PROTEIN AND AMINO ACID DIGESTIBILITY IN CEREALS AND PROTEIN MEALS BY PIGS

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If dietary protein is to be used with maximum economy in ration formulation, there is a need for information on amino acid availability figures both in animal feedstuffs and for animal requirement.

At present it is difficult to meet either of these requirements with accuracy because of the difficulties involved in measuring aminoacid availability. Various methods have been used and each method is probably satifactory for determining relative availability of particular amino acids between feeds. Difficulties in choosing correct values appear when several different methods are used, and in many cases it would seem that the use of total amino acid content would be better than the figures for individual amino acids now in common use. The most commonly known published values for amino acid availability for a range of feeds are probably those presented by Feedstuffs (1976), but these appear unsuitable for practical use. Not only are the figures for the different feeds determined by different methods in different laboratories but also only one availability figure is used for all amino acids in each feed. The use of one figure, although simple, is incorrect and often misleading as the availability of different amino acids does vary. Methionine is considerably and consistently more digestible than threonine (Purser 1976); Holmes et *al* (1974) reported values of 93 and 74 percent digestibility for methionine and threonine respectively in rapeseed meal. Moreover, the differences between amino acids are not always consistent; Soares and Kifer (1971) determined amino acid digestibility by ileal analysis in chicks and found lysine in cottonseed meal only 48% digestible compared to an average of 65% for all other amino acids, whereas in soyabean meal, lysine digestibility of 82% was slightly greater than the mean, of 79%.

Amino Acid Digestibility

No method of assessing amino acid availability is without criticism or complication but of the methods used in vivo digestibility studies appear both the simplest and the most meaningful. The apparent digestibility of nitrogen or amino acids is determined by analysis of feed and of either faeces or ileal digesta. True digestibility (TD) is calculated by correcting the apparent digestibility for the amount of endogenous nitrogen or amino acids present either in faeces or ileal digesta, usually determined with animals fed a protein free diet.

Faecal analysis has been employed to determine amino acid digestibility many times since Kuiken and Lymen (1948) first used the technique. Ileal analysis has only more recently been used to determine amino acid digestibility since Payne *et al* (1968) suggested that recovery of amino acids from the ileum, before being subjected to modifications by microflora in the large intestine, might be a more sensitive index of protein quality than that obtained by faecal analysis.

For most amino acids, faecal digestibility values are generally higher than ileal values, indicating that the balance between amino acid degradation and synthesis reached by hind-gut bacteria favours degradation, even though the net disappearance of amino acids is variable.

Salter and Fulford (1974) suggested that, from the pattern of amino acids that disappear, much of the N disappearing in the hind-gut is of endogenous origin. This is supported by evidence that the hind-gut bacteria have a broader range of digestive enzymes than the host animal and can ferment certain mucopeptides (Mason and Palmer (1973)) and other compounds which the host animal's enzyme system cannot. In fact, recent work of Mason *et al* (1976) indicated that because of bacterial activity in the hind-gut of pigs, faecal digestibility figures are likely to be a poor estimate of amino acid absorption from the small intestine. They found that bacterial N contributed more than 50% to total faecal N and that of the total disappearance of a-amino N from the alimentary tract 11% disappeared from the caecum and colon. Moreover, bacterial activity can be modified by dietary treatments, e.g. availability of dietary energy, as has been shown by differences in VFA concentrations and molar proportions (Ly 1974) and by differences in the apparent digestibility of ct-amino N and most dietary amino acids (Mason et *al* 1976).

This is contrary to findings with chickens in which the presence of microorganisms in the alimentary tract has little or no effect on protein or amino acid digestibility (Salter and Coates 1971). However, microbial activity should have a greater influence on digestion in pigs than chicks because of the relatively long time digesta is exposed to microbial action. In pigs, digesta remains for 20-30h in the hind-gut alone whereas in poultry it takes only 8 - 9 h (Siregar - pers. comm.) for 90% of the digesta to pass through the entire digestive tract.

There is no convincing evidence that any of the a-amino N disappearing from the hind-gut is absorbed as such and it is likely that any changes in the amount or nature of the nitrogenous compounds in the hind-gut are of minor nutritional significance. If amino acids are not absorbed distal to the ileum, then ileal digestibility values truly represent amino acid absorption. How closely amino acid absorption reflects amino acid availability depends on the extent to which absorbed amino acids are utilized for maintenance or growth.

Discrepancies between amino acid absorption and availability can occur as a result of the reactions of amino acids during food processing (see review by Carpenter 1973). Even under mild conditions the E-amino group of lysine residues can react with reducing sugars, leading to reduced availability of this amino acid. The reaction produces a derivative (E - N - deoxyfructosyl - lysine) with no nutritional value but which is partly absorbed and excreted in the urine. Similarly, Varnish and Carpenter (1975) found that with propionylated lactalbumin the reduced digestibility of lysine is only partly accounted for by low lysine availability estimated by chick growth assay. However, with increased severity of heating (as might be encountered in processing conditions) the availability of all other amino acids is affected and this is associated closely with decreases in amino acid digestibility;

e.g. Varnish and Carpenter (1975) found close agreement between growth assay values and ileal digestibility values of amino acids in autoclaved muscle samples. In fact as a measure of lysine availability, digestibility can be better than estimates of reactive lysine; e.g. in cases where reactive lysine is trapped inside indigestible peptides formed with severe heating with a limited supply of reducing sugars. Also, under some conditions with severe heating, the ε - amino group of lysine may be blocked by the formation of various atypical peptides with asparigine or glutamine. The γ glutamyl - lysine dipeptide is absorbed intact through the intestinal wall and is utilized as effectively as L-lysine (Waibel and Carpenter 1972).

Miller (1976) suggested that a comparison of amino acid digestibility figures for commercial plant protein concentrates with amino acid availability figures determined by various growth bioassays indicated that indigestibility of amino acids is indeed the major cause of unavailability. Also, for cereals, amino acid indigestibility is almost certainly the major cause of unavailability. Even where toxins such as tannin are involved their deleterious effect on amino acid availability is due to inhibition of digestive enzymes and reduced amino acid digestibility (Eggum and Christensen 1975).

Cannulation Technique

A range of techniques for studying digestion and absorption of nutrients from the gut has been reviewed by Laplace (1972). Digestion and absorption are relatively easily followed in chicks and rats by slaughtering the animals and recovering segments of the gut. However, for pigs the best method at present for studying protein digestion is by continuous sampling of digesta from the conscious animal through cannulaeinserted in the gut. Simple (T-shaped) cannulae, through which discrete samples of digesta can be obtained, are particularly useful in perfusion type experiments with gut segments. However, with 'spot' sampling of digesta, total flow can only be assessed indirectly by reference to markers and where there is differential flow of the solid and liquid phases of the digesta (e.g. out of the stomach) an accurate marker system for pigs has not been reported. A dual isotope marker system is used to estimate digesta flow with ruminants fitted with simple cannulae (Faichney 1975) but even so the results are more variable than those obtained with re-entrant cannulae which allow total collection of digesta (MacRae and Ulyatt 1972).

Several workers have successfully cannulated the small intestine of the pig using re-entrant cannulae. Cunningham, Friend and Nicholson (1963) were among the first to use a two-piece, T-shaped re-entrant cannula. The surgical method for inserting this cannula involves transecting the intestine, inserting and fixing the cannulae near the stumps, then exteriorizing the cannulae through small stab wounds. During non-collection periods the cannulae are joined by soft tubing but for collection of digesta a bag or a tube leading to a collection flask is attached to the proximal cannula while return digesta is fed through tubing to the distal cannula. Using these cannulae at U.N.E., most pigs have survived at least six months and have usually allowed at least fifty collections periods. Similar success rates have been reported by some

others e.g. Holmes et *al* (1974). Blockage in the cannula is *a* persistant problem with this technique although we found its incidence very much reduced with finely ground meals.

Recently at U.N.E., Ivan (1974) developed a different type of cannula which seemed to have some advantages over those previously used. This was a single re-entrant cannula which, for insertion, required only one incision in the intestine rather than transection. The intestinal tube of the cannula was inserted into the gut lumen and a strip of graft material around the intestine at each end of the cannula secured the intestine to the cannula and directed the flow of digesta through the intestinal tube. The cannula was exteriorized through a single stab wound. During non-collecting periods a maintenance plug placed in the cannula confined the digesta to the intestinal tube of the cannula. Compared to the conventional re-entrant cannula the Ivan type re-entrant reduces the distance digesta must travel through the cannula and also reduces the disturbance of the cannula against the metabolism crate because only a small part of the cannula protrudes outside the body. The Ivan type cannula has proven successful for use in the duodenum and jejunum but in the ileum where the digesta is more viscous we have found the conventional two piece cannula more reliable. Although the two piece cannula may block more readily the blockage is more evident and easily cleared than with the Ivan cannula which, if left blocked, may damage the pig permanently.

In the course of our studies with cereals, eight pigs were fitted with re-entrant cannulae in the ileum about 50 cm from the ileo-caecal junction, i.e. at about 97% of the total length of the small intestine. Three pigs did not recover from surgery but the remaining five animals were used in all planned collections.

Initially it was considered more practicable to feed pigs on a 12 hourly basis and to collect digesta over 12 h periods rather than 24 h periods. To test the effect of different feeding and collection periods on digestibility, four pigs were fed a wheat diet either in a single 2 kg meal/day and 24 h collections made, or as two 1 kg meals/day and 12 h collections made both day and night.

The disturbance to normal gut function caused by the continuous collection and return of digesta, can cause a reduction in the total flow of digesta. To correct for this, chromic oxide, Cr_2O_3, was included as an indigestible marker in all diets and its flow through the ileum was corrected to Cr_2O_3 recovery in the faeces which were collected over four days at the end of each period. Holmes, Bayley and Horney (1973) found recovery ratios for Cr_2O_3 at the ileum ranged from 64 *to* 112% with a mean of 90.3%; for the recovery ratios so far determined, our results appear similar to those of Holmes, Bayley and Horney (1973).

The Cr_2O_3 recovery ratios for this trial are not yet determined, but for these results (Table 1) the corrections are unlikely to influence the relative values.

TABLE 1. Apparent dry matter digestibility measured at the terminal ileum of a wheat diet determined by either 24 h or 12 h night or day collection periods.

Collection period.		Pigs Winnie	Moshe	Harry	Roger	Mean
24 h	A.	79.2	79.7	80.6	79.3	
	B.	77.5	79.8	80.9	78.7	
	mean	78.4	79.8	80.9	79.0	79.5
12 h Day	A.	78.7	74.6	79.0	78.5	
	B.	78.6	79.1	80.4	79.3	
	mean	78.7	76.9	79.7	78.9	78.5
Night	A.	74.5	75.6	77.4	76.5	
	B.	78.9	79.1	78.8	78.6	
	mean	76.7	77.3	78.1	77.6	77.4

Although most workers (e.g. Braude, Fulford and Low, 1976) reported considerable variation in flow rates and digestibility between pigs, there was surprisingly small variation between our pigs in these uncorrected data. The results indicated little difference in digestibility values between 12 h day and 24 h collections. In all other experiments pigs were therefore fed at 12 h intervals and all collections were made between feeds at 7 a.m. and 7 p.m.

Digesta was collected into small plastic bags attached to the proximal cannula. As each bag was filled with digesta it was replaced, weighed and stored on ice and a similar amount of warmed digesta was returned through the distal cannula. At the end of each three hour period the digesta samples were bulked and a 10% sample retained for analysis; the remaining digesta was then warmed to 37°C for later return.

Protein and Amino Acid Digestibility in Cereals

Most values of protein and amino acid digestibility for cereals have been derived by faecal analysis with rats, chicks and pigs. The values reported by various workers differ considerably, e.g. reported values for lysine digestibility in wheat range from 92.8% (Kuiken and Lymen 1948) to 67.3% (Sauer, Giovanetti and Stothers 1974), but the relative differences between cereals and between amino acids are generally consistent. Furthermore, Eggum (1973) found close agreement between digestibility coefficients determined with rats and those in young pigs.

Eggum (1975) studied a range of cereal grains and found that TD of protein varied widely, from 99.3% in rice to 77% in rye. The variation between other more common feed grains was less, but never-

theless considerable (Table 2), with Triticale protein the highest (92%) and 10% better than barley protein. The digestibilities of the individual amino acids vary in a similar manner but, whereas with a mixed food Just Nielsen (1968) found that the digestibility coefficients of most amino acids (particularly lysine) were similar to that of N, for cereals the TD of individual amino acids can differ markedly from the TD of N. Lysine is usually the least digestible and glutamic acid or proline the most digestible amino acid in cereal grains. Digestibility values determined by ileal analysis are as anticipated, lower than these figures derived by faecal analysis (Table 2).

TABLE 2. The digestibility by pigs of protein, lysine and glutamic acid in cereal grains.

Grain	Protein	Lysine	Glutamic acid
Faecal analysis- TD			
Wheat+	91.8	84.1	97.4
Barley+	82.4	72.3	90.4
Sorghum+	85.3	71.5	88.1
Maize+	90.2	89.3	92.2
Oats+	78.6	73.0	88.9
Triticale++	92.0	86.3	94.5
Ileal analysis - apparent digestibility			
Wheat°- hard	-	77.7	93.8
- soft	-	64.4	93.4
Maize°°	70.7	-	-

+ Eggum (1973) ; ++ Sauer *et al* (1974); ° Ivan (1974); °°Holmes, Bayley and Horney (1973).

Factors Affecting Amino Acid Digestibility in Grains

Cereal protein contains four major fractions - two of which constitute the protein from the endosperm (storage proteins) and two which are present mostly in the outer parts of the seed, mainly in the aleurone cell layer. The digestibilities and amino acid composition of the protein fractions vary considerably, characterized by the highly digestible storage proteins that are rich in glutamic acid and proline but poor in lysine and other basic amino acids, and proteins in the aleurone cells which are poorly digested but contain a relatively high concentration of lysine. The amino acid composition of the protein fractions tends to be similar for all grains, the amino acid content of a grain being determined largely by the proportions of the protein fractions in the grain. These proportions are determined genetically (e.g. one of the storage proteins, prolamin, makes up 50-55% of corn protein but only 10-15% of oat protein) but are also influenced by various factors during maturation. Generally, the total amount of protein from the structural protein fractions remains constant and the proportions are altered by varying amounts of endosperm protein in the seed. Thus, the storage proteins generally

make up a larger proportion of the total protein in a high protein cereal than a low protein cereal grown under similar conditions. As this fraction is a poor source of lysine, but is highly digestible, the higher protein grain generally has more digestible protein but the protein is of poorer quality (lower biological value).

One part of our cereal study is a comparison of protein and amino acid digestibility between wheats, two sound (wheats 1 and 2) and two off-grade samples (wheats 3 and 4). The contents of protein, lysine, threonine and glutamic acid are presented in Table 3. An analysis of the protein fractions in the grains is not yet available, but the lower proportions of lysine and higher proportions of glutamic acid in the total protein of the sound wheats suggests that they contain a greater proportion of the storage proteins than the off-grade wheats. Protein and amino acid digestibility has been assessed by ileal analysis both with rats and with pigs but only the rat data have been completely analysed.

The rats were fed each wheat at four levels of inclusion (100, 75, 50, and 25%) as the sole source of N in the diet and ileal samples were recovered following slaughter at 3½ h after feeding. The levels of N and lysine remaining in the ileum were related to dietary intake by the use of a marker concentration (Cr^{51}EDTA) and plotted against N or lysine concentration in the diet (Figs. 1 and 2). The slope of the lines is the true indigestibility of N or lysine, the intercept representing the endogenous level in the ileum. Significant linear regressions were established for each of the grains and for three of them the intercepts were close together and similar to the value determined by feeding a N-free diet. However, with one of the wheats the intercept was well below the others. For comparison, TD of protein and lysine for each wheat was also determined by the conventional method using the values obtained from rats fed the 100% wheat diets and the N-free diet. For lysine these digestibility values tend to be slightly less than by the regression method, while for N they tend to be slightly higher (Table 3).

TABLE 3. The contents of protein and some amino acids and the true digestibilities of N and lysine in four wheat samples.

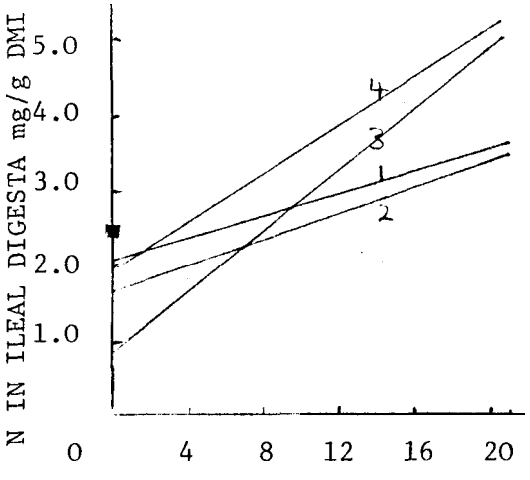
Wheat sample		1	2	3	4
Protein (Nx5.7) %		12.9	13.3	9.7	9.4
Lysine (mg/g protein)		27.9	29.4	33.0	36.2
Threonine (mg/g protein)		34.2	36.1	37.2	30.8
Glutamic acid (mg/g protein)		316.7	356.9	289.0	265.8
TD^{+} lysine %	a.	88.2	91.9	75.2	82.5
	b.	86.9	89.3	81.2	78.8
N %	a.	92.3	91.2	80.2	84.0
	b.	93.9	92.9	88.2	85.0

$^{+}$True digestibility determined by a. regression method and b. direct method.

The results show a difference in digestibility, particularly of lysine, between the sound, higher protein wheats and the poorer, lower protein wheats; the lysine in the poorest wheat was 10% less digestible than that in the best. However, although the lysine in the lower protein wheats is less available and the amount of available lysine per kg of grain is less (3.1, 3.5, 2.6, and 2.7 g available lysine/kg grain for wheats 1, 2, 3, and 4 respectively), the amount per kg of grain protein is slightly greater (24.2, 26.3, 26.8, and 28.5 g available lysine/kg grain protein for wheats 1, 2, 3, and 4 respectively) than in the higher protein grains.

The tendency in our results for N content to be positively related to protein digestibility is supported by Eggum and Christensen (1975) who measured protein digestibility in 29 barley samples (by faecal analysis) and found that N digestibilities

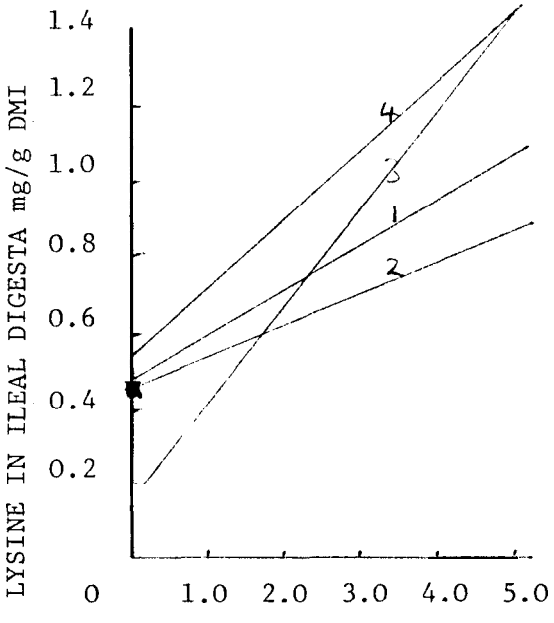
Figure 1. Relationship between N level in diet and N level in the ileum of rats fed diets containing wheats 1, 2, 3 and 4 and N-free diet (■)



N in diet mg/g DM

WHEAT NO.
1. Y=0.077X + 2.04
2. Y=0.088X + 1.61
3. Y=0.198X + 0.88
4. Y=0.160X + 1.93

Figure 2. Relationship between lysine level in diet and lysine level in the ileum of rats fed diets containing wheats 1, 2, 3 and 4 and N-free diet (■)



Lysine in diet mg/g DM

WHEAT NO.
1. Y=0.118X + 0.47
2. Y=0.081X + 0.45
3. Y=0.249X + 0.16
4. Y=0.175X + 0.54

ranging from 82.1 to 90.6% were positively correlated with N content. In the same experiment barley protein digestibility showed a significant negative correlation (r = -0.31) with tannin content. The tannin content of the barleys ranged from 0.55 to 1.23%. They found that additions of 0.5 and 1.5% tannin to a basal soya-bean meal diet reduced the TD of protein from 93 to 81 and 73% respectively. The TD of all amino acids in the diet were also reduced.

Sorghum also contains significant amounts of tannin and the apparent digestibility of N in high-tannin sorghums was considerably less than in low tannin sorghums, 49 and 80% respectively, and tannin extraction from the high tannin variety was found to increase digestibility to 77% (Featherston and Rogler 1975). Stephenson et *al* (1971) reported wide variation in amino acid digestibility in sorghums (variation in lysine digestibility from 58.3 to 92.5%) which is probably largely due to differences in tannin content.

Thus, for a number of reasons, amino acid availability does vary widely both between types of cereal grains and even within types of grain. To facilitate the broader monitoring of amino acid availability in grains, a further aspect of our work is involved in relating our TD values for amino acids to various laboratory estimates of amino acid availability. The TD of amino acids in barley, sorghum, maize, Triticale and the wheats (determined by both ileal and faecal analysis with pigs) will be related to estimates of amino acid availability determined by in vitro digestibility studies, as well as estimates of reactive lysine including the Silcock method and dye-binding techniques.

Certainly in the future, as the world demand for all food resources increases and the cereals available for pigs are increasingly poorer quality, differences in amino acid availability will have to be recognized. But, even now, differences in lysine availability in grains that can range from 50 to 90% (in sorghums) must be recognized in diet formulation if dietary protein quality is to be assured.

Protein and Amino Acid Digestibility in Protein Concentrates

Differences in amino acid availability are not usually any larger between protein concentrates than between cereals but they are more widely recognized because the proportion of their protein in the diet is greater.

Eggum (1973) found the digestibility of amino acids in protein concentrates was generally greater than in cereals, although our results of amino acid digestibility in protein concentrates are much lower than those reported by Eggum (1973). The digestibilities of N, lysine and threonine in some protein concentrates are presented in Table 4.

TABLE 4. N, lysine and threonine digestibility in some protein concentrates.

Protein source	Reference	Crude protein (%)	Digestibility of N (%)	lysine (%)	threonine (%)
Soyabean meal	1	48.3	79.9	90.7	82.2
	2	46.2	77.2	78.5	81.6
	3	51.3	90.2	91.7	89.6
Meat meal	2	52.3	61.1	76.9	73.0
	3	55.1	85.5	85.7	82.6
Fish meal	2	50.2	63.8	74.9	80.0
	3	78.9	93.2	95.6	95.3
Peanut meal	2	44.7	85.5	83.2	93.8
	3	53.2	91.2	88.1	86.9
Sunflower meal	2	36.0	78.0	71.6	83.2
	3	40.9	90.4	86.9	89.9
Rapeseed meal	1	34.5	67.7	81.3	73.5
Faba beans	4	36.3		81.7	75.3

+
1. Holmes *et al* (1974) - apparent digestibility, ileal analysis.
2. Alimon (present study) - apparent digestibility, ileal analysis.
3. Eggum (1973) - true digestibility, faecal analysis.
4. Ivan and Bowland (1976) - true digestibility, ileal analysis.

The variation in amino acid digestibility between experiments for each protein concentrate reflects not only the differences between the methods of estimating digestibility (i.e. TD estimated by faecal analysis compared to apparent digestibility estimated by ileal analysis), but also the extent of variation that can exist within concentrates. Differences in lysine availability of up to 10% have been reported before for meat meals, but 20% differences in lysine availability between fish meals is unusual. A recent survey of fishmeals available in Britain found so little variation in lysine availability that it was suggested total lysine values would adequately predict nutritional value (Carpenter and Woodham 1974). This is apparently not the case in Australia where some particularly low protein fish meals are available which are only poorly digested.

References

Braude, R., Fulford, R.J., and Low, A.G. (1976) - Studies on digestion and absorption in the intestines of growing pigs. Measurements of flow of digesta and pH. Br. J. Nutr. 36 : 497-510.

Carpenter, K.J. (1973) - Damage to lysine in food processing: its measurement and significance Nutr. Abstr. Rev. 43 : 424.

Carpenter, K.J., and Woodham, A.A. (1974) - Protein quality of feeding-stuffs. 6. Comparisons of the results of collaborative biological assays for amino acids with those of other methods. Br. J. Nutr. 32 : 647-660.

Cunningham, H.M., Friend, D.W., and Nicholson, J.W.G. (1963) - Observations on digestion in the pig using a re-entrant intestinal fistula. Can. J. Anim. Sci. 43 : 215-225.

Eggum, B.O. (1973) - A study of certain factors influencing protein utilization in rats and pigs. Beretn. 406. Forsøgslab. Copenhagen, Denmark.

Eggum, B.O., and Christensen, K.D. (1975) - Influence of tannin on protein utilization in feedstuffs with special reference to barley. In "Breeding for Seed Protein Improvement using Nuclear Techniques". Internat. Atomic Energy Agency, Vienna.

Faichney, G.J. (1975) - The use of markers to partition digestion within the gastro-intestinal tract of ruminants in Digestion and Metabolism in the Ruminant edit. I.W. Mc Donald and A.C.I. Warner (U.N.E. Publishing Unit) p.277.

Featherston, W.R. and Rogler, J.C. (1975) - Influence of tannins on the utilization of sorghum grain by rats and chicks. Nutr. Reports Internat. 11 : 491-497.

Feedstuffs (1976) - Yearbook 46

Holmes, J.H.G., Bayley, H.S. and Horney, F.D. (1973) - Digestion and absorption of dry and high-moisture maize diets in the small and large intestine of the pig. Br. J. Nutr. 30 : 401-410.

Holmes, J.H.G., Bayley, H.S., Leadbeater, P.A. and Horney, F.D. (1974) - Digestion of protein in small and large intestine of the pig. Br. J. Nutr. 32 : 479-489.

Ivan, M. (1974) - A nutritional evaluation of wheat for pigs. Ph.D. thesis, U.N.E.

Ivan M. and Bowland, J.P. (1976) - Digestion of nutrients in the small intestine of pigs fed diets containing raw and autoclaved faba beans. Can. J. Anim. Sci. 56 : 451-456.

Just Nielsen, A. (1968) - Digestibility of amino acids in relation to digestibility of nitrogen in bacon pigs. Royal Veterinary and Agricultural College, Copenhagen, Denmark, Yearbook p. 79-88.

Kuiken, K.A. and Lyman, C.M. (1948) - Availability of amino acids in some foods. J. Nut. 36 : 359.

Laplace, J.P. (1972) - Le transit digestif chez les monogastriques. 1. Les techniques d'etude. Ann. Zootech. 21 : 83-105.

Ly, J. (1974) - Caecal function in the pig : VFA content and utilization by the caecal wall, Cuban J. Agric. Sci. 8 : 247-254.

MacRae, J.C. and Ulyatt, M.J. (1972) - Comparison of spot and continuous sampling for estimating duodenal digesta flow in sheep. N.Z.J. Agric. Res. 15 : 98-106.

Mason, V.C., Just, A. and Beth-Anderson, S. (1976) - Bacterial activity in the hind-gut of pigs. 2. Its influence on the apparent digestibility of nitrogen and amino acids. Z. Tierphysiol., Tierernahrg. U. Futtermittelkde 36 : 310-324.

Miller, E.L. (1976) - Amino acid availability from plant protein : plant protease inhibitors, effects of heat treatments of proteins. in "From Plant to Animal Protein" edit. T.M. Sutherland J.R. McWilliam and R.A. Leng (U.N.E. Publishing Unit) p. 47.

Purser, D.B. (1976) - Protein digestion and amino acid absorption in "From Plant to Animal Protein" edit. T.M. Sutherland, J.R. McWilliam and R.A. Leng (U.N.E. Publishing Unit) p. 39.

Salter, D.N. and Coates, M.E. (1971) - The influence of the microflora of the alimentary tract on protein digestion in the chick. Br. J. Nutr. 26 : 55-69.

Sauer, W.C., Giovannetti, P.M. and Stotters, S.C. (1974) - Availability of amino acids from barley, wheat, triticale and soybean meal for growing pigs. Can. J. Anim. Sci. 54 : 97-105.

Soares, J.H. and Kifer, R.R. (1971) - Evaluation of protein quality based on residual amino acids of the ileal contents of chicks. Poult. Sci. 50 : 41-45.

Stephenson, E.L., York, J.D., Bragg, D.B. and Ivy, C.A. (1971) - The amino acid content and availability of different strains of grain sorghum to the chick. Poult. Sci. 50 : 581-584.