1 Introduction

There is a need to study and estimate the minimum nutrient need of farm species. This is in line with economic and environmental considerations. With regards to dietary nitrogen (crude protein and amino acids), the minimum nitrogen requirement is the inevitable losses. It forms an integral component of the total animal requirement that is used to maintain the nitrogen integrity of the animal. It can be readily understood, therefore, that it is fundamental to the total nitrogen requirement, and would have to be met before dietary nitrogen is diverted to growth or some other index of production. Estimates of inevitable losses are useful in modern feed evaluation where a distinction is made between the nitrogen requirement of the animal and the nitrogen supplying capacity of different feedstuffs (Short et al., 1999; Lemme et al., 2004). Again, inevitable losses may help to explain the discrepancies in amino acids and protein utilisation commonly observed among poultry species (Kluth and Rodehutscord, 2006). Similarly, research into inevitable losses is justifiable on the premise that estimates of inevitable losses are useful to determine the animal requirements based on the factorial approach (Rodehutscord, 2006). As reviewed by Owens and Pettigrew (1989) and D'Mello (2003), factorising the total requirement into its inevitable fractions and those for production (or growth) provides a non-static basis for extrapolation of requirements to animals differing in environment and production specifications. Moreover, since the modelling of requirement is tending to be more conventional (Moughan and Fuller, 2003) inevitable losses and indeed the total maintenance should no more be based on assumptions rather they should be systematically studied. Studies in these regards should as well include thorough investigations into possible physiological and dietary modulating factors. A review of terminologies and literature with special relevance to the overall title is hereby presented as an important prelude to the main objectives and reports on the experimental investigations undertaken.

2 Literature review, definitions and objectives

2.1 Theory of N maintenance

Generally, N maintenance is the amount of nitrogen needed by an animal under resting condition and in optimal environment to maintain zero nitrogen balance. In the maintenance state, nitrogen is neither gained nor lost, that is, a state of nitrogen equilibrium. Requirement for maintenance must first be satisfied before an animal can utilise any protein or amino acid for growth or production.

Although, maintenance is a hypothetical state, it is explained by experimental data (Fisher and Scott, 1954) showing that proportional needs for certain amino acids do not fully match animal body composition. Also it is demonstrated in the fact that protein accretion does not occur in matured animals yet there is still an obligatory protein requirement by this age group. These aspects have been reviewed by Owen and Pettigrew (1989), who noted that the use of carcass amino acid accretion to estimate net requirement is inadequate because maintenance portion is not considered.

2.2 Physiological basis of N maintenance and inevitable losses

During nitrogen (crude protein or amino acid, CP/AA) equilibrium, protein and amino acids metabolism nonetheless continue for the sustenance of life. This sustenance of life involves obligatory and irreversible processes (Table 1) that must continuously use nitrogen. These nitrogen (N) usages would not result in some ‘growth’ but in maintenance and therefore represent net loss to the overall nitrogen nutrition of the animal. Therefore, they are referred to as ‘inevitable losses’.

Current information regarding the sources of inevitable losses contributing to physiological maintenance have been summarised by Fuller (1994), Moughan (1994), and Moughan and Fuller (2003). Major processes are:

a. Body protein turnover (Urinary N excretion)
b. Urinary amino acid loss
c. Irreversible modification of amino acids
d. Gut endogenous N losses
e. Synthesis of non protein nitrogen (NPN)

Table 1. Physiological processes contributing to N maintenance requirement

Processes	Amino acid	Consumption routes
Synthesis of NPN	Methionine	glutathione, taurine, choline, creatine, methylation reactions
	Lysine	Carnitine
Neurotransmitters and hormones	Tryptophan	Serotonine, melatonin
	Tyrosine and glutamate	Neurotransmitters (Dopa, adrenaline), hormones (thyroxine)
	Histidine	Histamine, carnosine, anserine
	Glycine	Nucleic acid bases, haem, creatine
Replacement of irreversibly modified AA	Histidine	1-methylhistidine, 3-methylhistidine
	Lysine	Hydroxylysine, methyllysine

Adapted from Fuller, 1994 and D'Mello, 2003

Inevitable losses are excreted via different routes such as the urine and faeces. Based on their routes of excretion, inevitable losses may be simplified and mathematically described according to the following equations:

Inevitable losses = faecal (IFL) + urinary (IUL) + Scurf …..…….... equation 1
Maintenance cost = Inevitable losses / Efficiency of utilisation ..……...…. equation 2

Based on equation 1, three avenues of losses contribute to the total maintenance cost. These are inevitable faecal losses, inevitable urinary losses and inevitable scurf or integument losses.

Inevitable Faecal Losses (IFL): These are the nitrogen recovered from the digestive tract under condition of zero N intake. They are contributed by unreabsorbed enzyme residues, mucins, abraded gut linings and microbial debris.

Inevitable Urinary Losses (IUL): Sometimes referred to as minimum amino acid oxidation, or as inevitable catabolism (Black, 2000). It measures nitrogen resulting from inefficiency in basal body protein turnover. Free amino acids that are lost in the urine (O'Dell et al., 1960) may possibly be included in this scheme as well (Moughan and Fuller, 2003). A recent review of quantitative data on these losses concluded that they range between 126-207 mg/kg $BW^{0.75}$ with an average value of 155 mg/kg $BW^{0.75}$ in swine (Black, 2000). Leeson and Summers (2001) suggested that IUL in adult birds on a N free diet is about 140 mg/kg $BW^{0.75}$ and IFL is about 40 mg/kg $BW^{0.75}$

Inevitable Scurf Losses: these are the nitrogen losses originating from integument structures such as feathers, hairs, horns, nails, beaks and skin. In swine, scurf losses form only about 2 % of the total N maintenance (Fuller, 1991 as cited by Fuller, 1994). Moughan (1999) has provided information indicating that about 17 $mg/kg^{0.75}$ d^{-1} of nitrogen is lost via the integuments in growing pigs.

Efficiency of N utilisation: the efficiency value in the total maintenance requirement is necessitated by the fact that inevitable losses is a 'net' value, and that their supply must take cognisance of inefficiencies in utilisation of protein supplied either through body protein reserves or from dietary sources (Fuller, 1988). The inefficiencies in utilisation of protein in meeting inevitable losses mainly come from oxidative losses of absorbed dietary nitrogen (Heger and Frydrych, 1985) and to a lesser extent from incomplete digestion of dietary sources. Oxidative losses comes about because the array of limiting amino acids in the body protein to be replenished usually differ to those of the source of re-supply, meaning that their content of non-required amino nitrogen is not utilised.

2.3 Experimental approaches to study and estimate inevitable losses

The measurements of inevitable losses depend on the route of excretion. To do this, two basic and simple sampling approaches exist namely tissue carcass retention analysis (Fatufe and Rodehutscord, 2005) and N balance (Burnham and Gous, 1992; Xu et al., 2002). Between these methods, tissue carcass analysis is regarded as the more direct and accurate approach since N balance method might be more susceptible to errors, the most important being incomplete recovery of excreted nitrogen (Eggum, 1989; McNab, 1994). However, N balance assays provide more detailed information, flexibility and obviously less experimental efforts when working with large animals. With the N balance approach, it is possible to collectively study the total maintenance requirements, as well as individual aspects of inevitable losses according to schemes depicted by equations 1 and 2 (section 2.2).

In using the N balance method to study inevitable losses, it is noteworthy to mention that peculiar cases exist for the different livestock species. First, in poultry unlike in swine or ruminants, scurf losses may conveniently be categorised not as maintenance cost but as part of the productive or growth cost. This is for practical reasons because in poultry feathers preserve carcass value, are saleable products (see review of Pingel, 2004) and impacts energy utilisation in cold environments. Secondly, because urine and faeces are voided together as excreta in poultry, inevitable faecal losses (IFL) and urinary losses (IUL) may be measured together as inevitable losses (IL).

There is yet another variant of inevitable losses, in which case these losses are measured at the terminal ileum as inevitable precaecal losses (IPL). IPL is contained in IFL. IPL is useful for modern feed evaluation schemes (further discussed in the section on practical uses of inevitable losses, section 2.5). This is because current information indicate that precaecal rather than excreta digestibility more accurately reflects protein availability in poultry (Ravindran et al., 1999), and that amino acid absorption beyond the terminal ileum does not contribute to the protein nutrition of the animal (Jamroz et al., 2001).

In conjunction with both of the above mentioned sampling techniques of carcass analysis or the N balance, quantitative estimation of the above discussed aspects on inevitable losses can be undertaken by the construction of regression equations

describing the existing relationships between nitrogen (CP/AA) intake and some measure of N utilisation such as N accretion or N excretion. This is the basis of the so called regression approach (Nyachoti et al., 1997). This approach forms the basis of the experiments reported in this thesis and it is further explained in the next section under materials and methods.

In addition to the regression method, experimental efforts may also use an array of other common but different techniques as summarised on Table 2. All these methods operate on different assumptions, and have come under the scrutiny of reviewers (Nyachoti et al., 1997; Lemme et al., 2004). Some investigators have undertaken studies including comparative ones looking into their usability, validity and accuracy (Donkoh et al., 1995; Schulze et al., 1995; Angkanaporn et al., 1996a; Angkanaporn et al., 1997a). However, the accuracy and reliability of many of these techniques are still unclear (Angkanaporn et al., 1977b), although some are of the opinion that the enzyme hydrolised casein (EHC) is perhaps the most accurate and reliable for poultry simply because it overcomes most of the draw backs of the other methods (Lemme et al., 2004).

It is also clear from literature that each of these methods are suitable for different experimental conditions, an instance is the EHC which is restricted to semi purified diets and not usable to study proteins in practical diets containing high levels of anti nutritional factors (Nyachoti et al., 1997). Sometimes different quantitative interpretations are given to the different techniques. For example, estimates given by homoarginine and radioisotope dilution refer to total endogenous secretion of nitrogen (Lemme et al., 2004). On the other hands, the conventional methods such as the N-free diet, feeding highly digestible protein, peptide alimentation technique, and the regression techniques refer to inevitable losses. It should be noted therefore, that inevitable losses are integral part of the total endogenous losses, the latter containing an additional quantity referred to as specific endogenous losses which results directly from intrinsic effects of the diet components per se (Nyachoti et al., 1997). Specific losses may be obtained by subtracting inevitable losses from the total endogenous losses (Stein et al., 2007).

Table 2. Comparisons of experimental techniques used to study inevitable losses

TECHNIQUES	DESCRIPTIONS	STRENGHT	WEAKNESS
1). Fasting (Starvation)	Starving negative control birds during determination of true AA digestibility	-rapid -simple	-stressful -birds in negative N balance -losses can not be related to dry matter intake
2). Feeding N free diet. Kessler et al. (1981) and de Lange et al. (1989)		-rapid -simple -useful to study inevitable losses	-animal in catabolic state -can not be used to study practical ingredients
3). Zero Protein diets with parenteral infusion of amino acids. (de Lange et al., 1989b; Leterme et al., 1994)	Measurement of N flow in animals fed N free diets but intravenously infused with balanced solution of AA to limit disturbances to normal protein metabolism	-more physiological state -useful to study inevitable losses	-depresses digestive secretions in nonruminants -not useful for practical diets
4). Feeding of highly digestible protein diets. (Fuller and Cadenhead, 1991)	A low protein diet (e.g. 4 %) containing highly digestible casein as the only protein source is fed.	-animals are in positive N-balance -useful to study inevitable losses	- assumptions about 100% true digestibility of casein and synthetic AA may not be true -feed specific endogenous losses are not measured
5). Regression Method. (Fan et al., 1995)	- N flow is related to N intake. Inevitable losses are estimated using mathematical extrapolation to zero N intake.	-useful to study inevitable losses -estimates are used to calculate standardised ileal amino acid digestibility	-based on the assumptions that endogenous losses are constant at all level of N intake -feed specific endogenous losses are not measured
6). Enzymatically Hydrolysed Casein (EHC) or Peptide Alimentaion Ultrafiltration. (Donkoh, et al., 1995)	Peptides of molecular weight <5000 Dalton, is fed. Only proteins >10000 Da is assumed to be endogenous.	-give estimates that agree well with HA and ^{15}N Isotope dilution technique (^{15}N IDT) -suitable for animal proteins such as meat and bone meal	-applicable to only semi purified diets -feeding small peptides may not be comparable to intact protein sources -feed specific endogenous losses are not measured

Table 2. continuation

TECHNIQUES	DESCRIPTIONS	STRENGHT	WEAKNESS
7). ^{15}N-IDT Isotope infusion of body N-pool. (Schulze et al., 1995)	- body AA or precursor pool is labelled by long infused ^{15}N –leucine and then estimating the amount of ^{15}N in the gut relative to that in the precursor pool for endogenous gut protein synthesis	-suitable for practical diets -measures total endogenous losses	-^{15}N labelled materials are expensive -intensive in nature and require high technical skill -Success depend on correctly measuring isotopic enrichment in individual AA -uniform labelling of all sources of losses is required
8). Isotope labelling of diet. (Roos et al., 1994)	-endogenous N is differentiated by labelling of diets with ^{3}H or ^{15}N -Leucine	-measures total endogenous losses	-Underestimation of losses due to recycling of ^{15}N into body proteins
9). Homoarginine (HA) or Guanidination (Angkanaporn et al., 1996a)	Based on the conversion of protein lysine with o-methyl-isourea into homoarginine. Some assumptions include: a. metabolism of HA is not different from other AA b. HA is not used for protein synthesis c. HA does not affect endogenous N losses (ENL) d. HA is not broken down in the gut into lysine and urea	-allows a differentiation between exogenous and endogenous lysine -suitable to estimate true digestibility in commercial diets -indirect determination of true ileal digestibility of other AA apart from lysine -allows evaluation of ingredients specific factors -suitable for practical diets	-it is difficult to achieve optimum guarnidination -assumption of constant profile of AA in endogenous secretions may not be valid -possibility of microbial degradation of HA -may not be suitable for long term studies to avoid possible lysine deficiency because HA is not used for protein synthesis