

NUTRITIONAL IMPLICATIONS OF HEATING PROTEINS:
THE EFFECT OF HEATING PROTEIN CONCENTRATES ON THE DIGESTIBILITY
AND METABOLISM OF LYSINE IN GROWING PIGS

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SUMMARY

The effects of heating proteins, mechanisms of heat damage, and techniques for assessing the effect of heat on amino acid utilisation are unclear. Recent research suggests that a considerable proportion of the ileal digestible lysine from heat-processed meals is apparently absorbed from the digestive tract of the growing pig, but is inefficiently utilised. The reason for this is unclear. Accordingly, a series of experiments were undertaken to define the relationship between the effect of heating proteins and the ileal digestibility, availability and utilisation of lysine. Once these relationships were defined, attempts were made to identify the biochemical mechanisms underlying these responses. Field peas were chosen as the model protein concentrate for these studies with heat applied at levels of 110°, 135°, 150° or 165° for 15 min respectively using a forced-air dehydrator. The results from this series of experiments suggest 1) Heat applied to field peas, even at mild temperatures of 110°, renders lysine in a form that is apparently absorbed from the gastro-intestinal tract of the pig, but is poorly utilised; 2) Ileal digestibility values for lysine in heat-processed meals are unsuitable for use in diet formulations; 3) Lysine availability values determined using the slope-ratio assay are a close reflection of lysine utilisation and therefore are suitable for use in diet formulations; 4) Some of the poorly utilised ileal digestible N from heated field peas may be excreted as intact protein in the urine.

INTRODUCTION

Why heat protein concentrates used in pig diets ?

There is considerable variation in the motive for applying heat to protein concentrates, and hence, protein nutritional quality can vary substantially. Many protein concentrates of plant origin used in pig diets are processing by-products. Oil-bearing seeds such as soybean, linseed, rapeseed, sunflower and peanuts are primarily produced for vegetable oils for human foods or for paints and other industrial purposes. Hence, heat applied to these proteins is a consequence of processing to remove oil, rather than for reasons to improve the protein nutritional quality. Similarly, in the processing of meat meals, meat and bone meals and blood meals, heat is used to remove moisture, aid in fat extraction, and reduce microbial contamination of the meal.

Some protein concentrates are heated with the aim of improving their protein nutritional quality. Legumes, in particular, require heat to inactivate anti-nutritional factors such as lectins and protease inhibitors (Liener 1980). Likewise, the nutritional value of cottonseed meal is improved with heating that results in the binding of gossypol (Ascarelli and Gestetner 1962). In these cases, however, protein nutritional quality is often

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measured in terms of digestibility only, with little consideration given to amino acid availability and utilisation.

Implications of using heated proteins in pig diets

To date, the ileal digestibility of amino acids has been used **widely** in diet formulations in an attempt to match, as closely as possible, diet specifications to the pigs requirements, and hence optimise pig production. Recent research suggests, however, that a considerable proportion of ileal digestible lysine from heat-processed meals is apparently absorbed from the digestive tract of the growing pig in a form that is inefficiently utilised (Bellaver and Easter 1989; Batterham et al. 1990a; Moughan et al. 1991; Wiseman et al. 1991). For example, Batterham et al. (1990a) reported that 0.76 of the ileal digestible lysine was retained in the empty-body of pigs given soybean meal, whilst only 0.36 was retained by pigs given cottonseed meal. In addition, in a comparative study of ileal digestibility and availability of lysine, Batterham et al. (1990b) reported that ileal digestibility and availability values were similar for soybean meal, but for cottonseed meal, ileal digestibility values were considerably higher than availabilities. Subsequent testing of these values for lysine availability confirmed their applicability in diet formulations. Based on these findings, a hypothetical relationship between the ileal digestibility, availability and utilisation of lysine in heat-processed meals is presented in Fig. 1.

Due to the variable conditions inflicted upon cottonseed meal and other protein concentrates during processing, there is considerable uncertainty as to whether heat alone is responsible for the poor utilisation of ileal digestible lysine reported by Batterham et al. (1990a), Wiseman et al. (1991) and Moughan et al. (1991). To clarify this, the aim of this series of experiments was to apply graded levels of heat to a model protein and examine 1) total lysine composition, 2) ileal digestibility of lysine, 3) utilisation of ileal digestible lysine and 4) availability of lysine using the slope-ratio analysis. Having defined this relationship, attempts were then made to identify some mechanisms of heat-damage in proteins.

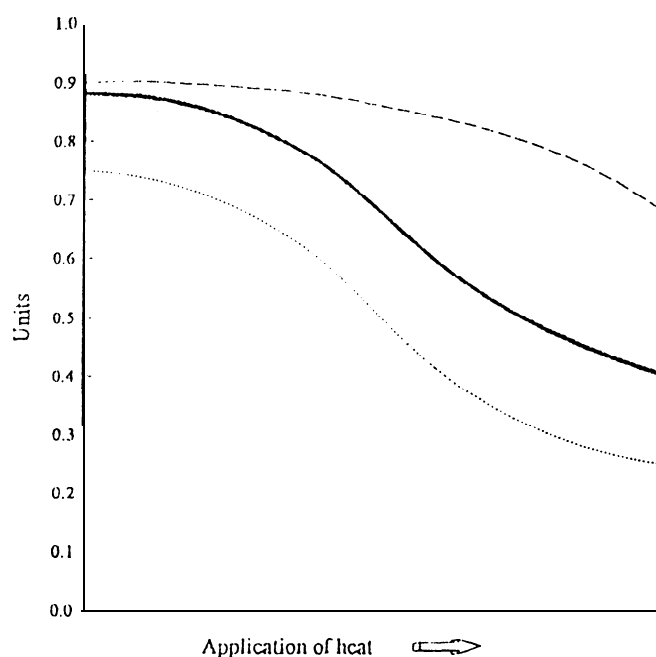


Fig. 1 Hypothetical relationship between the ileal digestibility (-----), availability (—), and utilisation (····) of lysine in heat-processed proteins

PROTEIN CONCENTRATES AND HEAT TREATMENTS

Field peas (*Pisum sativum* cv Dundale) were used as the protein concentrate. They are a good quality protein source in terms of digestibility and amino acid balance, and are free from most anti-nutritional factors, with the exception of low levels of **anti-tryptic** factors (Saini and Batterham 1989). They can therefore be fed raw following coarse crushing and provide an unheated control. Meals such as soybean meal, cottonseed meal, fish meal, meat and bone meal etc. are unsuitable for this work as they are all heated during preliminary processing, and thus the extent of heating prior to feeding is unknown.

Graded levels of heat at 110°, 135°, 150°, or 165° were applied to batches of field peas and maintained for 15 min respectively using a forced-air dehydrator. Dry heat was applied so that the effects of heat alone could be investigated, without influences from pressure or moisture associated with autoclaving and similar processes. The selected levels of heat application were chosen for the following reasons:

- 1) They are indicative of the levels of heat that can be obtained during the processing of commercial meals used in pig diets;
- 2) Heat application at 150° and 165° is likely to be sufficient to result in a significant reduction in the ileal digestibility of amino acids (Jagger 1987);
- 3) The heat levels should provide the field pea treatments with lysine availabilities ranging from 0.90 down to 0.30, similar to that reported in commercial meals by Batterham et al. (1984).

Heating was completed in 140 kg batches with 180, 270, 330 and 450 min required to reach 110°, 135°, 150° and 165° respectively. Any variation between heating batches was eliminated by mixing all batches within a heat treatment in a vertical mixer prior to rebagging and hammer-milling.

EFFECT OF HEAT ON THE TOTAL LYSINE COMPOSITION OF FIELD PEAS

Amino acids in the field peas were separated by ion-exchange chromatography. Analysis was completed in duplicate following hydrolysis at 110° for 24 h with constant boiling point hydrochloric acid (HCl) under nitrogen. The influence of heat on the reactive lysine content of heated peas was measured in a single analysis using the **Silcock**-reactive lysine assay (Roach et al. 1967).

The application of heat at 150° and 165° resulted in a 14 and 40% drop in total lysine respectively (Fig.2). These decreases are likely to be due to early and advanced **Maillard** reactions. That is, during heating some of the lysine in the peas heated to 150° and 165° is likely to have bound with a carbohydrate in the field peas to form a new compound (eg. Fructoselysine; Erbersdobler et al. 1989).

The Silcock-reactive lysine assay revealed that up to 25% of the total lysine remaining in the field peas heated to 165° had undergone some form of binding at the --amino group (Fig. 2) and therefore is thought to be unavailable.

Despite revealing that at least 55% of the lysine in the peas heated to 165° is not chemically available, current techniques used to measure total amino acids, 'reactive'

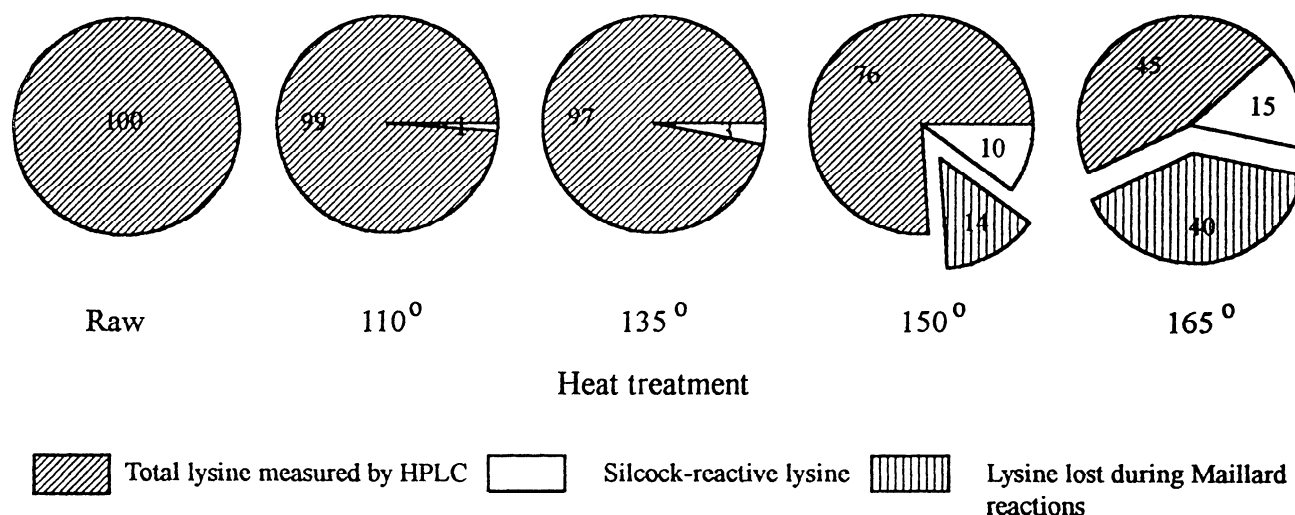


Fig. 2 Effect of heat on the total lysine content and proportion of Silcock-reactive lysine in field peas

amino acids and to identify heat-induced compounds have not been able to estimate the biological availability of amino acids in heated proteins (Varnish and Carpenter 1975; Batterham et al. 1979, 1981, 1986). It should be noted, however, that even if heat induced bonds and compounds could be identified, quantified and shown to influence availability, it is likely that there would be numerous variations in bond configuration or compound formation depending on the starting composition of the protein concentrate. As a consequence, the relationship between chemical changes within a protein source and biological availability would be very difficult to establish. There is a need to identify a suitable biological/animal response for the rapid, accurate determination of amino acid availability.

EFFECT OF HEAT ON THE DIGESTIBILITY, AVAILABILITY AND UTILISATION OF LYSINE

A series of experiments were conducted to define the relationship between heating proteins and the ileal digestibility, availability and utilisation of lysine.

The apparent ileal digestibility of amino acids in field peas was determined in pigs fitted with simple 'T'-piece cannulas and by direct ileal sampling. The respective field pea treatments represented the only source of amino acids in sugar-based diets. Chromic oxide was included in the diets as an indigestible marker. Results for lysine are presented in Table 1.

Table 1 The ileal digestibility of lysine in raw peas and peas heated to 110°, 135°, 150° or 165° for 15 min respectively

	Heat treatment				
	0°	110°	135°	150°	165°
Lysine digestibility (%)	92	97	92	93	84

Growth experiments were then conducted to determine the effect of heat on the utilisation of ileal digestible lysine from field peas fed to growing pigs. Five lysine-deficient sugar-based diets (0.36 g ileal digestible lysine/MJ DE) were formulated using

the raw field peas and field peas heated to 110°, 135°, 150° or 165° respectively. Additional diets were formulated with supplements of free lysine to verify that lysine was limiting in the diets containing the **raw** peas, and peas heated to 150° or 165°. The growth performance and retention of ileal digestible lysine by pigs given the diets was determined over the 20-45 kg growth phase prior to slaughter and empty-body analysis. The results were as follows:

Table 2 The effect of formulating diets containing raw and heated field peas on an apparent ileal digestible lysine basis on growth performance, protein deposition and lysine retention in growing pigs (20-45 kg liveweight)

Response	Heat treatment				
	0°	110°	135°	150°	165°
Gain (g/d)	498	482	477	450	315
Food conversion ratio	2.6	2.7	2.7	2.9	4.5
Protein deposition (g/day)	76	69	68	61	36
Lysine retained: ileal digestible lysine intake	0.85	0.65	0.68	0.63	0.45

The effect of heat on the availability of lysine in field peas was determined using the slope-ratio assay with growing pigs. To apply the slope-ratio assay, wheat-based experimental diets were formulated to contain graded levels (625 mg increments) of standard (free) lysine and graded levels of test lysine (that is, lysine from raw and heated field peas). In addition, the diets were equalised for fibre and digestible energy (DE), and attempts were made to maintain an equal amino acid balance across all diets. The dose levels of lysine from the test proteins were formulated to provide the same total lysine as that from the standard lysine doses, so that the estimates of lysine in the test doses were an expression of lysine availability. Linear regression coefficients of response (for example, FCE on an empty-body basis) to increasing dose level of standard lysine and lysine from the test proteins were calculated. Providing all statistical criteria were met, an estimate of lysine availability in the test protein was determined by calculating the ratio of linear regression coefficients, lysine in test protein : standard lysine. The following availability values were determined:

Table 3 The availability of lysine in raw field peas and peas heated to 110°, 135°, 150° or 165° for 15 min respectively

	Heat treatment				
	0°	110°	135°	150°	165°
Lysine availability (%)	96	71	77	56	47

The relationship between the ileal digestibility, availability and utilisation of lysine from heat field peas is presented in Fig. 3. Heat had little effect on the ileal digestibility of lysine in field peas. The application of heat to field peas at 110° increased lysine digestibility from 92 to 97%. It was not until the peas were heated to 165° that digestibility fell to 84%. The initial increase in digestibility suggests that at 110°, protein structure was modified in such a **way** that lysine was more susceptible to **enzymic** attack, with the reverse being true for the higher heat treatments.

Despite only small changes in ileal digestibility, the utilisation of ileal digestible lysine was substantially reduced, even at mild temperatures of 110°. As a consequence, it can be concluded that:

- 1) Heat applied to protein concentrates appears to render lysine in a form that is apparently absorbed from the gut of the growing pig, but inefficiently utilised;
- 2) Ileal digestibility values for lysine in heat-processed meals are unsuitable for use in diet formulations.

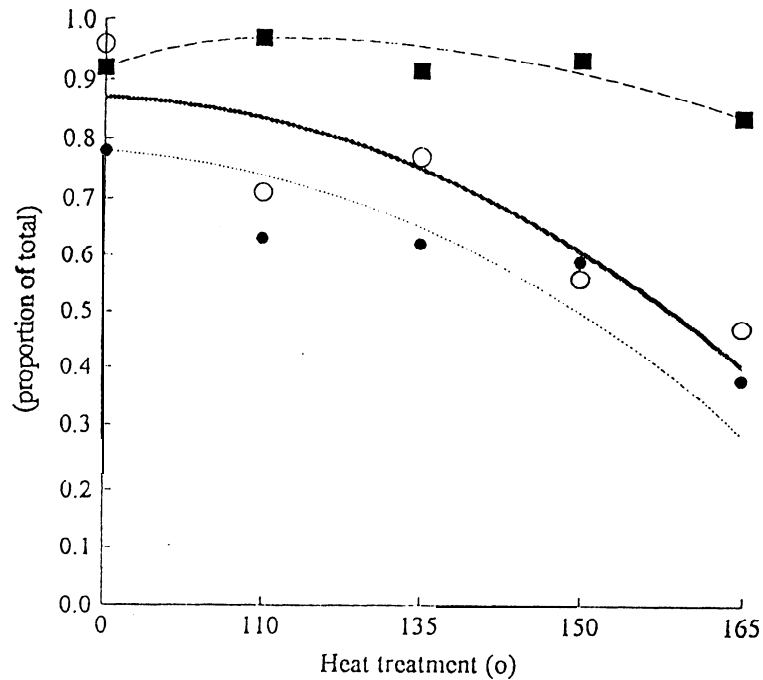


Fig. 3 Relationship between lysine digestibility (■---■), availability (○—○) and utilisation (●....●) in raw field peas and field peas heated to 110°, 135°, 150° or 165° for 15 min respectively using a forced-air dehydrator

The above conclusions are in agreement with the initial hypothesis (Fig. 1), however, significant reductions in the utilisation of ileal digestible lysine were not expected at temperatures as low as 110°. This has considerable implications if we consider that 110° is frequently achieved during commercial processing of proteins. The current results suggest that even the mild processing of say, soybean meal, may be inducing a reduction in the utilisation of ileal digestible lysine. Despite the motive for processing, there is a need to define the point at which digestibility and availability of amino acids and the reduction in anti-nutritional factors is maximised, and the economic implications of adjusting commercial processing systems to meet this level.

In contrast to ileal digestibility, estimates of lysine availability determined with the slope-ratio assay were a close reflection of the utilisation of ileal digestible lysine, and hence are more-suitable for use in diet formulations. The slope-ratio assay, however, is unsuitable for use in routine analysis for estimating amino acid availability due to the time and expense involved. A rapid, inexpensive and efficient amino assay is required. Such an assay may be developed if we can gain an understanding of the biochemical mechanisms associated with the poor utilisation of ileal digestible lysine from heated proteins.

THE FATE OF POORLY UTILISED ILEAL DIGESTIBLE LYSINE

Having defined the relationship between digestibility, availability and utilisation of lysine from raw and heated field peas, the nitrogen balance and urine, serum and plasma composition of growing pigs was studied. This was an attempt to identify biochemical pathways by which poorly utilised ileal digestible lysine was lost. Due to the fact that ileal digestible lysine was not retained in the empty-body, it was hypothesised that it was being excreted in the urine. In addition, it was thought that the site of loss of these **non-**utilisable compounds might be more easily identified if overall nitrogen metabolism was examined, rather than the fate of a single amino acid.

The **Dundale** cultivar of field peas was used in all previous experiments to define the relationship between digestibility, availability and utilisation, but as these supplies were diminished, the **Wirrega** cultivar was substituted for this nitrogen balance experiment. It was assumed that no major differences in ileal digestibility existed between these two cultivars, and that the chosen heat treatments would induce similar changes in chemical composition. There were sufficient peas to allow a single treatment of cv **Dundale** peas heated to 150° to be included for direct comparison with the **Wirrega** cultivar.

This experiment used raw field peas (cv **Wirrega**) and field peas heated to 150° (cv **Wirrega** and cv **Dundale**) or 165° (cv **Wirrega**). Sugar-based diets were formulated to contain 1.15 g ileal digestible nitrogen/MJ DE and 0.36 g ileal digestible lysine/MJ DE with an equal balance of all other essential amino acids based. Diet formulations were based on digestibility estimates for the **Dundale** cultivar determined previously. Total urine and faeces collection from pigs was conducted over 7 d collection periods with a 7 d diet adaption period. Pigs were fed frequently (3 hourly) to ensure full utilisation of the dietary amino acids (Batterham and Murison 1981). Serial blood sampling from the external jugular vein was conducted on the final day of each collection period.

Table 4 Nitrogen intake (g/d), urinary and faecal N output (g/d) and N balance for pigs fed diets containing raw field peas or field peas heated to 150° or 165°

Cultivar	Heat treatment				Statistics	
	0° Wirrega	150° Wirrega	165° Wirrega	150° Dundale	Treatment	SEM
<i>Intake</i>						
N intake	36.33	39.74	51.75	40.06	**	2.402
<i>Urine</i>						
Urine N	10.40	9.29	6.18	12.54	*	1.378
<i>Faeces</i>						
Faeces N	2.95	6.18	26.17	7.37	***	0.947
<i>N balance</i>						
Balance	22.98	24.26	19.40	20.15	NS	2.092

This experiment did not reveal any difference in the nitrogen balance of pigs fed raw and heated peas which is in direct contrast to previous growth studies. It appears that variability associated with this N balance experiment, due to only four replicates, may have reduced the sensitivity of this comparison.

Analysis of blood parameters revealed a significant decrease in serum urea levels for pigs fed peas heated to 165° (Fig. 4). In addition, urine nitrogen levels were also depressed in the diets of pigs fed heated peas. This was in contrast to our original hypothesis which suggested that poorly utilised nitrogen and amino acids may be deaminated at a higher rate and therefore increase blood and urine urea. An explanation for this response was that the ileal digestibility of nitrogen and lysine in the Wirrega cultivar of peas heated to 165° had been overestimated. A subsequent digestibility experiment revealed that cv Wirrega peas heated to 165° had a lysine digestibility of 35% compared to 84% for cv Dundale peas heated to 165°. As a result, the diets used in the above experiment did not contain equal levels of ileal digestible lysine and nitrogen. This result emphasises the way the effects of heat can vary depending on the starting composition of the protein concentrate.

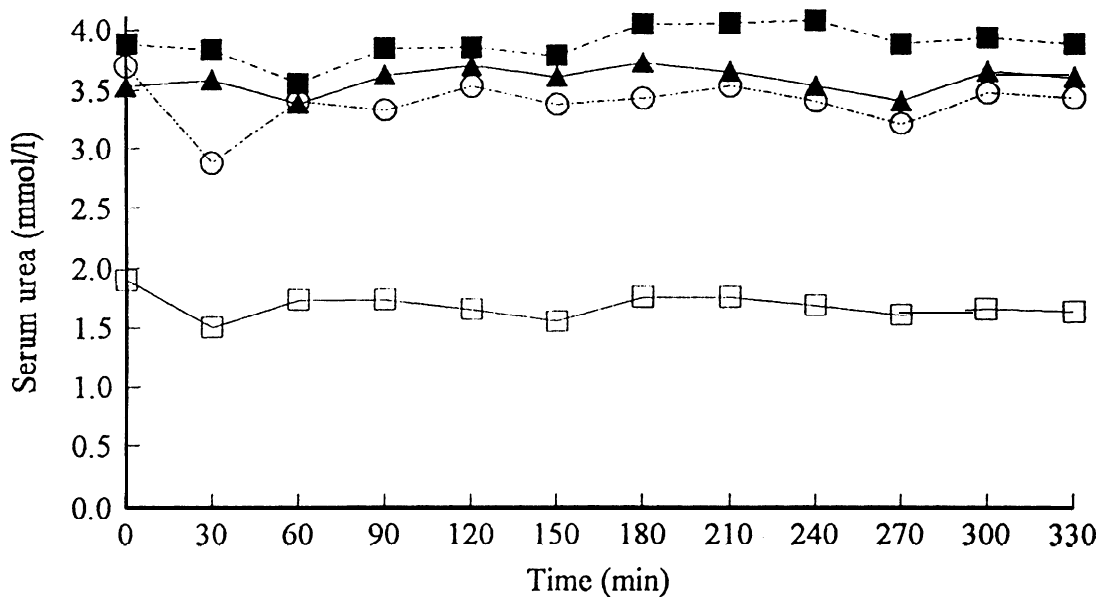


Fig. 4 Serum urea levels of pigs fed diets containing raw peas (cv Wirrega; ▲—▲) or peas heated to 150° (cv Wirrega; ○—○), 165° (cv Wirrega; □—□) or 150° (cv Dundale; ■---■)

Accounting for the overestimation of nitrogen and lysine digestibility, the proportions of ileal digestible nitrogen retained and excreted in the urine can be estimated (Table 5).

Table 5 Ileal digestible N intake, N retained: ileal digestible N intake and urine N: ileal digestible N intake in growing pigs fed raw and heated field peas

Cultivar	Heat treatment			
	0°	150°	165°	150°
	Wirrega	Wirrega	Wirrega	Dundale
Ileal digestible intake (g/day)	31.5	30.4	23.5	31.1
N retained: ID N intake	0.67	0.70	0.73	0.59
Urine N: ID N intake	0.33	0.30	0.27	0.41

There was still no significant difference in the proportion of ileal digestible nitrogen retained or the proportion of ileal digestible nitrogen excreted in the urine. As well as being in contrast to the original hypothesis, this result is further complicated if we consider that the overestimation of digestibility would have resulted in a greater dietary imbalance of amino acids, and hence we would expect the excretion of ileal digestible nitrogen in the urine to be further increased.

It is interesting to note, that despite the pigs fed diets containing peas heated peas receiving less ileal digestible nitrogen and lysine than pigs fed raw peas, there was a substantial increase in the daily excretion of protein in the urine of these pigs (Fig. 5). Although it does not account for all the poorly utilised ileal digestible nitrogen and lysine, these results suggest that some of the poorly utilised protein from heat-processed protein concentrates may be excreted as intact protein in the urine. There is a need to improve the accuracy of urine protein analysis and repeat the above experiment with diets containing equal levels of ileal digestible nitrogen and lysine from heated proteins.

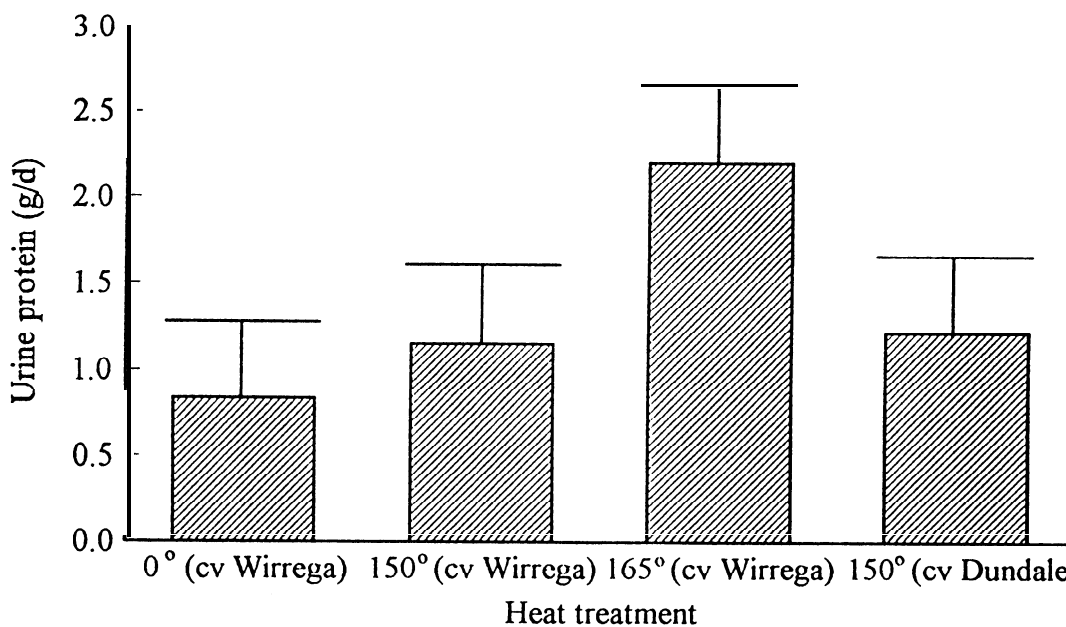


Fig. 5 Urine protein content of pigs fed raw peas (cv Wirrega) and peas heated to 150° (cv Wirrega), 165° (cv Wirrega) or 150° (cv Dundale)

GENERAL DISCUSSION

These experiments prove that heat is the causal factor depressing the utilisation of ileal digestible lysine in processed proteins. Of considerable concern was the effect of mild heat (110° for 15 min) on increasing the ileal digestibility of lysine, whilst at that same time significantly depressing lysine availability. This heat treatment is considerably less than is normally applied in the processing of protein concentrates, and if the result with field peas applies to other proteins, then the majority of heat-processed proteins may suffer depressed lysine availabilities.

A fundamental assumption in this series of experiments was that those amino acids removed from the digestive tract of the pig (and hence digested) are in fact absorbed across the gut wall. It is possible that following digestion, amino acids altered by heating may be metabolised in the gut wall, and hence not absorbed into the portal blood. There is a need to develop techniques to estimate gut wall metabolism of amino acids, and to quantify absorption of amino acids from heated proteins into the portal blood.

Despite being sensitive to the effects of heat on amino acid availability, the slope-ratio analysis is not suitable for use as a routine analysis due to the time and expense involved with such an undertaking. If amino acid availability values are to be used regularly in diet formulations, an assay that is inexpensive, rapid, repeatable, and able to be performed on a wide range of feedstuffs is required. Possible avenues that may be considered in the quest for a rapid amino acid availability assay include:

- 1) The use of microbiological techniques in *vitro*. To date, there is no evidence to suggest that these techniques should be discounted for use as a rapid availability assay;
- 2) A biochemical understanding of the mechanisms involved with the poor utilisation of ileal digestible amino acids from heated proteins. This may include investigation into the extent of gut-wall metabolism of amino acids from different protein sources;
- 3) The use of modern analytical techniques such as near-infrared reflectance spectrophotometry (NIR) to assess protein quality.

Having shown that lysine availability is a close measure of lysine utilisation in pigs fed heated proteins, the following benefits can be perceived if availability values are used in diet formulations:

1. Accurate matching of the diet to the pig's requirements resulting in:
 - * less nitrogen excretion and therefore less pollution;
 - * the ability to choose the most cost-effective ingredients for diets, and;
 - * the achievement of more even growth rates resulting in more reliable production.
2. Simulation models such as AUSPIG will become far more reliable as a management tool, due to the fact that simulations rely on amino acid availability data.

CONCLUSIONS

The main conclusions of this study are:

- 1) Heat applied to field peas, even at mild temperatures of 110°, renders lysine in a form that is apparently absorbed from the gastro-intestinal tract of the pig, but is poorly utilised;
- 2) Ileal digestibility values for lysine in heat-processed meals are unsuitable for use in diet formulations;

- 3) The availability of lysine in raw and heated field peas, measured by applying the slope-ratio analysis, was a close reflection of the utilisation of ileal digestible lysine from these peas. Amino acid availability values should therefore be used in diet formulations when dealing with heat-processed meals;
- 4) Some of the poorly utilised ileal digestible amino acids from heated field peas may be excreted as intact proteins in the urine.

In addition, the study indicates:

- 1) Heat can induce variable effects on the ileal digestibility of amino acids depending on the nature of the protein source prior to heating. The apparent ileal digestibility of amino acids in cv. Dundale field peas was only slightly altered by the application of dry heat up to 165°. Field peas (cv. Wirrega) exhibited a substantial decrease in the ileal digestibility of amino acids when heated to 165°;
- 2) The relationship between chemical changes within a protein source and biological availability of amino acids would be very difficult to establish. There is a need to identify a suitable biological/animal response for the rapid, accurate determination of amino acid availability;

There is a need to pursue an understanding of the biochemical mechanisms involved with the poor utilisation of ileal digestible lysine from heat-processed meals. This will greatly assist the development of a rapid amino acid availability assay.

ACKNOWLEDGMENTS

This work was completed as part of Robert van Barneveld's PhD research program. RJVB was in receipt of a Pig Research and Development Corporation Research Fellowship and the work was funded by grants from the Pig Research and Development Corporation.

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