CHAPTER II

LITERATURE REVIEWS

2.1 Overview of beef cattle production

It is widely stated that, following on from the Green Revolution of the late twentieth century we are now seeing the Livestock Revolution. Whereas the former was supply-led, the latter is demand driven. In many developing countries and especially South-East Asia (SE-Asia), livestock production is growing at unprecedented rates. This process is driven by the increasing demand of animal products that is a consequence of population growth, urbanization and income growth (Rowlinson et al., 2005). Beef cattle production is involved in this world Livestock Revolution, thus an overview of the beef cattle production situation is reviewed as follows:

2.1.1 Beef production situation in the world

Data of FAOSTAT (2010) (Table 2.1) indicates that the world cattle population is gradually increasing. Developing countries and especially those in Southeast-Asia (SE-Asia) show an increase in cattle population, while cattle production in developed countries such as Europe shows a decline over the last decade. The increase in demand for livestock products and the increased livestock numbers and feed requirements which follow, have been referred to as the Livestock Revolution (Delgado et al., 1999; Rowlinson et al., 2005).

Developing countries in SE-Asia show an increasing cattle population (Table 2.2). Data of FAOSTAT (2010) showed that Myanmar had the largest number of cattle through the last decade. Thailand and Viet Nam had similar cattle populations, followed by Cambodia, while The Lao People's Democratic Republic (Lao PDR) and Malaysia had a relatively small population of cattle in the region.

The global human population is increasing and it is believed that the food supply will become inadequate to meet the future demand. Consumption of beef and dairy products is expanding in the Indochinese peninsular nations because of lifestyle changes. It is expected that the expansion of demand will continue in the future.

Table 2.1 Cattle population in some regions of the world from 1999 to 2008 (million head)

Year	World	Africa	Americas	Asia	Europe	Oceania
1999	1314.14	228.65	456.51	442.25	150.61	36.11
2000	1315.88	227.92	460.33	443.33	146.96	37.34
2001	1317.53	231.90	468.17	437.15	142.56	37.75
2002	1325.88	237.60	478.17	430.98	140.90	38.23
2003	1336.64	241.01	492.65	427.37	138.49	37.11
2004	1344.22	243.15	501.67	427.10	134.51	37.79
2005	1350.57	251.51	503.13	426.90	131.01	38.01
2006	1362.05	255.42	507.90	431.61	128.40	38.73
2007	1360.61	261.48	504.48	428.44	127.77	38.45
2008	1347.47	269.96	480.94	430.95	127.15	38.47

Source: FAOSTAT (2010)

Table 2.2 Cattle population in some countries in South-East Asia from 1999 to 2008 (million head)

Year	Cambodia	Lao PDR	Malaysia	Myanmar	Viet Nam	Thailand
1999	2.83	1.00	0.71	10.74	4.06	4.76
2000	2.99	1.16	0.73	10.98	4.13	4.60
2001	2.87	1.22	0.74	11.24	3.90	4.64
2002	2.92	1.22	0.75	11.55	4.06	4.82
2003	2.99	1.24	0.75	11.73	4.39	5.05
2004	3.04	1.28	0.79	11.94	4.91	5.30
2005	3.18	1.27	0.78	12.12	5.54	5.61
2006	3.34	1.32	0.77	12.36	6.51	6.04
2007	3.37	1.35	0.79	12.63	6.72	6.48
2008	3.46	1.50	0.79	12.93	6.34	6.70

Source: FAOSTAT (2010)

2.1.2 Beef production situation in Thailand

Animal production should have a key role in nutrient recycling and should be one of the important factors in sustainable crop production, agriculture economy and creation of employment. Accompanying economic development, the demand for meat and milk have been rapidly increasing, consequently the enlargement of meat and milk production is an urgent necessity in Thailand.

As reported by Department of Livestock Development; DLD (2010) from year 1999 to 2008 (Table 2.3), cattle are the largest population of ruminants in Thailand through the last decade. The cattle population (summation of beef and dairy cattle) is dramatically increasing. On the other hand, the buffalo population is gradually decreasing, and the goat and sheep populations are relatively small compared to cattle and buffalo.

Table 2.3 Ruminant livestock population in Thailand from 1999 to 2008 (head)

Year	Cattle	Buffalo	Goat	Sheep
1999	4,918,396	1,799,606	132,845	39,485
2000	5,208,541	1,702,223	144,227	37,312
2001	5,571,283	1,710,095	188,497	42,720
2002	5,908,625	1,617,358	177,944	39,326
2003	5,916,323	1,632,706	213,917	42,883
2004	6,668,332	1,494,238	250,076	47,811
2005	8,275,108	1,624,919	338,355	50,779
2006	8,036,057	1,351,851	324,150	51,151
2007	9,337,985	1,577,798	444,774	50,963
2008	9,582,030	1,359,807	374,029	43,738

Source: DLD (2010)

Beef cattle population in Thailand in the last decade is shown in Table 2.4. The data from DLD (2010) indicate that the beef cattle population in various regions of the country and overall have tended to increase throughout the decade.

Furthermore, the data of import-export beef cattle in Table 2.4 shows that throughout the decade, the import of beef cattle tended to decreased while the export number fluctuated. However, the export number for the year 2008 was dramatically highest in the decade.

Table 2.4 The database of beef cattle population in various regions of Thailand from 1999 to 2009 (head)

Year	Central Region	North-Eastern Region	Northern Region	Southern Region	Total	Export	Import
1999	855,232	2,219,437	875,403	685,669	4,635,741	4,064	126,319
2000	849,237	2,522,961	943,251	585,165	4,900,614	2,160	104,661
2001	1,022,264	2,573,233	1,025,750	606,357	5,227,604	3,344	185,319
2002	936,075	2,910,823	1,132,292	570,995	5,550,185	3,955	133,114
2003	984,069	3,078,149	1,297,460	556,645	5,916,323	4,212	71,844
2004	1,001,425	3,693,782	1,326,987	646,138	6,668,332	4,739	102,589
2005	1,296,820	4,092,206	1,636,851	770,395	7,796,272	1,074	83,784
2006	1,315,270	4,316,949	1,564,797	839,041	8,036,057	814	51,782
2007	1,516,298	4,501,769	1,953,406	876,919	8,848,392	4,806	13,548
2008	1,553,668	4,931,389	1,847,601	779,435	9,112,093	68,974	13,191
2009	1,496,033	4,655,444	1,677,932	766,019	8,595,428	-	-

Source: DLD (2010)

Animals have been selected globally by farmers for their particular characteristics or cultural value, whilst they were also adapting genetically to local conditions, diseases, available feed, climate, predators and many other persistent variables imposed by the local environment. The result has been the development of breeds that contribute to local, national and eventually global needs and demands. In Thailand, beef cattle breeds are selected for the same reasons. With regard to breeding by farmers, it is difficult to collect a breed database. However, DLD (2010) reported the beef cattle breeds in Thailand may be divided into two types, thus, Thai native cattle and exotic purebred plus crossbred.

DLD (2010) reported the population of Thai native cattle and exotic purebred plus crossbred as shown in Table2.5. The population of both types of cattle throughout the years 2000 to 2008 tended to increase with the Thai native cattle population having the edge. However, in 2009, Thai native cattle population decreased while exotic purebred plus crossbred population were still increasing.

The trend of cattle production in the world and also SE-Asia, including Thailand, is increasing. This indicates that the demand for meat is increasing and suggests an economic opportunity for the beef cattle farmer. However, for an efficient beef cattle

production system the various factors such as breed of cattle, feed and feeding management should be integrated together.

Table 2.5 The database of Thai native cattle and exotic purebred plus crossbred beef cattle population in Thailand, from 1999 to 2008 (head)

Year	Thai native cattle		Exotic purebred and crossbred		Total	
	Head	No. of farmer household	Head	No. of farmer household	Head	No. of farmer household
2000	3,270,552	-	1,630,062	-	4,900,614	855,384
2002	3,637,640	-	1,912,545	-	5,550,185	962,377
2004	4,907,289	-	1,761,039	-	6,668,328	1,020,657
2006	5,655,470	944,453	2,380,587	351,202	8,036,057	1,226,005
2008	6,365,620	1,002,576	2,746,473	400,198	9,112,093	1,331,561
2009	5,442,415	953,913	3,153,013	488,122	8,595,428	1,369,718

Source: DLD (2010)

2.2 Ruminant nutrition and feeding

The food of ruminants, forage and fibrous roughages, consist mainly of β-linked polysaccharides such as cellulose, which cannot be broken down by mammalian digestive enzymes. Ruminants have a special system of digestion that involves microbial fermentation of food prior to its exposure to their own digestive enzymes (McDonald, 2002). A grazing ruminant optimizes its utilization of cellulose carbohydrates by virtue of the arrangement of its digestive tract, in which the fermentation chamber (reticulo-rumen) precedes the main site of digestion. In this way, the fermentation products are used most efficiently. The fermentation of carbohydrates as an energy source results in the production of heat and methane. Hence, ruminants have an advantage over nonruminants because digestive processes unlock the energy in large molecules (Van Soest, 1994). Ruminants benefit through their special symbiotic relationship with microbes in the rumen and their ability to utilize ruminal fermentation products.

The reticulo-rumen provides a continuous culture system for anaerobic bacteria, protozoa and fungi. Food and water enter the rumen and the food is partially fermented to yield principally volatile fatty acids (VFAs), microbial cells and the gases methane and

carbon dioxide. The gases are lost by eructation and the VFAs are mainly absorbed through the rumen wall. The microbial cells, together with undegraded food components, pass to the abomasum and small intestine; there they are digested by enzymes secreted by the host animal, and the products of digestion are absorbed (McDonald, 2002).

As the microbial mass synthesized in the rumen provides about 20 percent of the nutrients absorbed by the host animal, the composition of microorganisms is important. The bacterial dry matter is about 100 g/kg, but only 80 percent of this is in the form of amino acids, the remaining 20 percent being present as nucleic acid nitrogen (N) (McDonald, 2002).

Nutrient balance in the diet is an important way to optimize animal production. However, in the case of ruminants this can be complicated, because of the complexity of rumen fermentation. There are many factors involved in rumen fermentation such as ruminal pH (Firkins, 1996), ammonia concentration (Satter and Slyter, 1974; Song and Kennelly, 1990; Wanapat and Pimpa 1999), temperature in the rumen (Wanapat, 1999), types of feed and roughage (Wanapat, 2000) and rumen availability of protein and carbohydrate (Nocek and Russell, 1988).

2.3 Tropical feed resources and utilizations

Throughout the world a wide variety of feedstuffs is available for feeding animals, depending on what can be grown in particular regions (Kellems and Church, 2002). Tropical forages are low in protein and have high cell wall contents resulting in low digestibility (Mupangwa et al., 2000). Tropical forages and some concentrate feedstuffs have a large proportion of lignified cell walls with low fermentation rates and digestibility, leading to low digestibility rates and limited intake (Ibrahim et al., 1995; Hindrichsen et al., 2001). Thus, ruminant livestock farmers are facing a critical shortage of feed supplies. A major constraint to livestock production in tropical areas is the scarcity and fluctuation in quantity and quality of the year-round feed supply. Particularly during the dry season, the natural pastures drop in quantity and quality, especially in energy and nitrogen content, causing low productivity (Wanapat, 2004).

Forage grass is the major roughage source for ruminant livestock such as beef and dairy cattle in Thailand. There are many tropical forage grasses including natural pastures and cultivated forages. Ruminants may be fed a variety of feedstuffs ranging from very

poor quality forage, and grain straw to almost 100% grain diets, similar to a non-ruminant diet. The type of feed consumed by the ruminant influences the microbe ability to digest feedstuffs and overall ability of the animal to live and grow. Farmers need quality feedstuffs for the animals. However, nutritive values vary depending on the plant parts, stage of maturity, soil fertility, environment, season and plant varieties (Wanapat, 1999).

In general, feedstuffs for ruminants in Thailand are produced locally. However, some non-ruminant feedstuffs are imported, including corn, soybean meal and fish meal (Chumpawadee, 2006a). Farmers are able to purchase concentrate feedstuffs in bulk to prepare their own mixture and supply roughage from the farm.

Numerous data of feed source and utilization are available in Thailand and South-East Asia. In the past, the Animal Nutrition Division, Department of Livestock Development established a table of feedstuffs composition (DLD, 2004). After a few years, The Working Committee of Thai Feeding Standard for Ruminant (WTSR, 2008) established the resource "Nutrient Requirement of Beef Cattle in Thailand". The table of chemical composition and nutritive value of feedstuffs are included. The database was collected from various sources such as Department of Livestock Development (Animal Nutrition Division), Universities and private companies. However, some data are calculated using equations e.g. Haris et al. (1982), Manke et al. (1979) and Menke and Steingass's (1988) equations. Thus, more investigation of tropical feed resources and their utilization is needed for more accurate and locally appropriate data.

2.4 Ruminant feed evaluation system

The nutritive value of a feed is assessed by the amount of nutrients it contains (chemical composition), digestibility and level of voluntary feed intake or feeding (Ibrahim et al., 1995). Feed evaluation methods are use to determine the nutritive value of feed. A basic description of feeds enables a prediction of the performance of animals offered the feeds (Medsen et al., 1997). There are many methods used in feed evaluation such as chemical analysis, degradability measurement, digestibility measurement and feed intake prediction. The goal of this review is to briefly describe the various feed evaluation methods.

2.4.1 Chemical composition analysis

2.4.1.1 Proximate analysis

Proximate analysis is the old scheme of laboratory analysis that allows comparison of feeds on the basis of a specific nutrient and, to some extent, prediction of components of animal performance (Galyean, 1997). Proximate analysis was developed over 100 years ago by two German scientists, Henneberg and Stohmann (McDonal et al., 2002). Frequently, proximate analysis is called the Weende system, in honor of the Weende Experiment Station where the experiments were carried out (Crampton and Harris, 1969).

Proximate analysis divided the feed into six fractions: Water or moisture, ether extract (EE), crude fiber (CF), ash, crude protein (CP) and nitrogen free extract (NFE) (Crampton and Harris, 1969).

Water or moisture, the simplest of all substances in food, is not the simplest to determine (Crampton and Harris, 1969). However we can express values in terms of dry matter (DM). The dry matter of feedstuffs is usually determined by oven drying at 60 or 100 °C. Silages require special treatment (e.g. toluene) in DM determinations due to their high content of volatile organic acids; thus DM is usually determined by distillation (France et al., 2000).

Ash is the inorganic residue from the burning of the sample at about 600 °C (Crampton and Harris, 1969). The residue or ash can used to determine the content of individual mineral elements in the feedstuffs. Ash content can be converted to OM content by the following equation; % OM = 100 - % ash.

Crude fiber value is obtained by subjecting the sample to successive treatments with boiling acid and alkali solution; the organic residue is the CF. The CP content is calculated from the nitrogen (N) content, determined by Kjeldahl procedure, assuming that all nitrogen in the feed is present as protein and that all feed protein contains 160 gN/kg (McDonald et al., 2002), then CP= total N x 6.25. More recently, Dumas methods, involving combustion and determination of released gaseous N, are being use (France et al., 2000).

Ether extract fraction is determined by subjecting the food to continuous extraction with petroleum ether for a fixed period. As well as lipids such a fraction contains organic acid, alcohols and pigments.

Nitrogen-free extract is calculated by subtraction from 100 of the sum of moisture, ash, crude protein, ether extract and crude fiber, expressed in percentage (% NFE = 100 - (% moisture + % ash + % crude protein + % crude fiber + % ether extract)). The nitrogen-free extract is a practically useful index of the non-cellulose portion of feed carbohydrates, and is primarily a nonspecific source of energy to the animal (Crampton and Harris, 1969).

2.4.1.2 Detergent fiber analysis

This system is a modification of proximate analysis which uses improved methods for estimating the content of fiber. The insoluble fiber in feed includes the cross linkages of the plant cell wall (Van Soest et al., 1991). Hemicelluloses, cellulose and lignin are considered structural components of cell walls, and are defined chemically as neutral detergent fiber (NDF). Hemicelluloses and cellulose fractions are broken down into simpler sugar units and utilized by rumen microorganisms; lignin is indigestible.

It has recently been accepted as the standard technique for analysis of forages. The procedures have varied because of the use of different amylases in attempts to remove starch interference (Van Soest et al., 1991). Therefore Van Soest et al. (1991) recommended two procedures for NDF determination to remove starch interference; the first one uses heat-stable amylase (amylase, Number A3306; Sigma Chemical Co., St. Louis, MO) and the second one uses 8 mole urea plus amylase. Additionally, Van Soest et al. (1991) also recommended a procedure for acid detergent fiber (ADF) and acid detergent lignin (ADL) determination.

2.4.1.3 Near-infrared reflectance spectroscopy

Near-infrared spectroscopy (NIRS) is a physical method, which depends on the measurement of light absorption by the surface of a sample using wavelengths in the infrared region of the spectrum (1100-2500 nm) (Deaville and Flinn, 2000). NIRS is based on the assumption that the spectrum of radiation absorbed and emitted by the organic components of feed is similar between feeds of the same chemical and biochemical composition. It uses calibrations and computer prediction models which compare NIRS spectra of feeds to the chemical analysis (France et al., 2000). This technique is more rapid, more precise than traditional assays and relatively inexpensive. However, the technique ultimately relies on a set of standard samples whose composition has been determined by traditional methods.

Agnew et al. (2004) found that NIRS provides accurate predictions of chemical composition in dried grass analysis. NIRS reports usually include dry matter, crude protein, acid detergent fiber, neutral detergent fiber, total digestible nutrients (TDN), net energy for maintenance and lactation of dairy cattle, Ca, P, Mg and K. Moreover, this technique has been applied to prediction of dry matter digestibility (Russell et al., 1989), organic matter digestibility (Adesogan et al.,1998), crude protein degradability (Antoniewicz et al., 1995; Tremblay et al., 1996), and metabolizable energy (ME) concentration (Givens et al., 1992). Nousiainen et al. (2004) had success in using NIRS to predict indigestible neutral detergent fiber of grass silage.

2.4.2 *In vivo* feeding trials method

In vivo is the conventional method for feed evaluation in ruminants, and involves feed intake and digestibility of nutrients. The digestibility and intake are responsible for total animal response.

2.4.2.1 Feed intake

Feed intake is a factor of considerable importance in that the more food an animal consumes each day, the greater will be the opportunity for increasing its daily production. An increase in production that is obtained from higher food intake is usually associated with an increase in overall efficiency of the production process, since maintenance costs are decreased proportionately as productivity rises (McDonald, 2002).

Factors that affect and regulate dry matter intake (DMI) by ruminants are complex and not understood fully (NRC, 2000; Allen, 2000; Frobes, 2003). However, NRC (2000) suggest that several factors alter animal feed intake such as numerous physiological conditions (e.g. animal body composition, sex, age, growth stage, body weight or frame size), environmental conditions, and management factors have been identified as affecting feed intake. It is influenced by the interplay of external and internal factors. The external factors include some environmental and dietary cues. The internal factors are physical and physiological in nature. Physical factors include cow size and gut capacity as well as the fiber content of the diet. Forages of low quality, physical factors, for instance rumen capacity, contribute to decreased feed intake. Changes in intake can be related to lactation, where lactating cows consume more feed than non lactating cows of the same weight, and on the same diet (Chumpawadee, 2006).

2.4.2.2 Digestibility

The total collection of feces (digestion trial) is conducted to evaluate diets with regard to nutrient availability. The standard method proposed by Van Soest (1994) is as follows. Livestock are subjected to a preliminary period of at least two weeks to eliminate feed residues from the previous diet from the digestive tract. This period is used to establish the level of intake. The diet is then fed at a level at which orts are absent or their quantity controlled. The feed should be chopped to eliminate selection. The collection should continue for 5-10 days to ensure a constant average fecal production to minimize the effect of diurnal variations. Feces must be collected daily and mixed and sampled to represent an average for the whole collection period for each animal. Samples must be dried at temperatures below 65 °C to avoid formation of artifacts, and if nitrogen balances are to be accurately measured, a frozen sample is required to retain any volatile nitrogen, which would be lost on drying.

Determination of feed intake and digestibility by using *in vivo* methodology is time-consuming, laborious, expensive and requires a large quantity of feed. It is also unsuitable for large scale feed evaluation laboratories (Chumpawadee, 2006). Thus, many scientists have attempted to develop regression equations to predict feed intake and digestibility (Chemiti et al., 1996; Karsli and Russell, 2002). Blummel and Ørskov (1993) used *in vitro* gas production and nylon bag degradability of roughage to predict feed intake. Khazaal et al. (1993) also reported the use of *in vitro* gas production and nylon bag degradability to predict the apparent digestibility *in vivo* and voluntary feed intake of hays in sheep.

2.4.3 *In sacco* method

The intraruminal incubation or *in sacco* method of sampling in cloth bags is preferred by those who wish to avoid the details of anaerobic techniques. Cloth bags are inserted into the rumen through a fistula (Van Soest, 1994). This technique is a direct method of measuring the rumen degradation and involves suspending a small amount of feedstuff in an undegradable porous bag in the rumen and measuring the disappearance of feed components after incubation (Norziere and Michalet-Doreau, 2000). The site of incubation in the rumen must be controlled and is best near the bottom. A major problem has been the integrity of the cloth bag as an analytical filter. Improved methods utilize cloths of specific pore size and control the ratio of the sample weight to surface area of

the bag. Bag with a large ratio of surface area to sample size minimize the error. Smaller pore sizes retard the entry of organisms and thus inhibit optimum fermentation, while larger ones permit escape of lignified particles (Van Soest, 1994).

Chumpawadee (2006) reviewed that, initially, the method was successfully used to assess different feedstuffs and to determine the effects of formaldehyde treatment on degradation of protein supplement (Michalet-Doreau and Ould-Bah, 1992). Ørskov et al. (1998) have suggested the use of kinetics of fermentation data to improve the estimation of nutritive value of feeds when both *in vitro* and *in sacco* methods are considered.

The nylon bag technique or *in sacco* method is a very robust and powerful tool with which to study aspects of nutrition in ruminants. It is particularly useful in describing degradation characteristics of protein, roughages and also for rumen environment studies (Ørskov and Shand, 1997). Although the nylon bag technique is widely used to determine kinetics of degradation of feedstuffs, there are many factors which should be considered to standardize the methodology (Chumpawadee, 2006).

2.4.4 *In vitro* methods

The sequence of all *in vitro* rumen procedures is anaerobic fermentation of sample substrate with medium then filtered rumen liquor is taken followed by an end-point measurement. The medium is usually a buffer solution simulating ruminant saliva. Unlike the rumen, *in vitro* systems do not have continual supply of saliva, which might supply nitrogen. The time of batch fermentation is commonly 48 h for digestibility estimation, although other time periods from 3 h to several hundred hours have been used to estimate rate of fermentation (Van Soest, 1994).

In vitro methods for laboratory estimations of degraded feeds are important for ruminant nutritionists. An efficient laboratory method should be reproducible and should correlate well with actually-measured *in vivo* parameters. *In vitro* methods have the advantage not only of being less expensive and less time-consuming, but they allow one to maintain experimental conditions more precisely than do *in vivo* trials. Chumpawadee (2006) reviewed that there are three major biological digestion techniques currently available to determine the nutritive value of ruminant feeds: 1) digestion with rumen microorganisms as in Tilley and Terry (1963) or using a gas method, 2) *in situ* incubation of samples in nylon bags in the rumen, and 3) cell-free fungal cellulase. These

biological methods are more meaningful since microorganisms and enzymes are more sensitive to factors influencing the rate and extent of digestion than are chemical methods.

2.4.3.1 Tilley and Terry method

The Tilley and Terry system (Tilley and Terry, 1963) involves two stages: 48-h digestion with rumen organisms followed by 48-h digestion with pepsin in weak acid (about pH 2). The residue is composed of undigested plant cell walls and bacterial debris and gives digestibility values comparable to *in vivo* apparent digestibility. The success of the method is related to the recovery of indigestible cell wall matter and its similarity to the ruminant digestion sequence. The main disadvantage of Tilley and Terry system is the long time required to do the analysis and the number of steps (Van Soest, 1994). Nowadays many researchers have modified this method. Tessema and Baars (2004) use this method to evaluate *in vitro* dry matter digestibility of Napier grass mixed with different levels of Sesbania (*Sesbania sesban*). Melaku et al. (2003) used Tilley and Terry method as modified by Van Soest and Robertson (1985) to determine *in vitro* dry matter digestibility (IVDMD) of selected multipurpose trees, wheat bran and Lab Lab legume (*Lablab purpureus*). In both cases they suggested that this method could be an alternative to evaluate the nutritive value of feeds.

2.4.3.2 *In vitro* gas production technique

The association between fermentation and gas production has long been known. A competitive system was developed by Manke et al. (1979) and Menke and Steingass (1988) using large-bore syringes as the measuring device. This system has been successful in digestibility and metabolizable energy studies by relating gas production to organic matter fermented. *In vitro* cumulative gas production techniques were developed to predict fermentation of ruminant feedstuffs. A feedstuff is incubated with buffered rumen fluid and gas produced is measured as an indirect indicator of fermentation kinetics. When a feedstuff is incubated with buffered rumen fluid, it is first degraded and the degraded fraction may either be fermented to produce gas and fermentation acids, or incorporated into microbial biomass. When combined with measures of degradation, gas production techniques provide a measure of the proportion of feed that is fermented as opposed to that which is partitioned to microbial growth (Rymer et al., 2005).

Direct displacement of a plunger by fermenting a feedstuff within a glass syringe was developed by Czerkawski and Breckenridge (1975) and was the basis of the 'Hohenheim Gas Test' later developed by Menke et al. (1979). Blummel and Ørskov (1993) modified the technique by incubating syringes in a waterbath rather than a rotating incubator. The syringe technique was originally developed to determine end-point fermentability of feedstuffs, at 24 h. The *in vitro* gas method based on syringes appears to be the most suitable for use in developing countries (Blumel et al., 1997b). One of several methods of Menke and Steingass (1988) modified the gas production technique to describe the kinetics of fermentation based on the exponential model: $P= a+b(1-e^{-ct})$ of Ørskov and McDonald, 1979, where p describes gas production at time t, a the gas produced (ml) by instantaneous fermentation of the soluble and readily-available fraction of feed, b the gas produced (ml) by the fermentation of insoluble but slowly fermentable fraction and c the fractional rate (rate constant) at which gas is produced per hour (%/h).

Recently, the *in vitro* gas production technique was proposed for determining fermentation kinetics of ruminant feed (Menke et al., 1979; Menke and Steingass, 1988; Blummel and Ørskov, 1993). It has provided better predictions of the *in vivo* digestibility than other techniques (Khazaal et al., 1993). The technique is gaining popularity because it is a low cost, highly reproducible and easy method of obtaining a dynamic description of the nutritive value of feedstuffs, while at the same time allowing for more samples to be analyzed (Chumpawadee, 2006).

1) Factors affecting gas measurements

The gas production technique has advantages and disadvantages (Gatechew et al., 1998a). Therefore, before attempting to evaluate nutritive value some factors affecting gas measurement should be considered. Chumpawadee (2006) has reviewed the factors affecting gas measurements as follows:

(1) Sample size and preparation; there is a highly significant linear correlation between the amount of substrate and amount of gas produced. Digestibility measurement will require the use of either small samples or equipment able to handle large gas volumes. And also substrates should be milled using a 1 mm screen to allow more precise sampling and to discount the particle size effect on the grounds that chewing and rumination in the animal will produce a result similar to grinding.

(2) Buffer and inoculum: The buffer should neutralize the volatile fatty acids produced during fermentation in order to keep constant pH. Secondly, the buffer should supply all necessary minerals for optimal microbial activity. For the quantitative gas measurement using this buffer, it is important that the pH be held within the range 6.8-6.2. Therefore, the quantity of feed incubated in the *in vitro* system must be set in relation to the volume of buffered rumen fluid medium.

The inoculums also have a considerable influence on *in vitro* gas production. The composition of rumen fluid will vary from day to day and from animal to animal, and these variations may affect the *in vitro* digestion profile. The recommended time for taking the rumen fluid is before feeding, because it is most constant in its composition and activity. Moreover, at least two animals as donors of rumen fluid mixture is recommended as this guarantees a greater constancy of activity.

(3) Incubation condition and time of reading: Incubation vessels, i.e. syringes or bottles, should be kept in a water bath or an incubator at 39±0.5 °C. Time of reading can be selected to suit the type of substrate which is being incubated. For forages, it is generally accepted to read after 3, 6, 12, 24, 48, 72 and 96 h but, for concentrate type substrates it may be necessary to take more frequent readings in the first 24 h.

2) Applications of the *in vitro* gas production technique to estimate metabolizable energy

Researchers have attempted to apply this method to describe nutritive value of feedstuffs. Menke et al (1979) and Menke and Steingass (1988) reported a strong correlation between metabolizable energy (ME) values measured *in vivo* and predicted from 24 h *in vitro* gas production and chemical composition of feed. However Getachew et al. (2002) found that ME values predicted by the gas production technique by laboratories in different parts of the world cannot be considered absolute.

Nevertheless, this technique has also been used to assess the presence of anti-nutritive compounds in different feedstuffs, e.g. tropical forage plants and browse plants. Tropical forage plants may contain secondary compounds such as the poly phenolic tannins, the terpene or steroid-based and nitrogenous alkaloids (Schofield, 2000).

Presently, there is intense interest in describing the kinetics of fermentation from gas production profiles (Getachew et al., 1998). However the exponential model of Ørskov and Mcdonal (1979) seems to be the most popular.

2.4.5 Eating behavior study

The physical characteristics of feed have a significant effect on animal eating behavior. For ruminants such as beef cattle which are traditionally fed roughage as basal diet, roughages are the major fiber sources. Mertens (1997) stated that, biologically, NDF or its inverse, neutral detergent solubles, have been related to intake, feed density, chewing activity, digestibility, rate of digestion, and depression of digestibility associated with high levels of intake. These physical characteristics can influence animal health, ruminal fermentation and utilization, animal metabolism, and milk fat production independently of the amount or composition of chemically measured NDF.

Allen (1997) stated that ruminants require roughage in their diets to maximize production and to maintain health by sustaining a stable environment in the rumen. The ability of roughages to stimulate chewing has been investigated extensively because of the relationship between chewing and the flow of salivary buffers into the rumen, which are required to neutralize fermentation acids. The time spent chewing per unit of DM could be used as an index of roughage value, and many feedstuffs have been characterized for total chewing time. Luginbuhl et al (2000) found that steers sorting during eating and a shorter particle length also may be responsible for the higher DMI and higher intake rate of the silage. Hay consumption also may have been limited by the amount of saliva needed to moisten the forage for swallowing. In addition, steers spent more time eating hay/kilogram of NDF intake than silage. Luginbuhl et al (2000) surmised that total saliva output, ruminal buffering capacity, and ruminal pH were higher for animals consuming hay.

Krause et al (2002) reported that cows spent less time ruminating per day and per kilogram of NDF intake when forage particle size was decreased. Total time spent chewing per day and per kilogram of NDF intake per day increased with increasing forage particle size However, more research is needed to quantify the effects of ruminally fermentable carbohydrates on cow health and production, so that both fermentation acid production and physically effective fiber can be considered when formulating and evaluating cattle rations.

The time that cattle spend eating and ruminating amounts to 13 to 17 h/d when animals are given *ad libitum* access to diets that contain a high proportion of roughage. The capacity of ruminants for mechanically reducing feed particle size can be a

limiting factor for feed intake. In addition, the energy requirement for chewing accounts for a considerable proportion of the total energy requirement. In low-quality roughages (i.e., straw), the energy requirement for eating can amount to 25% of the ME of the feed in horses. Energy needed for chewing thus reduces the amount of ME available for production, and this can have a substantial effect on productivity, particularly at low levels of production. This fact might be mainly responsible for the lower efficiency of utilization of ME in roughages than in other feedstuffs. Therefore, when treatment methods used to improve roughage quality are assessed, the energy need for mechanical reduction of particle size should be considered in addition to other effects of roughage treatment (Susenbeth et al 1998).

Several researchers (Luginbuhl et al. 2000; Krause et al. 2002) have demonstrated that chewing activity is a characteristic that reflects the chemical and physical properties of feeds (NDF, particle size, intrinsic fragility, and moisture) and affected energy requirement (Susenbeth et al, 1998; Susenbeth et al. 2004). Chewing activity (the sum of eating and ruminating time) is also a function of the type, size or age, and DMI of the animal and perhaps measurement technique.

2.5 Energy system

Energy is defined as the potential to do work and can be measured only in reference to defined, standard conditions; thus, all defined units are equally absolute. The joule is the preferred unit of expressing electrical, mechanical, and chemical energy (NRC, 2000). An energy system is essentially a set of rules relating the energy intake of an animal to its performance or productivity. The simplest energy system consists of two sets of figures, one set for the energy values of foods and the other for the energy requirements of animals (McDonald, 2002).

2.5.1 Energy unit

The unit of energy is the joule, which is produced when a force of one Newton acts through a distance of one meter, a force of one Newton being required to accelerate a mass of one kilogram one meter per second squared (1 m/sec^2) (Blaxter 1989). The older unit of heat, a calorie (cal) is defined as the amount of heat required to raise the temperature of 1 g of water from 14.5 to 15.5°C. The conversion of the calorie to the joule has now been arbitrarily standardized as 1 cal (calorie) = 4.184 J (joule). In

practice, both the joule and the calorie are so small that nutritionists work with multiple units: Kilojoule (KJ) and megajoule (MJ) are 10^3 and 10^6 times greater than one joule, respectively (ARC, 1980; Blaxter, 1989; NRC, 2000; WTSR, 2008).

2.5.2 Energy partition

The animal obtains energy from its food. The quantity of chemical energy present in a food is measured by converting it to heat energy and determining the heat produced. This conversion is carried out by oxidizing the food by burning it; the quantity of heat resulting from the complete oxidation of unit weight of a food is known as the gross energy or heat of combustion of the food (Mc Donald, 2002). The utilization of food energy during digestion and metabolism by an animal is shown schematically in Figure 2.1.

Agricultural Research Council; ARC (1980), Blaxter (1989), NRC (2000) and The Working Committee of Thai Feeding Standard for Ruminant; WTSR (2008) described the maner of expressing energy value of feeds. Energy of the food minus the energy lost in the feces is termed digestible energy (DE). Metabolizable energy (ME) is defined as energy of the food minus fecal energy (FE), urinary energy (UE), and gaseous energy (GE) losses, or ME=DE-(UE+GE). The ME is an estimate of the energy available to the animal and represents an accounting progression to assess food energy values and animal requirements.

For most forages and mixtures of forages and cereal grains, the ratio of ME to DE is about 0.8 but can vary considerably depending on intake, age of animal, and feed source (ARC, 1980). However, this is only an approximation as the ME/DE ratio may vary considerably, being affected by the nature of the diet and the level of feeding (Garrett and Johnson, 1983; NRC, 2000). ME values are seldom determined in practice, however, because very few laboratories have the facilities and budgets to collect and analyze respiratory gases and urine (Van Soest, 1994; Pond et al., 2005).

The definition of ME and the energy balance identity indicate ME can appear only as heat production (HE) or retained energy (RE), that is, ME=HE+RE (NRC, 2000). As indicated by this relationship, one of the main values of ME is used as a reference unit and as a starting point for most systems based on the net energy (NE) concept. The value of feed energy for the promotion of energy retention is measured by determining the RE at two or more amounts of intake energy (IE). The NE of a feed or

diet has classically been illustrated by the equation; NE = ΔRE/ΔIE (NRC, 2000; Williams and Jenkins, 2003a).

By the nineteenth century it was generally recognized that feed value and animal requirements could be expressed in terms of available energy and protein. The principle of the total digestible nutrients (TDN) system expresses feed constituents on an equivalent basis relative to energy content (Van Soest, 1994). Nevertheless, TDN is similar to DE but includes a correction for digestible protein. TDN has no particular advantages or disadvantages over DE as the unit to describe feed values or to express the energy requirements of the animal. TDN can be converted to DE by the equation; 1 kg TDN = 4.4 Mcal DE (NRC, 2000).

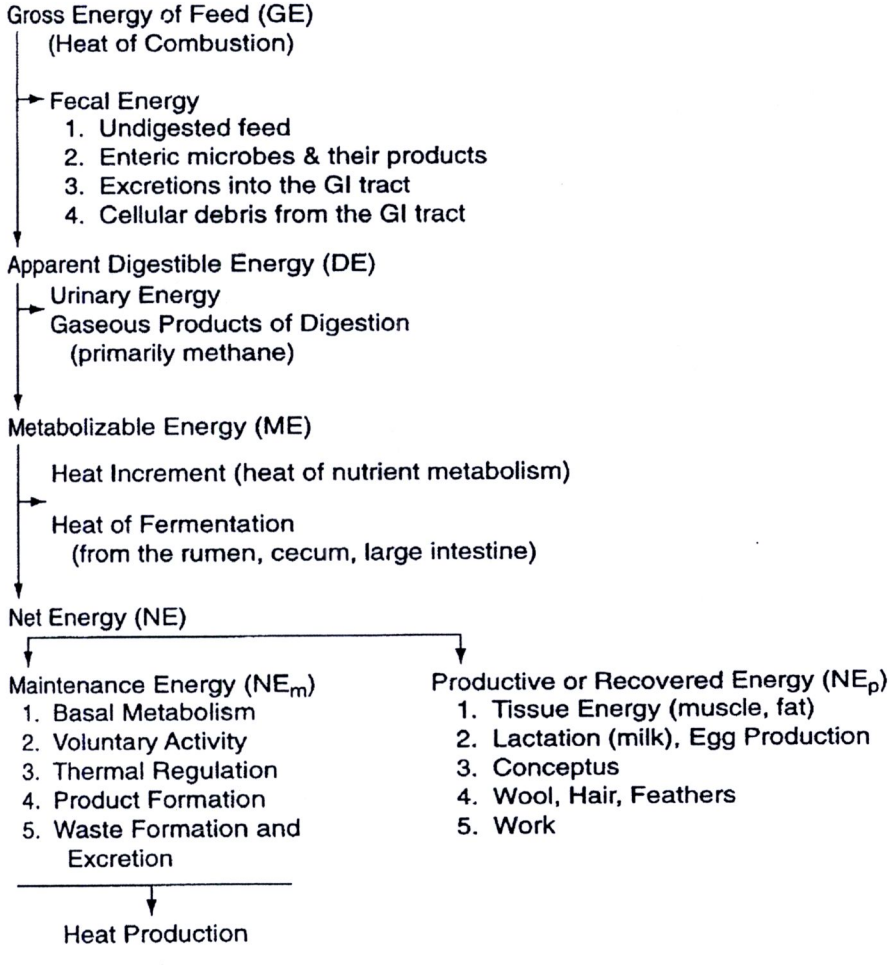

Figure 2.1 The utilization of food energy during digestion and metabolism by animal

Source: Pond et al. (2005)

2.6 Energy utilization in beef cattle

Beef cattle, as with other animals deprived of food, continue to require energy for functions of immediate necessity for life – for mechanical work of essential muscular activity, for chemical work such as the movement of dissolved substances against concentration gradients, and for the increased synthesis of body constituents such as enzymes and hormones (McDonald, 2002).

In the starving animal, the requirement for these purposes is obtained by the catabolism of the body's reserves, first of glycogen, then of fat and protein. In the fed animal, the primary demand on the energy of the food is in meeting this requirement for body maintenance and so preventing catabolism of the animal tissues. When the chemical energy of the food is used for muscular and chemical work involved in maintenance, the animal produces nothing and all energy is converted into heat. Energy supplied by the food in excess of that needed for maintenance is used for various forms of production (McDonald, 2002).

2.6.1 Energy requirement concept

The general relationship between MEI and RE is shown in Figure 2.7. The slope of the linear regression of RE on MEI provides an estimate of efficiency of utilization of ME for RE and in growing animals equates to k_g. By convention, the intercept of the regression of RE on ME intake is used to calculate fasting heat production (FHP), which equates to NE_m. The efficiency of utilization of ME for maintenance (k_m) is calculated as the ratio of NE_m to ME_m (NRC, 2000; McDonald, 2002).

When production is zero, RE would also be zero. In this case, if the animal is in weight equilibrium, this level of MEI would be referred to as the daily ME_m. When MEI < ME_m, body tissues will be mobilized to satisfy the energy deficit. In a productive animal, MEI is greater than ME_m. After accounting for ME_m, a part of the remaining MEI would be recovered as RE, and the remainder would be lost as heat energy. The term MEI – ME_m is the daily ME that is available for gain (ME_g) (Williams and Jenkins, 2003a). This system of partitioning of MEI is shown in Figure 2.2, with broken lines to show the summation of RE and heat increment of (H_iE_g) to give ME_g (ARC, 1980; McDonald, 2002; Williams and Jenkins, 2003a)

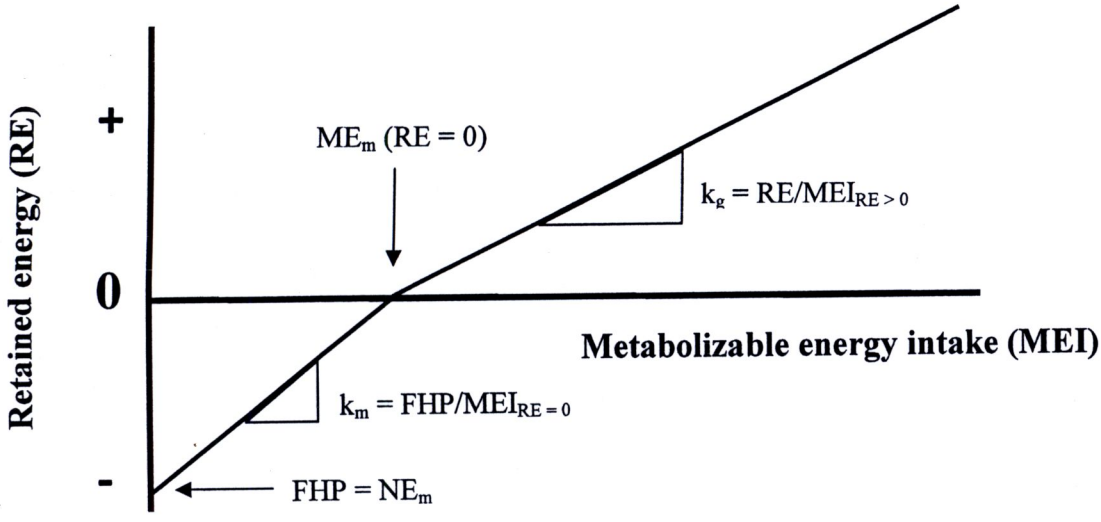

Figure 2.2 The relation between energy balance, metabolizable energy (ME) intake, fasting metabolism, maintenance, and gain.

Source: Adapted from McDonald et al. (2002)

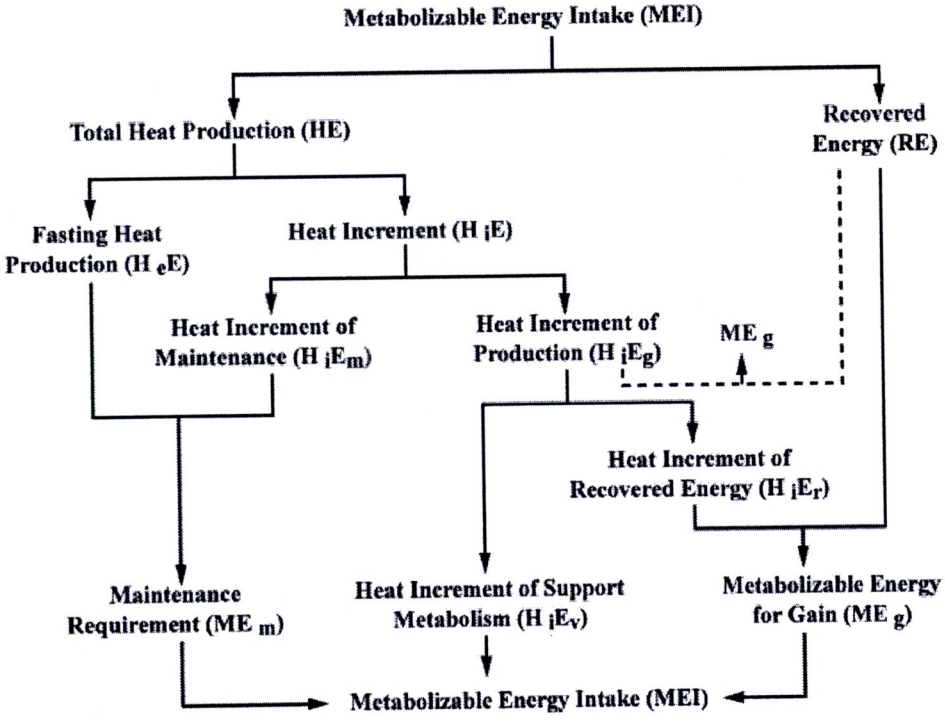

Figure 2.3 Partitioning of ME intake into recovered energy and heat production and the components of heat production.

Source: Williams and Jenkins (2003a)

The relationship between RE and MEI, the marginal efficiency is the ratio of an increment in RE to the increment in energy intake when two feeding levels are compared (Williams and Genkins, 2003c). ARC (1980) proposed that food intake be expressed in terms of ME to account for any effects of increasing food intake on digestibility, and suggested that no great error was involved if the continuous curvilinear relationship between daily rate of energy retention and rate of food intake expressed as ME was approximated by two straight lines, one applying from fasting to zero energy retention and the other for positive energy retention. Using a model in which incremental heat production above MEI at zero energy balance is treated as a single dynamic pool, RE would be represented by the following equation; $RE = k_g \times (MEI - ME_m)$ (Williams and Genkins, 2003c).

2.7 Method for measuring heat production and energy retention

Calorimetry means the measurement of heat. The methods used to measure heat production and energy retention in animals can be quite complicated, both in principle and in practice. So, animal calorimetry remains a specialized topic and few nutritionists become involved in it. The heat production of animals can be measured by physical methods.

2.7.1 Direct calorimetry

Direct animal calorimeters measure the total heat generated inside them and partition the heat into its two components, evaporative and non-evaporative. The latent heat of vaporization is derived mainly from the animal. This heat loss by the animal is transferred to the air in the form of increased humidity - the enthalpy of the air is increased. Heat loss can be measured directly (direct calorimetry) using either heat skin or gradient layer calorimeters for the chamber (Blaxter, 1967; Pullar, 1969; McLean and Tobin, 1987). However, due to the extremely high cost, both in construction and in operation, few direct calorimeters for farm animals are presently in use (McDonal et al., 2002; Pond et al., 2005).

2.7.2 Indirect calorimetry

Indirect calorimetry estimates heat production from quantitative measurement of materials consumed and produced during metabolism (McLean and Tobin, 1987). Indirect calorimetry is based on the principle that metabolic heat production is the result of oxidation of organic compounds (Blaxter, 1967; Flatt, 1969). For ruminants, the most commonly used equation to estimate total heat production (HE, heat energy) for indirect calorimetry is: $HE = 3.88\ 6O_2 + 1.200\ CO_2 - 0.518\ CH_4 - 1.431\ N$, where HE is in Kcal; O_2, CO_2 and CH_4 refer to gaseous exchange in liters; and N refers to urinary nitrogen in grams (Brouwer, 1965).

Most methods involve estimation of respiratory gas exchange and these may be classified according to their operating principles as confinement closed-circuit, total collection, and open-circuit systems (Abrams, 1961; Flatt, 1969; McLean and Tobin, 1987; Van Soest, 1994).

Firstly, in the closed-circuit type, the animal is enclosed in a temperature-controlled chamber. Air in the chamber is continuously circulated through an absorbent, which removes water and carbon dioxide. Oxygen use is determined as the amount of oxygen supplied to maintain pressure, and carbon dioxide production is determined from the amount collected by the absorbent. Methane production is calculated as the concentration difference between the beginning and the end of the test time multiplied by the volume of the system (Blaxter, 1967; McLean and Tobin, 1987; Johnson et al., 2003; Ferrell and Oltjen, 2008). In total collection system all the air expired by the subject is accumulated in order to measure subsequently its volume and chemical composition (McLean and Tobin, 1987).

Secondly, in the open-circuit, there are two major forms. In one the subject breathes directly from the atmosphere and by means of a non-return valve system, expires into a separate outlet line. In the second form, the subject inspires from, and expires to a stream of air passing. Oxygen, CO_2 and CH_4 concentration must be determined accurately in incoming and outgoing air. The rate of consumption or production of these gases is calculated as the concentration difference between incoming and outgoing air times air flow rate. Most calorimeters incorporate apparatus for measuring respiratory exchange and can therefore be used for indirect calorimetry as well (Blaxter, 1967; McLean and Tobin, 1987; Johnson et al., 2003; Ferrell and Oltjen, 2008).

2.7.3 Carbon-Nitrogen balance

It is also possible to estimate heat production approximately from carbon dioxide production alone. Alternatively heat production can be calculated accurately from the rate of turnover in the body of carbon and nitrogen (C-N balance) (McDonal et al., 2002).

The main forms in which energy is stored by the growing and fattening animal are protein and fat, for the carbohydrate reserves of the body are small and relatively constant (Pond et al., 2005). The methodologies for estimating heat production are based on this. The quantities of protein and fat stored can be estimated by carrying out a carbon and nitrogen balance trial. The energy retained can be calculated by multiplying the quantities of nutrients stored by their calorific values (Blaxter, 1967; McDonal et al., 2002). The C-N balance is frequently calculated in association with indirect calorimetric measurements. Whilst this eliminates the need of measurement of oxygen, it does involve measurement of the total quantity and chemical composition of all food consumed and excreta produced. Thus, the C-N balance is now not much practiced (McLean and Tobin, 1987).

2.7.4 Comparative slaughter

Because calorimetry experiments require elaborate apparatus and can be conducted with only small numbers of animals, numerous attempts have been, and are still being made to measure energy retention in other ways. Energy retention, however, can be measured in feeding trials if the energy content of the animal is estimated at the beginning and end of the experiment (McDonald, 2002). The comparative slaughter method (Lofgreen, 1965; Lofgreen and Garrett, 1968) is done by dividing the animals in two groups and slaughtering one (the sample slaughter group) at the beginning of the trial. Body composition and energy content of the animals slaughtered are determined. The latter are slaughtered at the end of the trial and energy content determined in the same manner as those in the sample slaughter group. Their energy gain or ER is then calculated as the difference in body energy contents between the initial and the final slaughter groups (Lofgreen and Garrett, 1968; McDonal et al., 2002; Ferrell and Oltjen, 2008).

The comparative slaughter method requires no elaborate apparatus, but is obviously expensive, laborious, and destructive. That is, each animal can be used only once, and is especially wasteful when applied to larger animal species (McDonald, 2002; Pond et al., 2005). Nevertheless, estimates of energy utilization obtained by comparative slaughter and specific gravity measurements have been used in the U.S.A. to establish a complete cattle feeding system (Chaokaur, 2009).

2.7.5 Feeding trials

In the feeding-trial method of measuring the energy requirement for growth, different levels of feed are fed to find the minimum one which will give a maximum rate of growth (Pond et al., 2005). The inclusion of slaughter tests to show the nature of the increase made provides valuable additional data in the case of meat animals, helping illustrate the feeding-trial method (ARC, 1980; NRC, 2000). The maintenance values obtained very simply from this method involves the determination of the amount of food required to hold adult animals at constant weight. Allowances can be made for changes in live weight by estimating the food equivalent of the losses or gains and correcting the observed intakes accordingly (McDonal et al, 2002).

Energy requirements for maintenance from feeding-trials are estimated by regression of average daily gain (ADG) and energy intake (EI). The linear regression equation is as follows; $EI = a + bADG$. The equation, when at the zero gain ($ADG = 0$), expresses the minimum of energy requirement for maintenance (Luo et al., 2004a, b). The maintenance values in the early feeding standards developed in the United States were based, for the most part, on data obtained in feeding trials. Also the recommended energy intakes for growth that are found in the feeding standards for various species have been arrived at primarily from feeding trial data (Pond et al., 2005).