 0.78

SEM 0.076

$n = 9$
Impact of pH on pepsin activity and stability (Piper and Fentone, 1965)
Resistant protein in soybeans
(Wang et al., 1995)

- High molecular fraction resulting from in vitro digestion of soybean protein with pepsin and pancreatin – when fed to rats
- Resistant to further digestion – high homology between AA pattern of HMF and AA in feces
- Bound to bile acids results in increased bile acid secretion
- Increased cholesterol excretion
- Increased N excretion
Influence of Age on Lipase, Amylase, and Protease Activities in Pancreatic Tissue and Intestinal Contents of Young Turkeys\textsuperscript{1}

ÅSHILD KROGDAHL\textsuperscript{2} and JERRY L. SELL\textsuperscript{3}

Department of Animal Science, Iowa State University, Ames, Iowa 50011

(Received for publication October 12, 1988)

ABSTRACT Day-old male turkeys were fed either a reference diet with 12\% sucrose or experimental diets with 12\% tallow or 12\% animal-vegetable blend (A-V fat) replacing sucrose until 56 days of age. Poult's were sampled at 1 day of age and every 2 to 7 days thereafter for determination of enzyme activities of pancreas and contents of the proximal one-fourth of the small intestine.

In pancreatic tissue, trypsin, protease, and lipase activities increased with age after a lag period of about 14 days. Amylase activity increased rapidly during the first 14 days. In intestinal contents, trypsin, protease, and amylase increased from Day 1 until Day 21. Development of intestinal lipase activity seemed to depend on dietary fat level. Low activities were observed with low fat diets throughout the study. With high fat diets, a lag period of about 3 wk was followed by a five-fold increase in lipase activity.

(Key words: turkey, age, lipase, amylase, proteases)
FIGURE 1. Development with age (in days) of relative pancreatic weight in poults, as a percentage of live weight. 

n = 12 on Days 2 through 56, 9 on Day 1.

In turkeys

Krogdahl and Sell, 1989
Changes with age (d) of pancreatic enzymes, in pancreas (and in digesta in the proximal ¼ SI). In percent of 8 week values (100%) and calculated per weight of tissue for pancreas and per DM weight in digesta. In turkeys - Krogdahl and Sell, 1989
Net daily secretion into duodenum/gram feed intake (measured in intestinal content) Arbor Acres (open bars) and Lohman (hatched bars) Enzyme activities were defined as amount of enzyme hydrolyzing 1 /umol of substrate/min under the specified conditions. Means ± SD from five chicks. Uni et al., 1995
Apparent ileal protein digestion (corn/SBM diet) – changes with age
Arbor Acres (open bars), Lohman (hatched bars)
Uni et al., 1995
# Sequential hydrolysis of gliadin

Caldwell, 1980

<table>
<thead>
<tr>
<th>Material analyzed</th>
<th>Ultrafiltrable N, ug/ml</th>
<th>Ninhydrin reactants, nmole/ml</th>
<th>Peptides, OD/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undigested gliadin</td>
<td>5</td>
<td>15</td>
<td>2.18</td>
</tr>
<tr>
<td>Gliadin digests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pepsin</td>
<td>35</td>
<td>43</td>
<td>5.20</td>
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<tr>
<td>Pepsin + trypsin</td>
<td>150</td>
<td>826</td>
<td>12.0</td>
</tr>
<tr>
<td>Pepsin + trypsin + pancreatin</td>
<td>860</td>
<td>4930</td>
<td>620</td>
</tr>
</tbody>
</table>
Impact of SBM heat treatment
Total N/yttrium$^{91}$
Shows net secretion proportional to RS in duodenum

digested N/yttrium$^{91}$

Bielorai et al., 1973
Net Digestion and absorption

Hurwitz et al., 1972

• Effect of dietary protein concentration
• Effect of SI segment
• SBM/Milo diet
• 20.4 vs 14.4 % protein diets
• White Rock chicks – 3 weeks of age
• Fed experimental diets for 5 days
Calculations of digestion and absorption (Hurwitz et al, 1972)

\[
\% \text{ Net absorption} = 100 \left[ 1 - \frac{(N_t/Y)_S}{(N_t/Y)_F} \right]
\]

\[
\% \text{ Net digestion} = 100 \left[ 1 - \frac{(N_t - N_u)/Y}_S/(N_t/Y)_F \right]
\]

where

\begin{align*}
N_t & \quad = \text{total nitrogen} \\
N_u & \quad = \text{ultrafilterable nitrogen} \\
N_t - N_u & \quad = \text{undigested nitrogen} \\
Y & \quad = \text{yttrium-91.}
\end{align*}

The subindices S and F denote “segment” and “feed”, respectively.
% digestion

Duodenum digestion @ 60%

% absorption

Duodenum absorption @ 10%

Hurwitz et al, 1972

14.4% protein
20.4% protein
Hurwitz et al, 1972

% digestion

- Duodenum Digestion @ 60%
- Upper Jejunum @ 20% more
- @ 93% digestion

% absorption

- Duodenum absorption @ 10%
- Upper Jejunum @ 48% more
- @ 85% absorption

14.4% protein
20.4% protein
Net Digestion and absorption

Hurwitz et al., 1972

- Effect of dietary Ascaridia galli infection (most parasites in upper SI)
- Effect of SI segment
- SBM/Milo diet
- 20.4 vs 14.4 % protein diets
- White leghorn males– Infected at 1 week of age
- Fed experimental diets for 5 days, starting at 5 weeks of age
Hurwitz et al, 1972

Duodenal digestion
- No infection @ 50%
- Infected @ 8%

Upper Jejunal digestion
- No infection @ 60%
- Infected @ 60%

Duodenal absorption
- No infection @ -7%
- Infected @ -57%

Upper Jejunal absorption
- No infection @ 40%
- Infected @ 40%
Rate limiting step

- Digestion rather than absorption (Austic, 1985)
- But Hurwitz et al., 1972 data would suggest otherwise
AA or peptide absorption

- Different mechanisms of absorption
- High competition for absorption of AA –
- More efficient and more rapid absorption of peptides
- Transporter systems mostly Na dependent
Undigested protein

• Impact of ProAct in different ingredients
Full fat soy

Reference method (apparent)
Bertichini et al 2009
NDF (True) Messias et al., 2010

Undig Subs Berchichini

- Lysine
- Glutamic Acid
- Tyrosine
- Phenylalanine
- Aspartic Acid
- Leucine
- Isoleucine
- Arginine
- Histidine
- Methionine
- Threonine
- Valine
- Cystine
- Alanine
- Total AA

%
Full fat soy

Reference method (apparent)
Bertichini et al 2009

NDF (True) Messias et al., 2010

-5 0 5 10 15 20 25 30

Undig Subs Berchichini
Undig Subs Messias

lysine  Glutamic Acid  Tyrosine  Phenylalanine  Aspartic Acid  Leucine  Isoleucine  Arginine  Histidine  Methionine  Threonine  Valine  Cystine  Alanine  Total AA
Reference method (apparent)
Bertichini et al. 2009
NDF (True) Messias et al., 2010

Full fat soy

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Reference Method</th>
<th>Messias Method</th>
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</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>18</td>
<td>13</td>
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<tr>
<td>Histidine</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>Threonine</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>Methionine</td>
<td>33</td>
<td>32</td>
</tr>
<tr>
<td>Valine</td>
<td>38</td>
<td>37</td>
</tr>
<tr>
<td>Cystine</td>
<td>43</td>
<td>42</td>
</tr>
<tr>
<td>Alanine</td>
<td>48</td>
<td>47</td>
</tr>
<tr>
<td>Total AA</td>
<td>100</td>
<td>100</td>
</tr>
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## SBM composition

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Bertichini et al., 2009</th>
<th>Angel, et al., 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (%)</td>
<td>3.59</td>
<td>2.88</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>10.74</td>
<td>8.8</td>
</tr>
<tr>
<td>C. Protein (%)</td>
<td>44.44</td>
<td>48.0</td>
</tr>
<tr>
<td>Urease, pH change</td>
<td>0.03</td>
<td>0.01</td>
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<tr>
<td>Solubility KOH, %</td>
<td>88.15</td>
<td>94.2</td>
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</table>
## SBM composition

<table>
<thead>
<tr>
<th>Amino Acids, % as is</th>
<th>US Evonik (46.95 CP)</th>
<th>Rostagno et al., 2011 (48% SBM)</th>
<th>Angel et al., 2010 (48% SBM)</th>
<th>Bertichini et al., 2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic Acid</td>
<td>5.37</td>
<td>5.35</td>
<td>5.44</td>
<td>5.44</td>
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<tr>
<td>Glutamic Acid</td>
<td>8.47</td>
<td>8.57</td>
<td>8.60</td>
<td>8.60</td>
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<tr>
<td>Serine+Glycine</td>
<td>4.33</td>
<td>4.74</td>
<td>4.32</td>
<td>4.32</td>
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<tr>
<td>Histidine</td>
<td>1.23</td>
<td>1.25</td>
<td>1.29</td>
<td>1.26</td>
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<tr>
<td>Arginine</td>
<td>3.40</td>
<td>3.47</td>
<td>3.61</td>
<td>3.64</td>
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<tr>
<td>Threonine</td>
<td>1.85</td>
<td>1.87</td>
<td>1.85</td>
<td>1.67</td>
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<tr>
<td>Alanine</td>
<td>2.03</td>
<td>2.12</td>
<td>2.05</td>
<td>2.05</td>
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<tr>
<td>Proline</td>
<td>2.34</td>
<td></td>
<td>2.30</td>
<td>2.30</td>
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<tr>
<td>Tyrosine</td>
<td>1.74</td>
<td>1.55</td>
<td>1.83</td>
<td>1.83</td>
</tr>
<tr>
<td>Valine</td>
<td>2.21</td>
<td>2.31</td>
<td>2.40</td>
<td>2.22</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.65</td>
<td>0.65</td>
<td>0.68</td>
<td>0.77</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.68</td>
<td>0.71</td>
<td>0.69</td>
<td>0.78</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2.11</td>
<td>2.26</td>
<td>2.30</td>
<td>2.20</td>
</tr>
<tr>
<td>Leucine</td>
<td>3.55</td>
<td>3.66</td>
<td>3.76</td>
<td>3.59</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.34</td>
<td>2.46</td>
<td>2.41</td>
<td>2.35</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.89</td>
<td>2.93</td>
<td>3.09</td>
<td>3.07</td>
</tr>
</tbody>
</table>
SBM- Amount of undigested AA (W/O ProAct)

- Bertechini et al., 2009 Ref method (apparent)
- Angel et al., 2010 NDF (True)
Reference method (apparent)
Bertichini et al 2009

SBM

-5
-5
0
0
5
5
10
10
15
15
20
20
25
25
30
30
35
35
40
40
45
45
50
50
55
55

Undigested Substrate
Amount of Undigested Substrate digested by ProAct

Lysine, Glutamic Acid, Aspartic Acid, Tyrosine, Phenylalanine, Isoleucine, Serine, Valine, Leucine, Arginine, Methionine, Threonine, Histidine, Alanine, Cystine, Glycine

NDF (True) Angel et al., 2010
SBM—percent of undigested – digested by ProAct

- Bertechini et al, 2009 Ref method (apparent)
- Angel et al 2010, NDF (True)
AA composition of Mucin

- Lysine
- Glutamic Acid
- Tyrosine
- Phenylalanine
- Aspartic Acid
- Leucine
- Isoleucine
- Arginine
- Histidine
- Methionine
- Threonine
- Valine
- Cystine
How can we improve protein digestion/absorption?

• What should be the target of a protease?
  – Unclear what the rate limiting step is in digestion
  – Ingredient/diet related

• Include factor to help in denaturing process

• Look for balance in aa and peptide residue for absorption (not looking for complete)

• Passage rate/digestion modifiers – particle size, light programs, feed accesses
How can we improve protein digestion/absorption?

• Feed back mechanisms of nutrients on enzyme secretion

• What is really the portion that is undigested (changes site of digestion/absorption)
  – Endogenous
  – Feed proteins
Adequacy of methodologies

• Impact of fasting on mucin production (Smirnov et al., 2004)
• Fasted birds had more mucin mRNA and mucin proteins in duodenum and jejunum
• Enlargement of globlet cells
• Mucus layer thickness decreased
• Change in microbial populations associated with mucin (Miller and Hoskins (1981))
Endogenous proteins variable

• Mucin/enterocytes > enzymes/bile secretions
• Affected by:
  – Presence of feed in GIT (time without) – age affect
  – Fiber – amount and type
  – Microbial populations
  – Nutrient content and feed back mechanisms
Thank you