

MANUAL AND REFERENCES

VERSION 3

**CORNELL NET CARBOHYDRATE
and
PROTEIN SYSTEM**

CPM MODEL
and
NRC DAIRY 2001

ADVANCED RUMINANT NUTRITION

ANIMAL SCIENCE 815.3

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Feed Classification According to the Cornell Net Carbohydrate and Protein System (CNCPS)

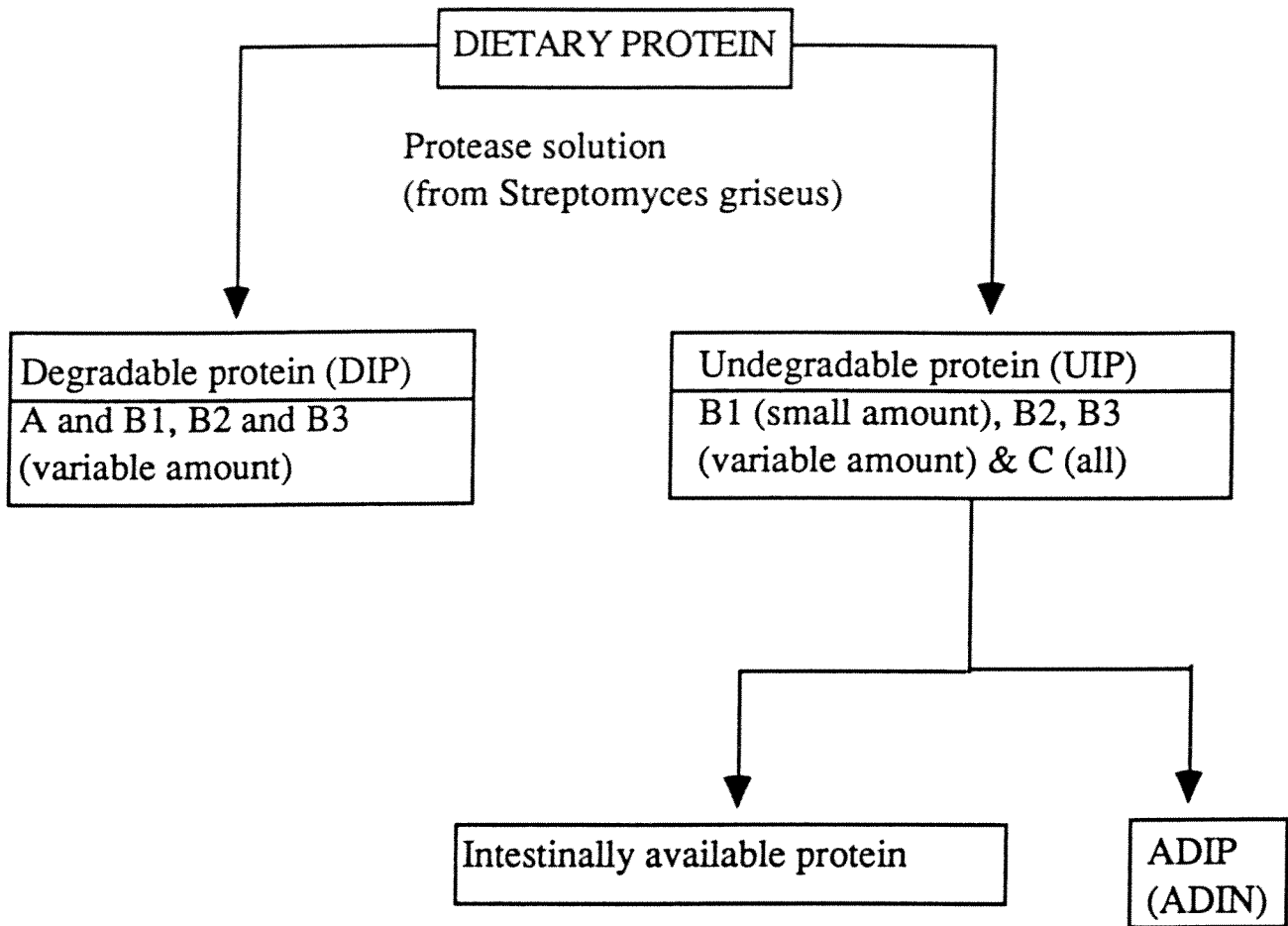
The CNCPS divides feed into 8 fractions:

- 1- Dry matter (DM).
- 2- Ash.
- 3- Ether Extract (EE).
- 4- Neutral Detergent Fiber (NDF) and Acid Detergent Lignin (ADL).
- 5- Total Crude Protein (CP).
- 6- Soluble Protein (SCP).
- 7- Neutral Detergent Insoluble CP (NDICP) and Acid Detergent Insoluble CP (ADICP).
- 8- Non Structural Carbohydrates (NSC).

Determination of DM, Ash, EE, CP and ADL:

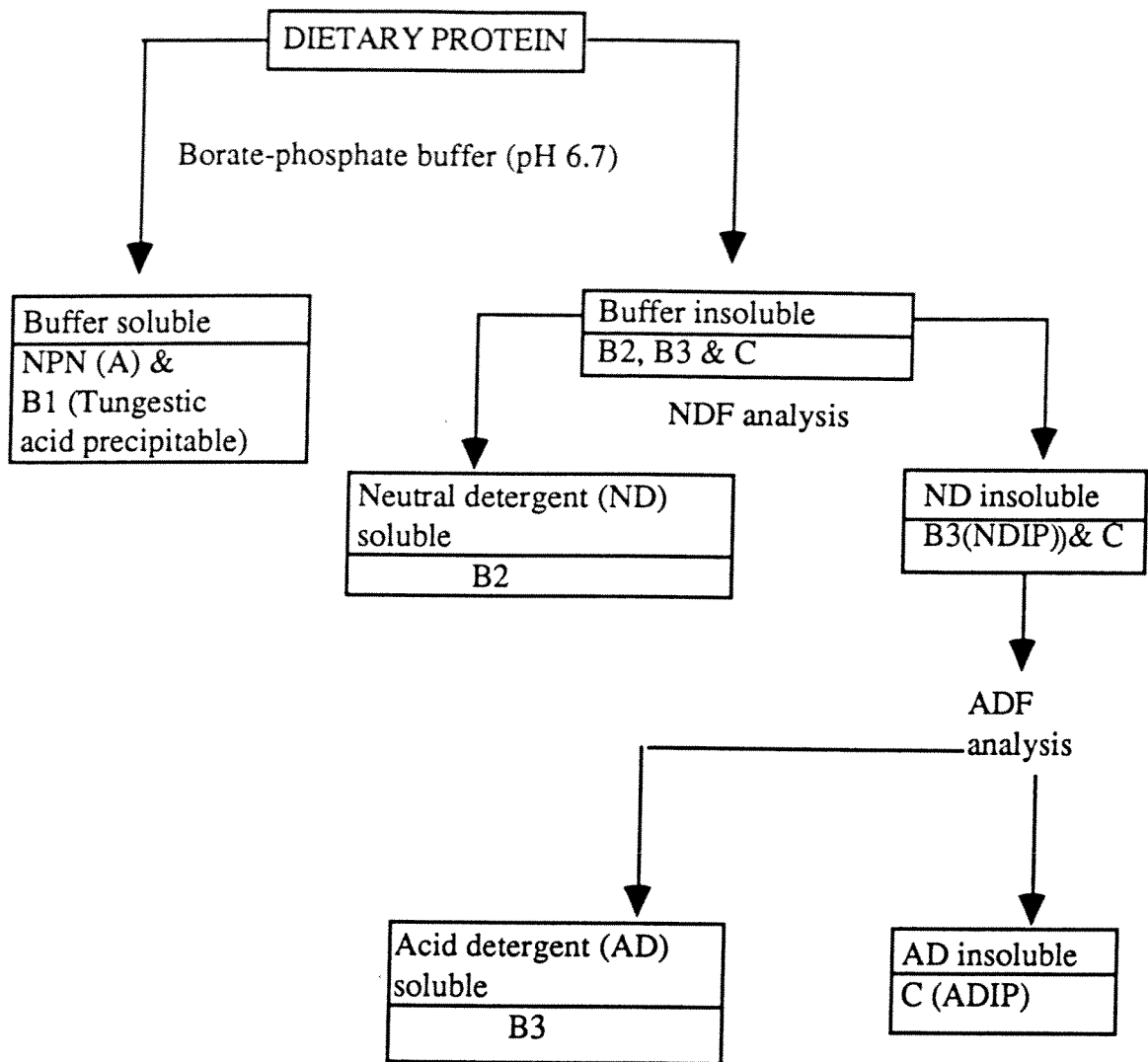
The methods of the Association of Official Analytical Chemists (1990) will be used to determine:

- Dry matter	Method No. 934.01
- Ash	Method No. 924.05
- Ether extract	Method No. 920.39
- Kjeldahl nitrogen	Method No. 984.13
- Acid detergent fiber	Method No. 973.18
- Acid detergent lignin	Method No. 973.18



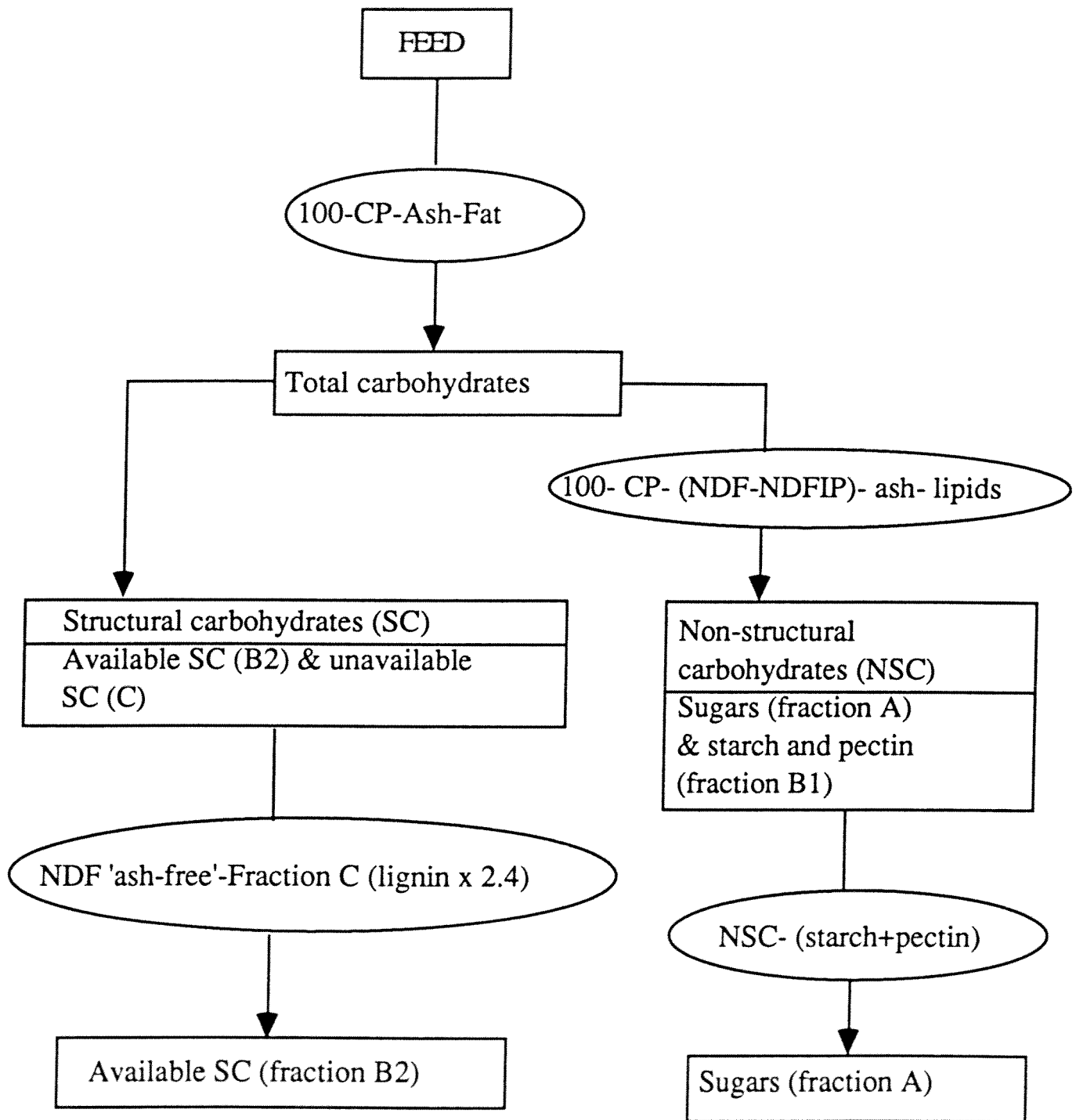
In vitro protease technique to estimate protein degradability.

(Roe et al.1990).



The CNCPS Protein Fractionation Scheme.

(Roe et al.1990).



The CNCPS scheme for feed carbohydrate fractionation.

(Sniffen et al., 1992)

Determination of Neutral Detergent Fiber (NDF)

Reference: Van Soest, P. J., Robertson, J. B. and Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

Neutral detergent fiber (NDF) in feed includes cellulose, hemicellulose and lignin as major components. However, there are some components which are removed by neutral detergent and are not part of NDF these include silica and some tannins.

Reagent:

- 1) Neutral detergent (ND) solution.

Procedure:

- 1) Weigh 0.5 to 1.0 g sample ground to pass 1 mm screen.
- 2) Boil with 100 mL ND solution for 1 h. In case of starchy samples, add 50 µl of heat stable amylase (dietary fiber kit; Sigma catalogue number A3306) before the beaker is placed on heat.
- 3) Filter through a preweighed #54 Whatman filter paper (if NDICP is desired) or a Coarse sintered glass crucible.

Calculation:

$$\text{NDF (\%)} = \frac{\text{Residual weight}}{\text{Sample weight}} \times 100$$

An alternative procedure for removing starch:

- 1) Treat the sample with 30 mL of 8 M urea plus 50 µL of amylase.
- 2) Stir with a rod to break any lumps. Incubate at room temperature for 4 h or overnight.
- 3) Add 100 mL ND solution and 50 µL amylase enzyme. Boil for 1 h and handle as above.

Determination of Acid Detergent Fiber (ADF)

Reference: Association of Official Analytical Chemists. 1990. Official methods of analysis, 15 ed. AOAC. Arlington, VA.

Acid detergent fiber (ADF) in feed includes cellulose, lignin and silica as major components. The extraction with acid detergent is used as a preparation step for determination of lignin and acid insoluble protein. Acid detergent solution also solubilizes hemicellulose.

Reagent:

- 1) Acid detergent (AD) solution.

Procedure:

- 1) Weigh 0.5 to 1.0 g sample ground to pass 1 mm screen.
- 2) Boil with 100 mL AD solution for 1 h.
- 3) Filter through a preweighed #54 Whatman filter paper (if ADICP is desired) or a Coarse sintered glass crucible.

Calculation:

$$\text{ADF (\%)} = \frac{\text{Residual weight}}{\text{Sample weight}} \times 100$$

Determination of Soluble Crude Protein

Reference: Roe, M. B., Sniffen, C. J. and Chase, L. E. 1990. Techniques for measuring protein fractions in feedstuffs. Proc. Cornell Nutr. Conf. p 81. Ithaca NY.

Soluble CP includes soluble true protein (fraction B₁) and non protein nitrogen (fraction A). Borate-phosphate buffer will be used to determine soluble CP.

Reagents:

- 1) Borate-phosphate buffer (pH 6.7).
NaH₂PO₄.H₂O 12.20 g L⁻¹.
Na₂B₄O₇. 10H₂O 8.91 g L⁻¹.

Procedure:

- 1- Estimate N in feed sample by Kjeldahl method.
- 2- Weigh 0.5 g dry sample into 125 ml Erlenmeyer flask.
- 3- Add 50 ml borate-phosphate buffer.
- 4- Incubate for 1 hour at 39 °C.
- 5- Filter through Whatman #54 filter paper. wash residues with 250 ml borate-phosphate buffer.
- 6- Dry filter paper at 105 °C for overnight.
- 7- Estimate N in residues by Kjeldahl.

Calculation:

$$\text{Soluble crude protein (\% CP)} = \frac{\text{Total CP} - \text{Residual CP}}{\text{Total CP}} \times 100$$

Determination of Non-Protein Nitrogen (NPN) Using Tungstic Acid

Reference: Licitra, G., Hernandez, T. M. and Van Soest, P. J. 1995.
Standardization of procedures for nitrogen fractionation of ruminant feeds.
Anim. Feed Sci. Technol (Submitted).

1996
LP 57: 347-358

In the CNCPS, NPN is designated as fraction A. The determination of NPN is based on precipitation of true protein using trichloroacetic acid or tungstic acid. The NPN content is then calculated as the difference between the total CP and residual (precipitated CP). The B1 protein fraction can be estimated by subtracting NPN from soluble CP.

Reagents:

- 1) Sodium tungstate ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$) 10% solution in water 0.30 M.
- 2) 0.5 M sulfuric acid.

Procedure:

- 1) Weigh 0.5 g dry sample in 125 ml Erlenmeyer flask.
- 2) Add 50 mL cold distilled water.
- 3) Add 8 mL of 10% sodium tungstate solution.
- 4) Let flask stand at 20-25 °C for 30 min.
- 5) Bring pH to 2 by adding 10 mL of 0.5 M sulfuric acid (check pH with pH meter).
- 6) Let flask stand overnight at room temperature.
- 7) Prepare Whatman #54 filter paper in a conical funnel. Thoroughly wet paper with distilled water before adding any sample. Filter by gravity or with mild vacuum. If first filtrate is cloudy, return to the filter funnel and refilter. Separate filter flasks must be used so that any cloudy filtrate can be recycled through the funnel.
- 8) Wash residue twice with cold distilled water.
- 9) Transfer paper to kjeldahl flask and determine residual N.

Calculation:

$$\text{NPN (\% CP)} = \frac{\text{Total CP} - \text{Residual CP}}{\text{Total CP}} \times 100$$

Determination of Neutral Detergent Insoluble CP (ADICP)

Reference: Licitra, G., Hernandez, T. M. and Van Soest, P. J. 1995.
Standardization of procedures for nitrogen fractionation of ruminant feeds.
Anim. Feed Sci. Technol (Submitted).

Neutral detergent insoluble N (NDIN) or CP (NDICP) represents the protein associated with cell wall and insoluble in neutral detergent solution. It includes slowly degradable true protein (fraction B₃) and indigestible protein (fraction C). Buffer insoluble CP minus NDICP is used to estimate fraction B₂.

Reagents:

- 1) Neutral detergent solution.

Procedure:

- 1) Follow the procedure of NDF analysis as described earlier.
- 2) Filter through pre weighed # 54 filter paper (if NDF is desired).
- 3) Wash with hot water and then acetone until acid-free. Place paper in a pre tarred crucible.
- 4) Dry at 105 °C for overnight and weigh if NDF is desired.
- 5) Transfer paper to Kjeldahl flask and determine residual CP.

Calculation:

$$\text{NDICP (\% CP)} = \frac{\text{Residual CP}}{\text{Total CP}} \times 100$$

Determination of Acid Detergent Insoluble CP (ADICP)

Reference: Licitra, G., Hernandez, T. M. and Van Soest, P. J. 1995.
Standardization of procedures for nitrogen fractionation of ruminant feeds.
Anim. Feed Sci. Technol (Submitted).

Acid detergent insoluble N (ADIN) or CP (ADICP) represents the indigestible protein (fraction C). It is associated with lignin and is insoluble in acid detergent solution. Difference between NDICP and ADICP is used to estimate slowly degradable true protein (fraction B₃).

Reagents:

- 1) Acid detergent solution.

Procedure:

- 1) Follow the procedure of ADF analysis as described earlier.
- 2) Filter through preweighed # 54 filter paper (if ADF is desired).
- 3) Wash with hot water and then acetone until acid-free. Place paper in a pretared crucible.
- 4) Dry at 105 °C for overnight and weigh if ADF is desired.
- 5) Transfer paper to Kjeldahl flask and determine residual CP.

Calculation:

$$\text{ADICP (\% CP)} = \frac{\text{Residual CP} \times 100}{\text{Total CP}}$$

In Vitro Estimation of Protein Degradation

Reference: Roe, M. B., Sniffen, C. J. and Chase, L. E. 1990. Techniques for measuring protein fractions in feedstuffs. Proc. Cornell Nutr. Conf. p 81. Ithaca NY.

Soluble CP includes soluble true protein (fraction B₁) and non protein nitrogen(fraction A). Borate-phosphate buffer will be used to determine soluble CP.

Reagents:

1) Borate-phosphate buffer (pH 6.7).

NaH₂PO₄.H₂O 12.20 g L⁻¹.

Na₂B₄O₇. 10H₂O 8.91 g L⁻¹.

2) Protease solution (protease type XIV from *Streptomyces griseus*, Sigma Chemical Co.).

a. 0.33 units per mL in borate-phosphate buffer [1000 mL solution = 330 units required divided by 5.4 units mg solid (units of activity in the bottle) = 61.11 mg per 1000 mL buffer].

b. Filter through Whatman # 54 filter paper, use filtrate.

Procedure:

1. Determine CP content of the feed by Kjeldahl.
2. Weigh 0.2 g feed protein of air dry sample into a 125 mL Erlenmeyer flask.
3. Add 40 ml borate-phosphate buffer and incubate at 39 °C for 1 h.
4. Add 10 mL fresh protease solution and swirl slightly to mix in enzyme.
5. Remove at 18 h (concentrate) or 48 h (forages).
6. Filter through Whatman #54 filter paper.
7. Wash residues with 250 mL distilled water.
8. Estimate residual N by Kjeldahl.

Calculation:

$$\text{Degradable CP (\% CP)} = \frac{\text{Total CP} - \text{Residual CP}}{\text{Total CP}} \times 100$$

Subtract ADICP from undegradable CP to estimate CP available in the small intestine.