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Endogenous amino acid losses (EAAL) in growing pigs influence on methodology and factors are affecting – A Review

Rab Nawaz Soomro[1, 2], Junhu Yao[1, *], Imtiaz Hussain Raja Abbasi[1], Mohamed Abdalla Elsiddig Mohamed[1], Saeed Ahmed Soomro[3], Bello Musa Bodinga[1], Xiaojun Yang[1]

[1] Department of Animal Nutrition, College of Animal Science and Technology, Northwest A & F University, Yangling Shaanxi 712100, P. R. China

[2] Livestock and Dairy Development Department Quetta, Balochistan 87300, Pakistan

[3]Department of Physiology and Biochemistry, Sindh Agriculture University Tando jam 70060, Pakistan

***Corresponding Author**
Name: Professor Junhu Yao
Email: yaojunhu2004@sohu.com

Abstract: Endogenous amino acid losses (EAAL) in growing piglets are affected by dietary supplementation of ingredients and feed stuffs are observed. In early age of pigs the feeding practices with environmental factors are highly affected on endogenous losses. There are numerous techniques are used to determined actual value of endogenous amino acid production and losses. The nitrogen free diet (NFD), regression, homoarginine, enzyme hydrolyzed casein (EHC) and 15N-isotopes techniques are used to evaluate the endogenous nitrogen losses, but all consequences were little bit adverse so still all methods are not uniform. Whereas methods, but there are several factors are affecting on EAAL in pigs. These factors are including methodological, environmental, feeding and sample collecting in animals. Dietary feed stuff sources are major contributions in endogenous losses such as dry mater intake (DMI), dietary protein, fibers, bacterial microflora and other anti-nutritional factors (ANFs) are increases EAAL. The present review highlights the EAAL by various techniques, factors and procedures to apply for collecting ileal digest and accurate measurement of endogenous losses. There are numerous procedures to collect samples from pigs, by ileorectal anastomosis, slaughter technique, reentrant canulas, simple T canulas, and post valve T caecum canulas. The criticism of in this review, it's discussed all aspects briefly in young pigs which are affecting on EAAL.
Keywords: Endogenous amino acid losses; methods; factors; collection; pigs

INTRODUCTION:

The nutritional value of feedstuffs for pigs is largely determined by their content of available nutrients, in particular amino acids and other nutrients that supply energy. Availability of nutrients is defined as the portion of ingested nutrients that is absorbed from the digestive tract in a form that renders them available for metabolism by the animal [1]. Because it is expensive and time consuming to routinely measure amino acid availabilities, apparent ileal amino acid digestibility's are usually determined and used as reasonable estimates of amino acid availabilities [1, 2]. It should be noted, however, that for certain feedstuffs apparent ileal digestibilities may underestimate availabilities because amino acids may react with other constituents present in the feedstuff to form complexes that are absorbed in a form which renders them unavailable to the animal [3]. In general, it is accepted that in swine feed ingredients, ileal amino acid digestibilities provide better estimates of amino acid availabilities compared with faecal digestibility's [4]. Amino acids that disappear from the hind gut are not available to the animal [5]. Furthermore, faecal digestibilities are confounded by the modifying and apparent equalizing effect of the microflora in the hind gut on amino acids recovered in faeces [6]. In conventional digestibility studies, it is not possible to distinguish between non-digested dietary amino acids and non-reabsorbed endogenous amino acids in ileal digesta or in faeces. The obtained digestibility values are therefore referred to as apparent digestibilities. True digestibilities can only be estimated when the recovery of endogenous amino acids in the ileal digesta is determined. Recent studies [7-10] have suggested that endogenous gut amino acid losses were previously underestimated. These studies indicate that observed differences in apparent ileal amino acid digestibilities between feed ingredients can largely be attributed to differences in the amount of EAAL recovered at the distal ileum, rather than to differences in true ileal amino acid digestibilities. Given that there will be metabolic inefficiencies associated with the production of EAAL, the actual amino acid and energy losses to the animal will be larger than what is measured in EAAL recovered at the end of the small intestine or in faeces.

It is now widely accepted that amino acid digestibility in the pig is better determined based on ileal rather than faecal measurement [11]. However,

digesta collected at the terminal ileum contain large quantities of endogenous protein. During the process of digestion in the upper tract considerable amounts of endogenous protein enter the gut lumen [12]. This secretion and an accompanying degree of reabsorption of amino acids have a significant influence on the amount of protein lost from the terminal ileum, and thus on determined values of ileal protein and amino acid digestibility. To be able to devise an approach for the accurate determination of digestible amino acids in feed mixtures, a better understanding of dietary influences on endogenous protein loss, and how these affect the amino acid digestibility, is essential. Studies in which gut endogenous protein losses were determined in the growing pig have been reviewed by [13], and discussed in several recent theses on the topic [14].The aim of the present review is to focus on topical aspects pertaining to the measurement of endogenous ileal protein and amino acid losses in the pig, as related to the determination of digestibility coefficients for use in practical feed formulations. The present review has focused on methodology and factors those are affecting and highly influenced on EAAL in growing pigs.

VARIOUS TECHNIQUES AND METHODS ARE USED IN DETERMINATION OF EAAL

NFD (nitrogen free diet)

Endogenous protein losses in the pig has traditionally been determined after feeding the animals a semi synthetic NFD diet, typically consisting of purified starch and/or sucrose (or glucose) supplemented with vegetable oil, 3-5% purified cellulose and a vitamin/mineral mixture. Data from such experiments have been collated by [15] in Table. 1 and endogenous protein losses were found to be higher at the ileal level than at the faecal level (13.9 5.5 vs. 8.5 f 2.0 g per kg DM intake). Subsequent to the latter review, several other determinations of ileal endogenous protein loss have been made [16] in which a relatively high excretion of 19.8 g crude protein per kg DM intake was recorded for pigs given a traditional NFD. The endogenous protein loss increased to 22.5 g kg.' DM intake, and was increased from 19.8 to 24.0 g kg-' DM intake with the addition of pectin (4% of diet). Studies have been undertaken with rats and pigs [17]. Where the sole source of dietary N was industrial free amino acids and endogenous ileal amino acid loss was determined directly. In these studies, the animals grew normally and had positive nitrogen balances, yet the endogenous amino acid flows were similar to those found after feeding an NFD. This shows that the protein free diet, which may lead to an unbalanced body protein metabolism, did not influence the endogenous protein loss.

Table 1: Evaluation of endogenous amino acid losses (EAAL) various methods are used

Methodology	N	References
N-free diet method	18	Wiinsche *et al.;* (1987)
	1	Leibholz & Mollah (1988)
	1	Furuya & Kaji (1989)
	2	De Lange *et al.;* (1989a, b)
	1	Leterme *et al.;* (1990)
	1	Hennig *et al.;* (1991)
	1	Butts *et al.;* (1993a)
	20	Jondreville *et al.;* (1995)
	2	De Lange *et al.;* (1989a)
	1	De Lange *et al.;* (1989b)
	6	Adedokun *et al.;* (2007a, b, c)
Regression method	3	Taverner *et al.;* (1981)
	2	Leibholz & Mollah (1988)
	1	Furuya & Kaji (1989
	4	Adedokun *et al.;* (2007a, b, c)
Peptide alimentation method	1	Butts *et al.;* (1993a)
	8	Butts *et al.;* (1993b)
	1	Schulze *et al.;* (1995a)
^{15}N dilution method	4	De Lange *et al.;* (1990)
	3	Huisman *et al.;* (1992)
	1	Schulze *et al.;* (1995a)
In vitro/in vivo digestibility	15	Boisen & Fernandez (1995)
	1	Boisen & Fernandez (1995)

N = number of studies.

Regression method

The regression method has been claimed to give more correct values for endogenous protein losses as induced by the individual feedstuffs in comparison with the protein free diet, given that the results are based on feeding the pig diets containing graded levels of the feedstuffs and then determining endogenous losses by mathematical extrapolation. A study was

reported in growing rats[15], five levels of dietary crude protein ranging from 80 to 160 g per kg DM were obtained by progressively diluting a meat and bone meal with an N-free semi-synthetic mixture. The Table.1 result obtained by the regression procedure was almost identical with that obtained after feeding the rats the N-free mixture used for dilution of the investigated protein source. Similar agreement between the two approaches has been reported by other scientists [18-20]. Although there are likely to be specific effects on endogenous loss related to the individual feedstuff it is likely that they are proportional to the amount of ingredient included in the mixed diet. It can be inferred from this that there should be a linear relationship between ileal protein output and dietary protein input, but the estimated endogenous loss is not always constant for the different experiments.

EHC (enzyme hydrolysed casein)

Using a new method [21], which has become known as the EHC method it has been shown [22] that feeding pigs a semi synthetic diet containing peptides and free amino acids (thus simulating natural products of digestion by hydrolyzing casein or some other protein) significantly increases endogenous ileal protein loss. With the latter technique pigs are fed a semi synthetic diet containing the EHC as the sole source of nitrogen. Digesta are collected and centrifuged and then ultra-filtered. The precipitate plus retentive is a measure of endogenous loss. Any unabsorbed dietary peptides or free amino acids are discarded in the ultra-filtrate. A similar effect of peptide alimentation to that found by [1, 6] with pigs has been observed in several studies with rats [23]. Furthermore, [24] recently compared all three methods EHC, NFD and Regression in one study and found that EHC led to significantly higher ileal endogenous protein flow in the rat compared with the other two methods, respectively. The EHC method gives minimum estimates of endogenous loss because some endogenous peptides and free amino acids are discarded in the ultra-filtrate along with those of direct dietary origin. However, this effect is thought to be limited [25, 26]. The work with enzyme hydrolysed casein diets fed to rats and pigs suggests a direct effect of dietary peptides on endogenous protein secretion or reabsorption.

Homoarginine method

The specific feed induced endogenous protein losses can in principle be measured using the homoarginine method as described by [27].The resultant homoarginine is assumed to be liberated from the protein and to be absorbed at the same rate as the original lysine would have been. Accordingly the real digestibility of lysine and thus endogenous lysine losses can be determined. The technique has been applied in several laboratories [28, 29]. Assuming a constant amino acid composition for endogenous protein, endogenous losses of protein and other essential amino acids, as well, can be based on the calculation of endogenous lysine loss. However, it has not been fully established that the guanidination process itself does not influence the digestibility of the protein, for example by inactivating anti-nutritional factors present in the feedstuff or by possible induced changes in the protein structure. On the contrary, recent results by [30] indicate a general decrease in the protein digestibility of different milk products after guanidination. Further, if the guanidination is not complete, then it cannot be excluded that the un-reacted lysine may have a different digestibility compared with the modified lysine; though, in this respect, [31] found similar endogenous lysine flows determined with completely or partially guanidinated gelatin.

Isotope dilution techniques (^{15}N)

Another method for determining specific feed induced endogenous protein losses is the tracer technique, by which endogenous protein can be distinguished from feed protein after labelling either the food or body protein using radioactive or stable isotopes. There are several isotopes that may be used, but ^{15}N dilution technique is the most common procedure. The ^{15}N dilution method [32] determined significantly higher endogenous protein losses when feeding common vegetable feedstuffs such as wheat, barley, soya bean meal and rapeseed meal protein per kg DM intake. In agreement, [33] also using ^{15}N dilution demonstrated that endogenous protein loss increased when the content of dietary fibre was increased. As the ^{15}N dilution technique measures the endogenous protein loss pertaining to the particular feedstuff investigated, it has been considered to give rise to “real” digestibility coefficients. There is confusion in the literature concerning terminology, however, and the term “true” digestibility is now also often used for values obtained by this approach. The endogenous losses determined with this technique are usually higher than found using the traditional methods, resulting in higher calculated true or real digestibility coefficients. Using the ^{15}N method in Table.1, [34] determined values of 31 and 34g per kg DM intake for two varieties of peas. The isotope dilution technique is a promising approach for allowing a direct measure of the feed dependent endogenous ileal protein losses. However, the method does not allow direct estimation of all the endogenous amino acid flows and there is debate as to what constitutes an appropriate precursor pool [35]. Recently [34, 36] compared endogenous ileal N flow in the pig obtained using the 15N approach with that for the same pigs but using the EHC. These two methods gave similar results in endogenous N flow and endogenous amino acid losses in pigs. The method of 15N-Leucine single injection has been introduced by [36], which applied in broiler for evaluation of EAAL.

Digestibility in vitro and in vivo methods

As an alternative to the above described approaches, endogenous ileal protein and amino acid losses can be estimated from the difference between in

vivo values of apparent ileal digestibility and in vitro digestibility values [37]. An in vitro technique has been developed [38] that seems to give accurate estimates of "real" digestibility. Thus, and assuming that in vitro digestibility assay does indeed give reliable estimates of real digestibility, values of endogenous protein loss can be calculated by barley, wheat, rye, oats, soyabean meal, rapeseed meal, sunflower meal, grass meal, peas, barley grits, barley meal, barley hulls and skim milk powder g/per kg DM intake [39]. The results from several studies in Table.1 on endogenous protein losses in the growing pig used the above discussed methodologies. The data demonstrate a high variation in published results. This variation is not only due to differences among methods but presumably also reflects real differences between feedstuffs. Only a few determinations directly relating endogenous loss to specific feedstuffs have been performed. This has been attempted using ^{15}N dilution and by calculation of in vitro and in vivo digestibility difference. As expected, the values obtained using these two methods are generally higher than values obtained with the semi synthetic and in particular the NFD diets. The values based on these two distinct approaches are in good agreement with each other. The value given for skim milk powder may seem somewhat high as milk powder does not contain fibre or anti-nutritional factors, though, interestingly, the value is identical to that given by[40] for casein using 15N dilution and similar to those reported for the EHC method.

ENDOGENOUS PROTEIN LOSSES BY DIETARY SUPPLEMENTATION FACTORS

During digestion, large quantities of endogenous protein enter the digestive tract from secretions in the mouth (saliva), stomach (gastric juice) and small intestine (pancreatic juice, bile and intestinal juice). Considerable amounts also enter as muco polysaccharide secreted from the epithelial cells in the digestive tract and epithelial cells that are continuously sloughed into the lumen and are replaced by new cells. Endogenous protein entering the digestive tract is digested and reabsorbed in the small intestine, with more being reabsorbed by the end of the digestive tract overall [41]. It has already been noted that the losses is higher in animals given a protein containing diet relative to that for a protein free diet. However, several other dietary factors are also known to influence endogenous losses. Feed induced variations in endogenous protein losses may be caused by changes in the rate of secretion or degradation and reabsorption of N compounds, but also indirectly by changes in intestinal bacterial activity.

DMI (Dry Matter Intake)

Under normal feeding situations there seems to be a close relationship between the daily loss of endogenous protein and metabolic body weight, even between different species [42]. Also, and at a given live weight, there appears to be a significant influence of feed dry matter intake on endogenous loss. Thus, the results of [43] from feeding pigs increasing daily amounts of the same feed clearly demonstrated a linear relationship between endogenous protein losses and dry matter intake. The primary influencing factor would appear to be feed dry matter intake rather than metabolic body weight. Furthermore, for use in feed evaluation, it is most suitable to express endogenous protein losses in relation to dry matter intake. However, readily digestible nutrients such as wheat starch have little influence on ileal N flow [44], and endogenous secretions into the gut and the reutilization of endogenous protein are dependent on the protein source [45]. The specifically feed induced endogenous protein loss may be better predicted from undigested dry matter [46], assuming that the undigested portion of the diet has the main influence on endogenous loss.

Dietary Protein (DP)

A higher endogenous ileal protein loss consequent upon using diets containing peptides (EHC) relative to the protein free situation is significant. The mechanism for this effect of dietary peptides has not yet been identified. However, a similar magnitude of effect on endogenous ileal lysine loss from feeding pigs the intact but lysine deficient protein [47] suggests a common effect for added peptides or those arising from the natural digestion of a protein. The EHC method may give a more realistic measure of the basic endogenous protein loss pertaining to the feeding of normal protein containing diets than the N-free and regression methods. However, the effects of hydrolysed proteins other than casein, the degree of hydrolysis and the amount of dietary hydrolysed protein have not yet been systematically investigated. On the latter point, however, the dietary inclusion level of 10% EHC as routinely used by Moughan and co-workers and an inclusion level of 18% EHC as used by [48] resulted in almost identical endogenous ileal protein losses. In general, there seems not to be a direct relationship between dietary protein level and endogenous ileal protein losses because these losses are primarily in dyed by the non-protein part of the feeds. It follows from this that expression of endogenous protein loss in relation to protein intake [49-51] is usually not relevant as a characteristic of the ingested diet. There is also some limited information that the type of protein per se has little direct effect on endogenous ileal protein losses [52, 53].

Influence of Dietary fibre (IDF)

Most non-starch plant polysaccharides (NSP) are known to have a significant influence on endogenous ileal protein loss, but the type of fibre is important. The commonly added preparations of purified wood cellulose to semi synthetic diets for simulating the influence of fibre on endogenous protein loss seem to have only a minor influence on this property [54-56]. This has often led to the assumption that fibre in general has little influence on endogenous

protein loss. However, the adsorptive capacity of cellulose is very low and negligible compared with that of naturally occurring dietary fibre, for example bran fibre, alfalfa fibre and lignin [57, 58]. How fibres mediate their effects on endogenous ileal protein losses is not well understood. The [59] have recently shown an effect of dietary fibre viscosity per se and the effects of abrasion and adsorption have been discussed [60]. A close linear effect of purified NDF from wheat bran on endogenous protein loss has recently been reported [61]. Moreover, a similar relationship is found between calculated endogenous ileal protein loss and undigested dry matter for samples of barley and barley products with a varying amount of fibre, is closely related to NDF, the results from the two investigations are both included in the figure. Apparently, barley fibre induces less endogenous ileal protein loss than wheat fibre. On the other hand, the effect of wheat fibre (analyzed as NDF) seems to be very similar to that of UDM in most other feedstuffs. At least for the range of dietary fibre concentrations encountered in practice, it appears as though there is a linear relationship between endogenous ileal protein flow and dietary fibre.

Anti-nutritional factors (ANFs)

The presence of anti-nutritional factors (ANFs) in the ingested feed may also have a significant influence on endogenous ileal protein loss [62, 63]. All vegetable feed stuffs contain ANFs but the content in the common cereals and most other feedstuffs is generally low [64]. However, seeds from legumes (beans, peas, lupins) may contain high amounts of ANFs if the protein sources have not been properly processed. Thus, the extremely high losses of endogenous ileal protein reported by [65] when feeding common beans to the pig were most likely caused by the action of ANFs. The effects of ANFs are even more complex than the effects of fibre and are quite different for the various ANFs. Thus, trypsin inhibitors and other inhibitors of hydrolytic enzymes (other proteases, amylases and lipases) bind specifically to the enzymes at their active sites. This in turn leads to a stimulation of pancreatic secretion [66]. At high concentrations of inhibitors in the ingested feed, an increased secretion may not completely compensate for the inhibitions, leading to a decreased digestion and absorption of endogenous as well as of feed protein. Tannins bind less specifically to the digestive enzymes and feed proteins and can thereby reduce digestion in the small intestine [67], although some types of tannin seem not to have this effect as measured in rats [68]. Also, tannins may induce secretion in the saliva of large amounts of a proline rich protein that binds specifically to the tannins and thereby neutralizes their effect [46, 69]. This complex passes relatively unchanged into the faecal matter [70]. However, this has been demonstrated only in rats, and pigs may not have a similar adaptive mechanism. Lectins, on the other hand, are glycoprotein that bind specifically to receptors on the surface of the epithelial cells and thereby induce changes in their metabolism, resulting in increased cell turnover and protein secretion [45.61].

Bacterial influence in Gut (BIG)

Bacteria present in the hindgut extensively degrade endogenous protein sourced from the small and large intestine. However, foregut bacterial activity may also play an important role in the metabolism of endogenous protein, because the non-absorbed endogenous nitrogen at ileal level may already be incorporated in bacterial protein [71]. Thus, muco polysaccharides, which are not degraded by mammalian enzymes, may be partly degraded in the small intestine by bacterial enzymes. Also, it seems that the degradation of pancreatic enzymes in the distal part of the small intestine is greatly promoted by the presence of bacteria [72]. The bacterial activity in the foregut, mainly in the distal part of the small intestine, may be influenced by factors such as the protein status of the animal (availability of N from the urea flux between blood and intestinal tract) and the availability of energy from dietary fibre. Although synthesized bacterial protein is usually included in the endogenous protein, the small intestinal bacterial net degradation or synthesis of amino acids does impair the accuracy of determined dietary amino acid digestibilities. The effects on endogenous ileal protein loss of the above mentioned specific factors may in general be considered to be proportional to the amount in the feed. Therefore, there is likely to be considerable variation in endogenous protein loss among different types of diets. It is also possible that some N free mixtures can induce higher endogenous protein losses than common feedstuffs with low levels of dietary fibre and ANFs. Finally, it should be noted that the values for endogenous protein loss obtained by some of the methods (isotope dilution & homoarginine), do not refer directly to the pure feedstuffs but refer to the experimental diets used in these studies. Therefore, the protein rich feed stuffs, which are diluted with N-free mixtures, in most cases underestimate the endogenous protein loss specifically induced by the investigated feed stuffs.

Synthesizes Amino Acid (AA) from endogenous protein

Estimates of the amino acid composition of endogenous protein determined in some recent studies using either the N free method or the EHC method. The results indicate that the position may vary considerably, although for most of the studies it seems to be rather consistent. The results in demonstrate that EHC protein determined in some recent studies. These results mention had little influence on the amino acid composition, and, furthermore, no effect of animal live weight on the amino acid composition of endogenous protein loss could be discerned. Comparing the mean values for amino acid composition based on current estimates of endogenous protein loss determined by the N free method with the earlier overall mean values

given by [73,74], and also the overall mean values for the EHC method as well as those obtained from the difference method, shows a rather high degree of consistency. These results indicate that the magnitude of endogenous protein loss has little influence on amino acid compositions. Endogenous protein and fat sources are increased and decreased production of AAs in mono gastric animals [75]. However and also as indicated in, the ileal output of proline may vary considerably. Intravenous infusion of a balanced amino acid mixture to pigs receiving a protein free diet reduced these losses dramatically [76], which led to the conclusion that the high endogenous loss of proline with protein free feeding was caused by disturbances in protein metabolism. However, in some studies with the EHC method relatively high proline losses were also found. The variation in proline losses influences the relative contribution of the remaining amino acids. If the amino acid composition is expressed relative to lysine, the influence of proline is removed. This makes it possible to, first estimate endogenous protein loss from the endogenous lysine loss determined by the homoarginine method, and second estimate the endogenous loss of the essential amino acids from protein loss determined using I5N dilution or similar isotope dilution. On the other hand it does also indicate that patterns are very sensitive to changes in the lysine concentrations whether these occur from experimental inaccuracies or real variations. The composition of essential amino acids in endogenous protein is of particular interest.

COLLECTING OF ILEAL DIGESTA WITH VARIOUS METHODS FROM THE TERMINAL ILEUM IN GROWING PIGS

In order to determine apparent ileal digestibilities, digesta need to be sampled at the end of the small intestine. Various sampling methods have been used, including the use of surgically modified (cannulated or anastomosed) animals or the slaughter of intact animals. The general principles of these procedures including their advantages and disadvantages are discussed; numerous cannulation and various procedures are used to facilitate collection of ileal digesta. These could be classified into general categories, ileorectal anastomosis, slaughter technique, reentrant canulas, simple T canulas, and post valve T caecum canulas.

Ileorectal anastomosis (IA)

As a means of avoiding problems with canulas, the ileorectal anastomosis (IRA) technique has been suggested by [88]. The small intestine is completely transected either at the terminal ileum or just after the ileocaecal sphincter and then joined to the rectum such that ileal digesta are easily collected from the anus. A modified version of this technique in which a T canula is placed in the colon to evacuate fermentation gases, has been suggested by [89]. Pigs with IRA are relatively easy to maintain and can be fed diets of any texture [90]. However, as with most surgical procedures, the impact of IRA on the physiology and nutrition of the animal may pose some concerns. This is particularly so with long term protocols, as the functional role of the small intestine is changed to make up for the missing colon. This may alter the amino acid composition of the chyme and observed digestibilities compared with that obtained when using other methods [91]. Anastomosed animals suffer considerable discomfort due to the continuous outpouring of digesta and this is likely unacceptable from the animal welfare standpoint.

Slaughter method (SM)

As the term implies, this technique involves slaughter of experimental animals so as to be able to collect digesta from the small intestine. The animals are killed at a certain time interval (usually 6 - 9 h) after feeding and digesta are recovered from the last 20 - 40 cm of the small intestine. The major criticisms of this method concern the fact that animals cannot be used as their own controls since only one measurement can be obtained per animal, and the possible difficulties of obtaining representative digesta samples. In particular, digesta can only be sampled at one instant in time when the slaughter technique is used. As a result, any diurnal variation in nutrient digestibility due to feeding schedules, and interactions between feeding schedules and diet compositions, is generally not considered when this method is used. However, in direct comparisons no differences were observed between digestibilities obtained using the slaughter technique and from pigs fitted with simple T-canulas [92]. The biggest advantage of this technique is that minimum disruption of the digestive function occurs since no manipulation of the digestive tract is involved and it is possible to collect digesta from several parts of the gut. The slaughter method, unlike cannulation techniques, poses no problems with the texture of the feed thus allowing practical diets to be tested. It takes a shorter time to complete an experiment with less labor input, and requires no special facilities such as metabolic crates.

Reentrant Canulas (RC)

The use of the re -entrant canula technique in pigs was first suggested by [77-80], but since then many variants including the ileo, ileocaecal and ileocolic post valve re-entrant canulas have been developed in an attempt to overcome the problems of the initial ileo procedure. The major problems with the initial re-entrant cannulation procedure are the complete transaction of the small intestine which disrupts its normal functioning and the high incidence of blockage particularly with normally ground feeds [81]. The incidence of blockage is increased at high feed intakes, increased dietary fibre levels and factors that increase digesta viscosity. Infusion of a physiological salt solution at the proximal part of the ileocaecal re-entrant canula to make the digesta thinner so that they flow smoothly has been suggested as a possible solution to blockage problems [82], although the disadvantages are that this is more laborious and increases the need to

restrain the pigs while infusing the salt solution. The ileocolic post valve cannulation technique, developed by [83], maintains the integrity of the small intestine and preserves the functional role of the ileocaecal sphincter thus maintaining a more physiological state. It also allows collection of digesta as they arrive normally at the colon. However, this technique is quite laborious as well; digesta are collected continuously and need to be returned to the hindgut at regular intervals [84]. Re-entrant cannulation has advantages in that it allows for quantitative collection and representative sampling of digesta and it does not rely on the use of digestibility markers.

T-Canulas (TC)

The simple T-canula technique uses a T-shaped canula that is placed at the distal ileum about 5 - 10 cm anterior to the ileocaecal valve without transecting the small intestine [85] This allows maintenance of a fairly normal physiological state since the migrating my electric complexes necessary for normal digesta passage are able to pass along the site of cannulation [46, 86]. During collection, only a fraction of the digesta that pass by the T-canula are collected. Therefore, this technique relies on the use of digestibility markers and natural forces to drive digesta from the intestinal lumen through the canula. This may pose problems regarding the limitations of using markers (e.g. the need for uniform mixing of markers in the diet and in the digesta, analytical problems, uncertainties concerning the marker's absorbability) and obtaining representative samples [85,87]. Other concerns of using the T-canula technique include the internal diameter of the canula, flow of digesta from diets of different composition and particle size and the amount of digesta collected to give a representative sample [88, 89]. If the T-canula is used for a long time, it may be outgrown by the animal. However, the T-canula technique is the most commonly used procedure, partly because it involves less invasive surgery than re-entrant canulas and it compares favorably with the use of intact animals [90].

Post valve T caecum Canulas (PVTC)

The post-valve T-caecum canula (PVTC) technique involves removal of the entire caecum with the exception of the area surrounding the ileocaecal sphincter which is prepared for placement of a T-canula permits quantitative collection of ileal digesta. Once the canula is opened, the ileocaecal sphincter moves into the barrel of the canula allowing a free flow of digesta passing through the ileocaecal valve to the collection tube attached to the external part of the canula. It also allows for determination of ileal digestibility of coarser diets and those containing high fibre levels. Furthermore, the PVTC method involves a simple surgical procedure and causes less discomfort and minor negative physiological effects [77, 91]. The major concern with this method relates to the possible physiological effects of caecetomy on ileal digestibility and the fact that its superiority over the T-canula has not been established [92]. A modified version of this technique has been proposed by [93]. Rather than removing nearly all of the caecum, these researchers suggest that a ring be placed around the ileocaecal sphincter and that this ring is used to steer the ileocaecal sphincter towards the caecal canula when digesta are collected.

CONCLUSION

The present review is concluded that dietary supplementation, methodology, environmental factors and the procedure of samples collecting in pigs are highly influenced on EAAL. The demonstration of procedures may improve the accurate estimation of endogenous losses. Endogenous protein loss at the terminal ileum of the pig is primarily dependent on dry matter intake but may be significantly influenced by the chemical and physical composition of the feed. Semi synthetic protein free diets with a low content of dietary fibre without anti-nutritional compounds result in minimal endogenous ileal protein losses. Protein and naturally occurring dietary fibre and ANFs in the diet all increase the endogenous protein loss above that found for a protein free diet. Determined values of endogenous protein loss obtained for protein rich feedstuffs that have been diluted with an N free mixture are, therefore, often underestimated. The effect on the endogenous protein loss of the specific inducing factors in the feed can generally be considered to be proportional to the amount in the feed. The endogenous ileal protein loss can be divided in a basal and an extra loss. The basal loss is nonspecific and related to the dry matter intake, whereas the extra loss is specifically related to inducing factors in the feedstuff dietary fibre and other ANFs. The amino acid composition of endogenous protein is relatively constant for different diets and different methods of determination and seems not to be influenced significantly by the amount of loss and live weight of pig. For practical purposes, and lacking evidence to the contrary, the composition can be considered to be constant. It is further suggested to evaluate the specific procedure applying for measurement of EAAL with including dietary feed stuffs supplementation in growing pigs.

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