NET ENERGY OF DRY EXTRUDED EXPELLED SOYBEAN MEAL FOR GROWING PIGS DETERMINED BY INDIRECT CALORIMETRY AND VALIDATION OF NET ENERGY SYSTEM USING A TYPICAL WESTERN CANADIAN GROWER-FINISHER DIET

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ABSTRACT

The objectives of this research were to determine the net energy (NE) of dry extruded expelled soybean meal (DESBM) using an indirect calorimetry (IC) in growing pigs, to determine the effect of multi-enzyme supplementation on the NE content of DESBM fed to growing pigs and to validate the NE system for feed formulation. In Exp 1, the values obtained with the IC method were consistently greater than those obtained with prediction equations. The discrepancy between the determination technique used was about 1% when diets were formulated with a constant protein content or corn:soybean meal ratio (1.0% and 0.7%, respectively), however, this was 4.1% when diet was formulated with simple substitution technique. Thus the NE value of DESBM was evaluated to be 2,548 kcal/kg DM when diets were formulated on a constant ratio between the other energy yielding components. In Exp 2, addition of enzyme increased the NE value of both the diet and the test ingredient; DESBM. Supplementation with multi-enzyme complex (Superzyme-OM, Canadian Bio-System Inc., Calgary, Canada) at 0.05% and 0.1% of the diet improved NE values of DESBM by 4.9 % and 3.7%, respectively. In Exp 3, the results indicate a better growth performance (ADG and G:F) when diets were formulated on NE basis compared to the DE system of diet formulation. Moreover addition of enzyme improved the performance for the NE system of diet formulation.

DEDICATION

This thesis is dedicated to my daughter Sasha, my wife Chandini Herman and my parents, Vanaja Velayudhan and Ettungalpadi Shanmughan Velayudhan.

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FOREWORD

This thesis was written in a manuscript format and it is composed of three manuscripts. Manuscript I was published as a short communication in the Energy and Protein Metabolism and Nutrition in Sustainable Animal Production – EAAP134. Manuscript II was presented at 2013 The American Dairy Science Association and The American Society of Animal Science (ADSA/ASAS) Joint Annual Meeting, Indianapolis. Manuscript III will be presented at 2014 ADSA/ASAS/CSAS Joint Annual Meeting, Kansas. All manuscripts were written according to the guidelines for the Journal of Animal Science manuscript preparation. Authors to manuscript I and II are D. E. Velayudhan, J. M. Heo and C. M. Nyachoti, while authors to manuscript III are D. E. Velayudhan and C. M. Nyachoti.

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LIST OF ABBREVIATIONS

AA	Amino acids
ADF	Acid detergent fibre
ADFI	Average daily feed intake
ADG	Average daily gain
AME	Apparent metabolizable energy
AME_n	Nitrogen corrected apparent metabolizable energy
ATP	Adenosine triphosphate
BW	Body weight
C	Carbon
CF	Crude fiber
CH_4	Methane
CH	Chemical composition method
CO_2	Carbon dioxide
CP	Crude protein
CS	Comparative slaughter
d	Day
DADF	Digestible acid detergent fiber
DEc	Digestibility coefficient
DCP	Digestible crude protein
DDGS	Dried distillers grains with soluble
DE	Digestible energy
DESBM	Dry extruded expelled soybean meal
dEE	Digestible ether extract
DM	Dry matter
DMI	Dry matter intake
DRES	Digestible residual
EDDM	Enzyme digestible ileal dry matter
$EUDM_i$	Enzyme undigested ileal dry matter
EE	Ether extract

Exp	Experiment
FC	Fermentable carbohydrate
FU	Feed unit
FUgp	Feed unit for growing pigs
FUgs	Feed unit for gestating sows
FUp	Feed unit for pigs
FHP	Fasting heat production
g	Gram
GE	Gross energy
G:F	Gain to feed ratio
H	Hydrogen
h	Hour
HI	Heat increment
HP	Heat production
IC	Indirect calorimetry
J	Joule
IDC	Ileal digestible carbohydrate
K	Potassium
kcal	Kilocalorie
kg	Kilogram
kJ	Kilojoules
MC	Multi enzyme complex
ME	Metabolizable energy
MEm	Energy required for maintenance
N	Nitrogen
Na	Sodium
NDF	Neutral detergent fiber
NE	Net energy
NEm	Net energy for maintenance
NEp	Net energy for production
NSP	Non starch polysaccharides

O_2	Oxygen
P	Phosphorus
RE	Retained energy
RQ	Respiratory quotient
SD	Standard deviation
SEM	Standard error of the mean
ST	Starch

1.0 GENERAL INTRODUCTION

Feed is a major expense in any swine production system representing about three quarters of the variable costs. At least 50% of this cost is mainly attributed to supplying energy to the animal thus making energy financially the most vital component. A detrimental impact on performance, product quality, environment, and overall profitability is observed when the energy supplied to the pig goes above or below the requirement (Chiba, 2000). Consequently, for a cost efficient feed or for a better regulation of animals' energy requirements to the feed supply, it becomes crucial to estimate the accurate energy value of feeds. So, high priority should be given to feed formulation especially when various feedstuffs are used in a diet. Practical diet formulations need to be adequately flexible to accommodate price and feedstuffs available while maintaining the required nutritive balance and adequacy (van Heugten *et al.*, 2000). This signifies the importance of formulation of swine rations utilizing the most precise information available. So, information about the exact energy content of feed ingredients is critical in formulating diets with specified energy concentration. Thus, it becomes crucial to put across the energy value of feeds on an appropriate basis; both energy supply through feed and requirement by the animal being expressed in the same system (Noblet and van Milgen, 2004) so as to provide adequate nutrients rather than surplus and thereby achieving profitable production .

The energy content of a feed can be expressed in several different ways; namely the gross energy (GE), the digestible energy (DE), the metabolizable energy (ME) and the net energy (NE). Gross energy can be defined as the energy released when a sample of feed is subjected to complete combustion in a bomb calorimeter under a pressurized oxygen atmosphere. Gross energy is not totally accessible for meeting the requirements of the animal since some energy is lost in feces, in urine, as gas of fermentation and as heat. Thus the gross energy of the feed consumed minus the gross energy of the feces excreted is referred to as the DE. Digestion is accompanied by the production of gases in both foregut and hindgut and by the urinary excretion of metabolites. Therefore ME is the difference between digestible energy and energy lost as gases and urine. Most of the energy lost in gases is due to methane production. Although the energy content of feed, faeces and urine can be measured with pigs kept in metabolism crates, the measurement of methane production necessitates the pig to be housed in a respiration chamber. As a result, most of the ME values reported overlook energy losses as methane (Noblet, 2007). Finally NE is defined as ME minus the heat increment (HI), which is the heat produced during digestion of feed, nutrient metabolism and excretion of waste. Net energy is therefore the true energy available to the animal for maintenance, reproduction and production purposes. In addition, NE is the only system in which energy requirements and diet energy values are expressed on a same basis, which should theoretically be independent of the feed (Noblet *et al.*, 2004). Therefore, for formulation of swine diets, the three main energy systems adopted are the DE, ME and the NE systems.

On a comparative basis it should be noted that the energy content of protein and high fibre ingredients are normally over-estimated by DE or ME system, whereas those for starch and high fat ingredients are under-estimated when expressed using the DE systems (Noblet and van Milgen, 2004). This is because, for diet rich in protein more heat is lost during catabolism and excretion of excess nitrogen which is not taken into account by the DE or ME system when compared to the NE system. Similarly, because less heat is produced in metabolizing fat into energy, it is given a higher value when expressed using the NE system

(Noblet *et al*., 1994a). Also it's only the NE system which accounts for the reduction in the efficiency of utilization of energy from the hindgut for high fibre diets. Diets formulated on NE system have lower CP when compared to those formulated on DE or ME system (Canh *et al*., 1998; Dourmad *et al*., 1993; Le Bellego *et al*., 2000, 2001; Kerr *et al*.. 2003) which reduces the nitrogen excretion. This in fact has revealed to improve the animal's performance, as reduced nitrogen excretion leads to decreased ammonia emissions and odor in the barns (Canh *et al*., 1998). Such diets have even shown to reduce the feed cost involved. Despite all the benefits, North American nutritionists continue to formulate diets using DE or ME systems as opposed to more advanced NE systems.

Soybean is one of the most important and commonly used protein source for livestock all over the world. The nutritional quality of soybean products widely varies with the method of processing which they go through. Owing to limitation involved in the conventional solvent extraction due to increasing hexane prices, new techniques were adopted. One such process is dry extrusion followed by expelling which produces a meal called dry extruded expelled soybean meal (DESBM) high in oil when compared to the conventional solvent extraction. DESBM being high in protein and fat, the energy component would be precisely evaluated by the NE system for more economical use of such products in swine diets.

Determining the NE content of either ingredients or diets is much more complex than either DE or ME estimation. The comparative slaughter (CS) technique gives the most accurate estimate of NE but is an expensive process and also requires a large numbers of animals whereas in indirect calorimetry (IC) method, heat production (HI) is correlated to oxygen consumption and carbon dioxide production. Compared to CS technique, IC method is much faster and requires only lesser number of animals allowing frequent measurement of energy balance. Yet another method of NE determination is by prediction equations developed by several researchers which require the data for various nutrients or digestible nutrients in the feed as an input. Each technique has its own merits and demerits which should be considered by any person using them.

Therefore, it was hypothesised that the NE values of dry extruded expelled soybean meal (DESBM) for growing pigs as determined by IC method will give similar values when calculated using the prediction equations developed by Noblet et al. (1994a) and diets formulated on the basis of the NE system will produce better performance in pigs. To test this hypothesis, three experiments were conducted with the overall objective of determining the NE of DESBM. The specific objectives of this study, therefore, were (1) to determine the NE value of DESBM for growing pigs using the IC method and prediction equations, (2) to compare the IC method and prediction equation for determining NE of feeds and feedstuffs, (3) to determine the effect of a multi-enzyme complex (MC) on the NE content of DESBM fed to growing pigs and (4) to validate the NE system of feed formulation.

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

Energy is what an animal derives from its food, through the process of cellular respiration which involves a set of metabolic reactions and processes that take place in the cells to convert biochemical energy from nutrients into energy units called adenosine triphosphate (ATP); which is the fundamental “currency” of energy in tissues (Brafield and Llewellyn, 1982; Burrin, 2001; van Milgen and Noblet, 2003). In other words the energy enclosed in the feed as chemical energy is released by partial or complete oxidation following digestive and absorptive mechanisms in the gastrointestinal tract (Pond *et al*., 1995) and can only be measured in its transformation from one form to another (Kleiber, 1975).

Feed contains ingredients which the animal’s body can use as fuel. But even fuel is not yet energy. It depends on the metabolically active components present in the feed such as sugars, fibers, fats and proteins. As per the first and second laws of thermodynamics, all forms of energy are quantitatively convertible to heat (Baldwin and Bywater, 1984) and therefore all measurements of energy are made and conveyed in terms of heat energy or calories (cal) (Armsby, 1917). Although International System of Units measures energy in joules (J), calorie which is a metric system unit of energy, is also widely used in contexts. Calorie can be defined as the amount of heat required at a pressure of one atmosphere to raise the temperature of one gram of water by one degree Celsius (Pond *et al*., 1995). For diets and feed ingredients, energy content can be expressed as calories (cal), kilocalories (kcal), or megacalories (Mcal) of gross energy (GE), digestible energy, metabolizable energy, or net energy (NRC, 1998).

Majority of the pig's caloric needs are supplied by carbohydrates and fats present in the feed. Carbohydrates are macromolecules consisting of carbon, hydrogen and oxygen atoms. Dietary carbohydrates constitute a major fraction of the diet for pigs and can be divided according to glycosidic linkages into sugars (mono- and disaccharides), oligosaccharides and two broad classes of polysaccharides starch and non-starch polysaccharides (NSP) (Bach Knudsen and Jorgensen, 2001). The bulk of disaccharides and starch is broken down by the action of pancreatic and mucosal enzymes in the small intestine, while there are no enzymes capable of cleaving some types of oligosaccharides (i.e. a-galactosides, fructooligosaccharides) and NSP (Bach Knudsen and Jorgensen, 2001). Therefore, degradation of NSP is performed by the microflora, mainly present in caecum and colon. However, studies have indicated that NSP have a negative effect on the intestinal digestion and absorption process of nutrients (Bakker, 1996). For simple sugars, such as glucose, one mole releases 2.80 MJ of energy equivalent to 3.7 kcal per gram, known as the "caloric content" of sugar which for practical use is taken as 4 kcal/g. Sucrose is basically just two simple sugar molecules linked together, while starch is a long chain of many such simple sugar. The cellulose (fiber) that makes up the cell walls of plants is also made of linked sugar molecules, but the pig's digestive tract is unable to unlink them and hence degraded by microbial fermentation in the hindgut (Bach Knudsen, 2001). Fat molecules, on the other hand, are made almost entirely of carbon and hydrogen, with very little oxygen which when metabolised yields approximately 9 kcal/g, more than twice the energy released from carbohydrates. Proteins are very complex molecules containing considerable amount of nitrogen in addition to carbon, hydrogen, and oxygen. They serve a variety of nutritional needs, but can be metabolized for energy when needed, extracting approximately 4 kcal/g, the same as from carbohydrates. Carbohydrates, protein and fats have an average caloric value of 4.1, 5.7 and 9.4 kcal/g, respectively (Brafield and Llewellyn, 1982; Pond *et al*., 1995).

2.2 ENERGY SOURCES FOR SWINE DIETS

A single feed ingredient cannot be practically used to supply the animal's requirement for nutrients, since a particular ingredient may be excess of one or more nutrients and be deficient in others. Hence it is always a combination of different ingredients which make up a swine diet. As a result, there has been an intensive effort to quantitatively depict the energy value of the vast array of feed ingredients available for selection in practical swine diets. Pigs have a relatively simple digestive system which makes them inefficient to utilize vast quantities of hay, silage, or pasture grasses. Therefore, swine rations are made up primarily of grains, along with protein supplements and other vitamins and minerals. Cereal grains make up to 50 to 85% of the ingredients in swine rations, which in turn provide much of the energy to the animal (Myer and Brendemuhl, 2013). Corn grain is among the leading cereal used in the swine feed industry; which has a greater energy density than other cereal grains. Because of its abundance and high energy concentration, corn is the base to which other cereal grains are compared. Small grains, such as barley, wheat, oats, rye, and triticale form other practical ingredients in swine feeding programs. On many occasions, pigs fed balanced small grain-based diets can perform well compared with those fed corn-based diets (Sullivan *et al*., 2005).

Nutritionally, small grains are comparable to corn in some aspects, but there are variations depending on the grain. The crude protein in small grains are higher than that in corn especially the lysine which is the first limiting amino acid in cereal grain based swine diets (Sullivan et al., 2005). In addition, small grains have a higher digestible phosphorus level than corn, but tend to be lower in energy content.

With the rise of the ethanol industry, the quantity and availability of grain processing co-products have increased in recent years. Corn distiller dried grains with solubles (DDGS) from the fuel ethanol industry is a major co-product used in swine feed (Stein *et al.*, 2006). Corn gluten feed and corn gluten meals are co-products of the corn wet-milling industry. The wheat milling co-products include bran and middlings. The nutrient composition of these co-products differs from the original grain source (NRC 1998).

The chemical composition of the feed ingredient has a major impact on its energy contend. Therefore an accurate estimate of the energy content of each ingredient is necessary for proper diet formulation.

2.3 ENERGY METABOLISM

The term metabolism refers to all the chemical processes in a living organism by which nutritive material is built up into living matter, or by which complex molecules are broken down into simpler substances during the performance of special functions (The Oxford Companion to the Body, 2002).

Metabolism is usually divided into two categories; catabolism and anabolism. Catabolism breaks down organic matter, releasing energy used up in other biochemical reactions or dissipated as heat. Anabolism uses energy to construct components of cells such as proteins and nucleic acids and when anabolism exceeds catabolism, it could be defined as growth or weight gain. In a fasting animal, loss of body weight represents a loss of energy equivalent to fasting heat production (FHP) from the body, while a gain in body weight represents energy retention (Blaxter and Boyne, 1978).

2.4 ENERGY REQUIREMENTS IN SWINE

The energy supplied through diets is utilized by the animal for two main functions; maintenance and production. Maintenance includes basal functions and involuntary activities, such as, muscle tone, feed digestion, blood circulation, tissues replacement (Wenk *et al*., 2000; Vestergen, 2001), cellular ion transportation for maintaining membrane potential and acid-base homeostasis (Baldwin and Bywater, 1984; Milligan and Summers, 1986) along with breaking down of complex chemical substances into simpler form that can be eliminated as waste products from the body. In addition, energy is also necessary to maintain the body temperature irrespective of the environment in which the pig is placed, which is otherwise known as the homeothermal functions (Cole, 1995). At times when thermoregulation, detoxification, immune, fever and stress responses are lacking, energy for maintenance is distributed into four equal proportions for physical activity, cellular ion (Na^+, K^+) transport activity, protein turnover and other maintenance activity like the waste elimination (Verstegen, 2001).

2.4.1 ENERGY FOR MAINTENANCE

Maintenance is the requirement of nutrients for the continuity of vital functions within the body so that the net gain or loss of nutrients by the animal as a whole is zero (ARC, 1981). But this definition is not always applicable to growing pigs; wherein they tend to deposit protein at the expense of fat when fed to maintain constant weight (Black, 1974; Campbell, 1988; Wiesemuller *et al*., 1988; Kolstad and Vangen, 1996). Hence, growing pigs will have an inconsistent energy balance in view of the higher heat of combustion of fat that is lost in exchange for protein gain.

Though exact measurement of maintenance energy may be complex (van Milgen *et al.*, 2000), it has been extensively adopted by animal nutritionists in an effort to break up the energy cost of maintenance versus that of production and to ease the additivity of the two processes (van Milgen and Noblet, 2003).

These energy requirements for maintenance (ME_m) are generally expressed on a metabolic basis, which is defined as body weight raised to the power of 0.75 ($BW^{0.75}$). However, studies show that the exponent function is significantly less than 0.75 ranging from 0.54 to 0.75 (Tess, 1981). It has been recommended that the appropriate exponent is closer to 0.60 (Noblet *et al.*, 1999) rather than 0.75 as the later underestimates ME_m for growing pigs (Tess *et al.*, 1984; Thorbek *et al.*, 1984).

2.4.2 ENERGY FOR GROWTH

Once the energy requirements pertaining to maintenance have been fulfilled, the pig can divert the energy to build body tissues (lean and fatty tissues) and grow. During normal growth phase, the first priority of the pig is for lean tissue deposition. Both lean tissue (ham, shoulder, loin, all without subcutaneous fat; Walstra, 1980) and fatty tissue deposition rate increase at a similar pace until the maximum genetic potential for lean growth is reached (Van Lunen and Cole, 2001).

The relationship between energy (feed) intake and tissue growth is that, lean tissue and growth rate respond in a linear fashion to energy intake up to a point where the protein deposition rate is at a maximum (Figure 2.1; Close, 1996; Van Lunen and Cole, 2001). This point corresponds to the genetic capacity of the pig for lean tissue growth. Any additional energy supplied beyond this point will lead to a huge increase in lipid deposition with modest increase in lean, if any. Alternatively, lipid deposition increases at a bigger rate above the 'capacity point' than below it due to the larger proportion of the energy required to fuel protein metabolism below the 'capacity point' (Close, 1996).

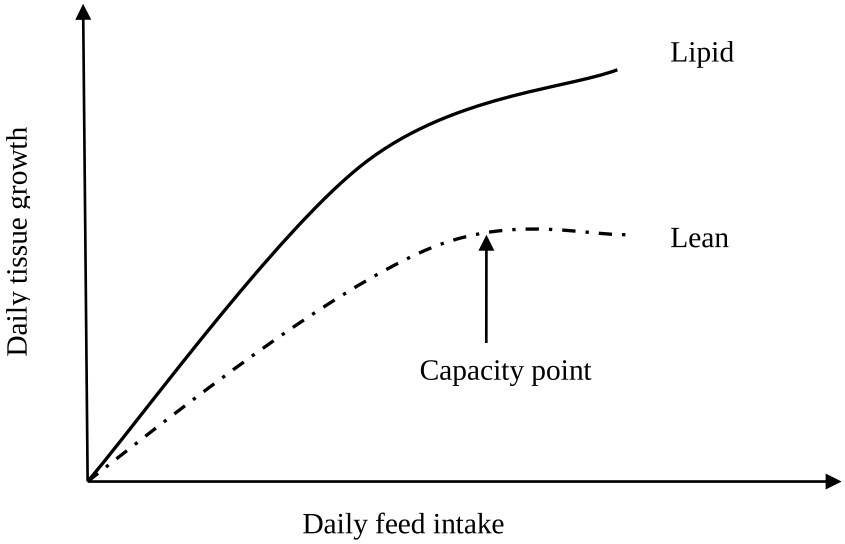


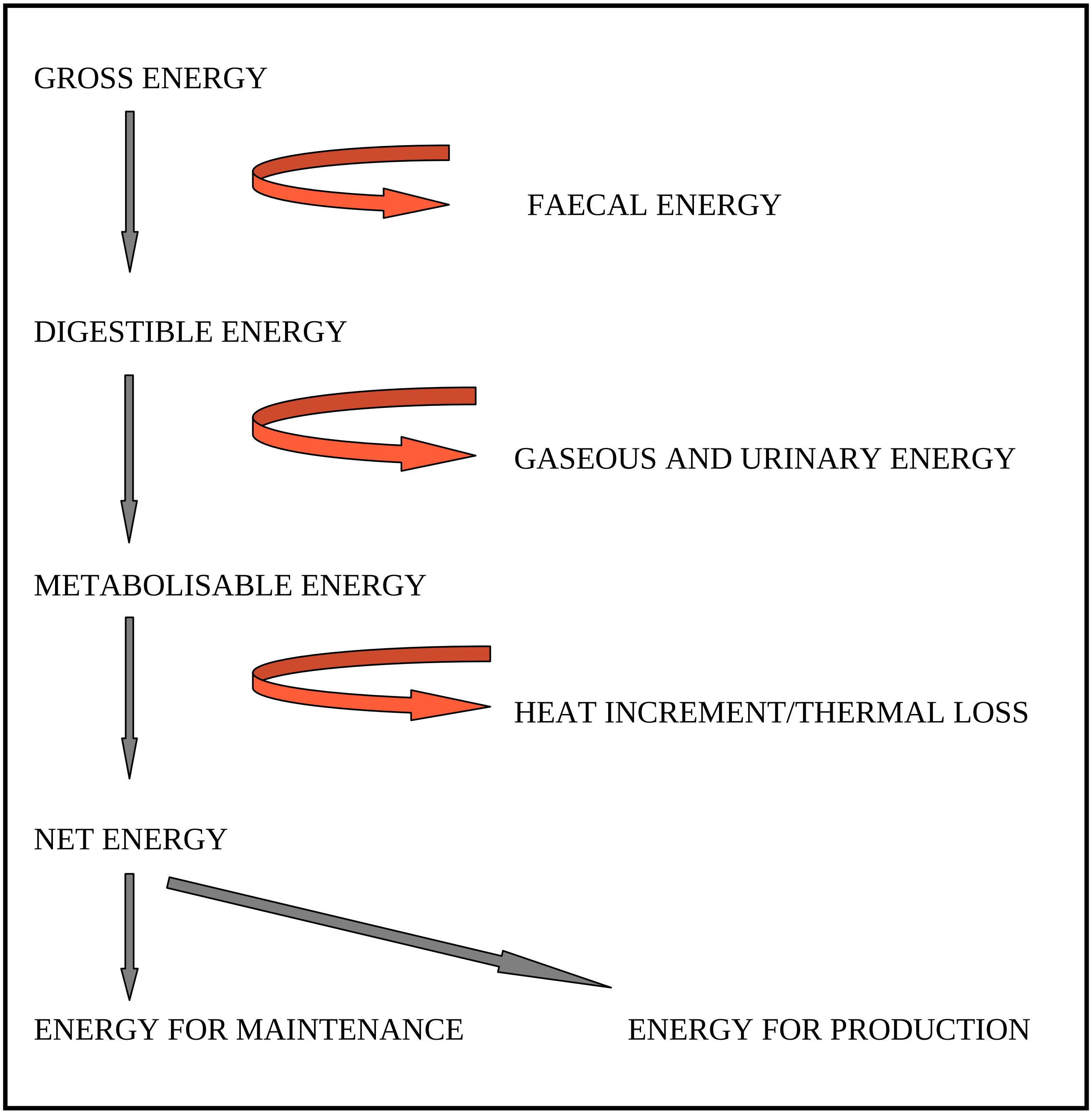
Figure 2.1 Influence of daily feed intake on tissue growth. Adapted from Close (1996)

2.5 ENERGY SYSTEMS FOR SWINE

Energy being one among the key factors governing the economics in animal production, rigorous efforts has been made to develop methods and systems for assessing the energy content of feed, metabolic utilization of energy and the animal's basic requirements for energy. The principal focus of energy based feed evaluation systems is the amount of energy that can be derived from ingested nutrients to sustain the animal's maintenance and productive functions. The essential qualities of a practical energy system is that they should be precise, should include unconventional rations and high production levels and should be simple to use and applicable in general (Van Es, 1980)

All energy systems follow the common pattern of energy utilization in pigs (Figure 2.2). In most of the practical energy systems, energy value is based on the ability to deposit a certain unit amount of energy in the body per unit amount of extra feed. Energy systems in livestock nutrition are intended for the following basic purposes; to attribute energy values to a feed ingredient or a mixture of feed ingredients that could be used to estimate the amount of a given diet needed to meet the performance of the individual animal (Emmans, 1999) and to determine the requirements for maintenance, production, diet formulation and to develop feeding programs. Eventually, the value of such a system lies in its ability to predict the performance of animals (Noblet, 2000).

Figure 2.2 Classical description of energy utilization. Adapted from Pilliner, 1999



2.5.1 GROSS ENERGY

Gross energy or heat of combustion is the energy released by burning a sample of feed in excess oxygen. It is usually determined in an adiabatic bomb calorimeter. As such it provides no information on the amount of that energy that is available to the pig through the digestive procedure or lost during metabolism. So GE is rarely used in feed formulation except for computational purposes.

The GE contend of an ingredient depends upon the amount of carbohydrates, fat and protein it contains. For carbohydrates, the GE value varies since monosaccharides (such as glucose) yield 3.75 kcal/g and polysaccharides (such as starch) yield 4.16 kcal/g (Wenk *et al*., 2000). Likewise, the GE content of protein and fat depends on the amino and fatty acid composition, respectively, with an average of 5.64 kcal/g for protein (Wenk *et al*., 2000) and for fat the widely acknowledged GE value is 9.51 kcal/g (Brouwer, 1965). Consequently if the nutrient composition of feed ingredients and/or diets is known, the GE content can be estimated using existing prediction equations (Ewan, 1989; Noblet and Perez, 1993).

2.5.2 DIGESTIBLE ENERGY

Digestible Energy is the energy in feed after subtracting the energy lost in feces. Since it is not a true measure of the energy values of the nutrients absorbed from the digestive tract, it is often referred to as apparent digestible energy. Moreover, a small fraction of the energy in feces is supplied by endogenous sources like digestive secretions and intestinal cell debris (Just, 1982).

Digestible energy is usually determined from the GE in the feed consumed and the GE of fecal matter excreted. Alternatively, DE can be measured by mixing non-absorbable indicators (e.g. acid insoluble ash, chromic oxide or titanium dioxide) into the diet. In pigs, up to 25% of ingested energy is found in faecal matter (Boisen and Verstegen, 2000); though, for swine diets the digestibility coefficient of energy (DE$_{c)}$ is known to fluctuate between 70 and 90%, with a much wider variation, 0 to 100% for the ingredients (Noblet and Henry, 1993). One of the factors affecting the digestible energy content in pigs includes dietary fiber levels, which is less digestible than other nutrients and reduces the apparent fecal digestibility of other dietary nutrients such as crude protein and fat (Noblet and Perez, 1993). The animal's ability to digest fiber also varies with the age. Hence DE values obtained from older pigs will overestimate DE values for nursery pigs, especially in feeds with high fiber content (Shi and Noblet, 1993). Fiber is not digested in the small intestine but passes to the large intestine where micro-organisms convert part of the fiber to volatile fatty acids, which are then absorbed. However, digestion in the large intestine is less efficient when compared to direct absorption from the small intestine. In addition, the digestive utilization of fiber is also variable with its botanical origin (Chabeauti *et al*., 1991).

In studies by Le Goff and Noblet (2001), where apparent energy digestibility was evaluated in growing pigs and adult sows fed the same diets; apparent digestibility was greater in adult sows compared with growing pigs and accordingly, a 4% greater DE contend was determined. In other terms, although dietary fiber is partly digested by the young growing pig, it supplies very little available energy to the animal (Noblet and Perez, 1993). Thus, it would be relevant to have separate energy requirements for different physiological stages of growth.

2.5.3 METABOLIZABLE ENERGY

Metabolizable energy could be defined as the gross energy in the feed minus the gross energy of the feces (Armsby, 1917), and is estimated as the DE minus urinary energy and gaseous energy (GE_{gas}; mostly CH_4). In pigs, the GE_{gas} is generally overlooked because it represents only a small fraction of DE, between 0.1 and 3% (Verstegen, 1971; Wenk *et al*., 2000).

In case of sows fed at maintenance level, methane production represents a much higher proportion of DE intake when compared to growing pigs (Noblet and Shi, 1994). In general, methane production increases with pig body weight and dietary fiber level. So, depending on the amount of plant cell wall content in the diet and the age of the animal the estimated ME values are usually 0.5 to 3% higher than the real value (Van Es and Boekholt, 1987).

Urinary energy losses represent a variable percentage of DE, since the urinary energy is very much dependent on the amount of nitrogen in urine. Urinary nitrogen in turn mainly depends on the amount of digestible protein and for that reason, on the crude protein content of the diet. Consequently, the ME:DE ratio is linearly related to dietary protein content (Le Goff and Noblet, 2001). Higher protein levels in diet leads to an increase in the catabolic processes in the animal, and therefore, a greater excretion of urinary nitrogen in protein-rich diets (Morgan *et al*., 1975). Since the urinary nitrogen loss is not accounted for while determining DE, the energy value of protein-rich ingredients is exaggerated with the DE when compared with ME (Morgan *et al*., 1975). Moreover, the amount of energy in the urine is dependent on the quality and quantity of the protein in the diet relative to requirement (NRC, 1998). By and large, the ME:DE ratio of complete feeds is relatively constant and equivalent to about 0.96. But, this mean value cannot be applied to single feed ingredients (Noblet and van Milgen, 2004).

Metabolizable energy is further used to meet different energy requirements of the pig namely maintenance, growth, protein or lipid gain, milk production, and so on. There is a marked variation observed in the average efficiency of utilization of ME for these different purposes: approximately 80% for fat gain (k_f) or maintenance (km), 60% for protein deposition (k_p) , 75% for weight gain (k_g) during growth, and 70% for milk (k_l**)** (Noblet et al., 1994a)

2.5.4 NET ENERGY

Net energy (NE) is defined as ME minus heat increment (HI) (Birkett and de Lange, 2001). The energy left after such losses is the energy actually available to the animal for maintenance (NE_m) and for production (NE_p). Heat increment is the heat produced from metabolic utilization of ME and the energy cost of ingestion, digestion and physical activity (Rijnen *et al*., 2004) and is mainly utilized for maintenance of body temperature in cold environments. The energy used for maintenance is also dispersed as heat, so that total heat production could be defined as the sum of HI and NE_m (NRC, 1998).

The NE content when expressed as a percentage of ME content (k) is otherwise known as the efficiency of utilization of ME for NE (Noblet *et al*., 1994a). Apart from variations due to the final utilization of ME (e.g., maintenance, protein gain vs. fat gain vs. milk production), k varies according to the chemical characteristics of the feed as all nutrients are not used with the same efficiencies (Noblet and van Milgen, 2004; Chudy, 2006). This variations of k, due to differences in efficiencies of ME utilization between nutrients are 90, 82, 58 and 58% when ME is provided by digestible ether extract, starch, digestible crude protein and digestible fiber, respectively (Noblet, 1999). Therefore it is evident that heat increment (per unit of energy) associated with metabolic utilization of energy is higher for crude protein and dietary fiber than for starch or ether extract (Noblet *et al*., 1994a). Therefore, it is evident that an increase of dietary crude protein results in increased heat production.

Net energy system could be described as the only system that depicts the energy that is actually available to the pig. Net energy accounts for the differences in metabolic utilization of ME between nutrients; consequently NE is the only system in which energy requirements of the animal and energy supplied by the diet are expressed on the same basis which is independent of the feed composition (Noblet and Henry, 1993; Noblet and van Milgen, 2004). However, NE is much more difficult to determine and more complex than DE or ME, which may be a reason why it is not as widely used as it should be.

2.6 HEAT PRODUCTION AND ENERGY RETENTION IN SWINE

Determination of NE value requires measurement of energy retention or heat production (HP) and an estimate of maintenance requirements. As reviewed by van Milgen and Noblet (2003), all ME not retained by the animal is lost as heat. The retained energy can be measured with the comparative slaughter (CS) technique or by measuring the carbon-nitrogen balance and the metabolic processes responsible for the energy supply to the body can be determined by measuring the HP of the animal which can be determined by direct or indirect calorimetry by measuring the gas exchange from the animal.

2.7 METHODS FOR DETERMINATION OF ENERGY RETENTION AND HEAT PRODUCTION IN SWINE

Energy retention, which is the actual fraction of energy in the feed retained by the body, may be measured by either the comparative slaughter (CS) technique or by carbon-nitrogen balance (Adeola, 2001). Though, the CS method involves simple techniques, it is laborious and gives an estimate of the average energy retention over a longer period of time (van Milgen and Noblet, 2003). Heat production may be measured by direct or indirect calorimetry.

2.7.1 COMPARATIVE SLAUGHTER TECHNIQUE

Comparative slaughter has been a method of preference to determine the NE of feed (Ayoade *et al*., 2012). Determination of body composition of an animal at the beginning and again at the end of a period of time is practically unfeasible (Blaxter, 1989). Hence, the possible alternative would be to determine the body composition of an exactly similar experimental animal at the beginning of the period and at the end of the period (Blaxter, 1989). This method of determining energy retention is termed the comparative slaughter method. Energy retention is the difference between the body energy contents of the initial and final slaughter groups (Kil *et al*., 2011). However, the CS method is labor intensive and requires a large number of animals. Moreover the delay in determining the body composition reduces the opportunity to use data in real- time situations (Salas *et al.*, 2012). As an alternative, a non-invasive technique; the Dual-energy X-ray Absorptiometry (DEXA) is being used recently to measure body composition in animals (Black *et al*., 2001). DEXA offers the added advantage of using the same animal over an extended period of time without any detriment to its health or performance because of a low radiation dose per scan (Salas *et* *al.,* 2012). In addition the amount of time needed per scan is lower compared to the time spent on sample preparation for chemical analysis.

2.7.2 CARBON-NITROGEN BALANCE TECHNIQUE

In this technique, carbon and nitrogen in feed, feces, urine and gaseous output are measured on the assumption that protein and fat are the only form of energy yielding component stored in the body and that these have fixed chemical composition and enthalpies of combustion (Blaxter, 1989). The C-balance provides the total amount of C retained in the body and the amount of C retained in fat is be calculated by subtracting the amount of C retained in protein as determined by the N-balance.

2.7.3 INDIRECT CALORIMETRY

Indirect calorimetry (IC) calculates heat that living organisms produce by measuring their consumption of oxygen, production of carbon dioxide and nitrogen excreted. Animals produce heat due to metabolic reactions associated with maintenance, production (growth, milk and eggs production) and other "non-productive" functions such as physical activity, thermoregulation or immune response. Heat production (HP) is closely correlated to O_2 consumed, CO_2 produced, CH_4 produced, and urinary N produced (Adeola, 2001). The coefficients to predict heat production was derived from the complete oxidation of carbohydrate, fat and protein. The concept was based on Hess's law, according to which the heat produced in a chemical reaction is independent of the pathway between the initial and final states (Blaxter, 1989). This implies that it does not make a difference whether a substrate undergo a complete direct oxidation, or whether intermediate products such as lactic acid, fatty acids, ketone bodies are produced which are subsequently transformed and oxidized at a later stage.

Direct measurements of HP in animals require expensive installations; so usually indirect methods are used to determine HP (Christensen *et al.*, 1988). Generally for indirect calorimetry technique, HP is calculated using the formula published by Brouwer in 1958 and later adapted by the "Sub-committee of Constants and Factors", published in 1965. The approach is based on O_2 consumed, CO_2 produced and heat released upon combustion of 1 g of fat, carbohydrate and protein and the method involves measurements of O_2 intake, CO_2 and CH_4 production and N excretion in urine.

$$HP = 16.18 \times O_2 + 5.023 \times CO_2 - 2.17 \times CH_4 - 5.989 \times UN \quad \ldots\ldots\ldots\ldots\ldots \quad (2.1)$$

Where HP is in kJ; O_2, CO_2, CH_4 in litres; UN is urinary N in g. The UN corrects for the catabolism of protein and CH_4 corrects for the incomplete oxidation of carbohydrates in the digestive tract that produces CH_4. This method is also termed the respiratory quotient (RQ) method expressed as RQ = litres CO_2 per litres O_2, and has been used more often in reduced form (without correction for CH_4 and UN) (Christensen *et al.*, 1988). Another indirect method is to calculate HP as the difference between metabolizable energy (ME) and total energy retained in the body:

$$HP = ME - RE \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.2)$$

Where HP = heat production, ME = metabolizable energy and RE = retained energy and all parameters are in kJ. Retained energy is based on measurements of the C and N balances, assuming that all energy is retained either as fat or protein.

2.8 FASTING HEAT PRODUCTION IN SWINE

The energy expended in the fasting animal is represented by the fasting heat production (FHP). In other words, FHP is the sum of basal energy requirement and energy needed to produce available energy from body nutrient stores, and is expected to be least affected by the animal's production level (de Lange *et al*., 2006). In fasting, energy from body reserves is mobilized so as to produce adenosine triphosphate (ATP) for important functions. However, normally-fed growing animals will seldom mobilise body reserves (other than glycogen) in order to supply energy for essential functions. NE systems use FHP as an estimate of the maintenance energy requirement (Noblet *et al.*, 1994a). Determination of FHP on non-producing adult animals is the basis for the calculation of minimum quantity of NE, which must be supplied to the animal to keep it in energy equilibrium (Chandramoni *et al.*, 1999). Calculated values of activity-free FHP in growing pigs vary between 700 to 800 kJ/kgBW$^{0.60}$/d (Le Bellego *et al.*, 2001; van Milgen *et al.*, 2001; Le Goff *et al.*, 2002).

2.9 NET ENERGY SYSTEMS

Major NE systems for pigs were developed in France (INRA), the Netherland (CVB) and in Denmark.The French system of NE being widely used has been described by Noblet (2000). The system used in the Netherlands was adapted from the equations proposed by Schiemann *et al*. (1972). Boisen and Verstegen (1998) proposed new concepts for estimating the NE value of pig feeds (so-called physiological energy) and is based on the combination of in vitro digestion methods for estimating digestible nutrients and biochemical coefficients for evaluating the ATP potential production from the nutrients. The French and the Dutch systems are based on NE values from animal experiments and prediction equations, whereas the Danish system is based on the potential physiological energy (PPE) released from ATP bonds at the cellular level of pigs (Stewart, 2005). However, all published NE systems for pigs combine the utilization of ME for maintenance and for growth (Noblet *et al*., 1994a) or for fattening by assuming similar efficiencies for maintenance and energy retention.

2.9.1 THE FRENCH SYSTEM

The system was proposed by Noblet *et al*. (1994a) based on a large set of measurements (61 diets). Digestible energy, ME, and NE values of 61 diets were measured in 45-kg growing Large white boars. Net energy was determined using IC technique. The amounts of DE before the end of the ileum and in the hindgut were measured for each diet. Regression equations for predicting dietary NE content was calculated and a total of 11 prediction equations were developed which can determine a correct hierarchy among feeds for both growing pigs and pregnant or lactating sows. The equations used for predicting NE are given in Table 2.1. The equations are all based on information available in conventional feeding tables and are applicable to single ingredients and compound feeds and at any stage of pig production (Noblet, 2006). However reliable data on the digestibility of energy or of nutrients is necessary for the prediction of NE content which could be serious limiting factor for predicting energy values of pig feeds (Noblet and van Milgen, 2004).

Table 2.1 Equations for prediction of NE (kcal/kg DM) of diets for growing pigs (Noblet *et al.*, 1994a)

Equation	
1	$\mathrm{NE} = 2.73 \times \mathrm{DCP} + 8.37 \times \mathrm{DEE} + 3.44 \times \mathrm{ST} + 0 \times \mathrm{DADF} + 2.93 \times \mathrm{DRES2}$
2	$\mathrm{NE} = 2.69 \times \mathrm{DCP} + 8.36 \times \mathrm{DEE} + 3.44 \times \mathrm{ST} + 0 \times \mathrm{DCF} + 2.89 \times \mathrm{DRES3}$
3	$\mathrm{NE} = 0.843 \times \mathrm{DE} - 463$
4	$\mathrm{NE} = 0.703 \times \mathrm{DE} + 1.58 \times \mathrm{EE} + 0.47 \times \mathrm{ST} - 0.97 \times \mathrm{CP} - 0.98 \times \mathrm{CF}$
5	$\mathrm{NE} = 0.700 \times \mathrm{DE} + 1.61 \times \mathrm{EE} + 0.48\ \mathrm{ST} -0.91 \times \mathrm{CP} -0.87 \times \mathrm{ADF}$
6	$\mathrm{NE} = 0.870 \times \mathrm{ME} - 442$
7	$\mathrm{NE} = 0.730 \times \mathrm{ME} + 1.31 \times \mathrm{EE} + 0.37 \times \mathrm{ST} - 0.67 \times \mathrm{CP} - 0.97 \times \mathrm{CF}$
8	$\mathrm{NE} = 0.726 \times \mathrm{ME} + 1.33 \times \mathrm{EE} + 0.39 \times \mathrm{ST} - 0.62 \times \mathrm{CP} - 0.83 \times \mathrm{ADF}$
9	$\mathrm{NE} = 2{,}796 + 4.15 \times \mathrm{EE} + 0.81 \times \mathrm{ST} - 7.07 \times \mathrm{Ash} - 5.38 \times \mathrm{CF}$
10	$\mathrm{NE} = 2{,}790 + 4.12 \times \mathrm{EE} +0.81 \times \mathrm{ST} - 6.65 \times \mathrm{Ash} - 4.72 \times \mathrm{ADF}$
11	$\mathrm{NE} = 2{,}875 + 4.38 \times \mathrm{EE} + 0.67 \times \mathrm{ST} - 5.50 \times \mathrm{Ash} - 2.01 \times (\mathrm{ADF} - \mathrm{ADF}) - 4.02 \times \mathrm{ADF}$

CP, crude protein; EE, ether extract; CF, crude fibre, ST, starch; SG, sugar; ADF, acid detergent fiber; DCP, digestible CP; DEE, digestible ether extract; DADF, digestible acid detergent fibre; DRES, digestible residual = digestible organic matter – (DCP + DEE + ST + DADF) all in g/kg DM.

2.9.2 THE DUTCH SYSTEM

The system was developed by Central Bureau Livestock Feeding (CVB) in the Netherlands using a variation of one of NE prediction equations developed by the French system (Stewart, 2005). The system uses the concentrations of digestible nutrients in feed ingredients to estimate the NE values of feeds and feed ingredients in a way that is consistent with the French NE system (Rijnen *et al*., 2004). However, the Dutch system separates total digestible carbohydrates (i.e., starch and sugar) into an enzymatically-digestible fraction and a fermentable fraction owing to differences in energetic utilization of carbohydrates between the small and the large intestine of pigs (Kil *et al*., 2013).

The equations developed by the CVB are presented below:

NE_{CVB} (kcal/kg) = (28.0 x % digestible CP) + (85.4 x % digestible EE) + (33.8 x % starch-e) + (30.5 x % sugar-e) + (23.3 x % FCH) ………………………………… (2.3)

Where energy and chemical components are expressed on a DM basis; starch-e = enzymatically digestible starch, sugar-e = enzymatically digestible sugar, FCH (fermentable carbohydrates) = fermentable starch (starch-f, zero value except for potato starch) + fermentable sugar (= total sugar – sugar-e) + digestible NSP; digestible NSP = digestible OM - digestible CP - digestible EE - starch-e - 0.95 x total sugar.

2.9.3 THE DANISH SYSTEM

A new concept called “Potential Physiological Energy (PPE)” was proposed by Boisen and Verstegen (1998) for estimating the NE value of pig feeds. This concept was based on the combination of in vitro digestion methods for evaluating the ATP potential production from the components and biochemical coefficients for evaluating the ATP potential production from components (Noblet, 2000). The value for PPE of nutrients is the potential energy value for ATP production if digestible nutrients are completely oxidized by animals (Boisen, 2007). The PPE of different nutrients is assumed to be independent of their metabolic utilization (e.g., oxidation or retention), and as a result, the PPE calculated from various feed ingredients or digestible nutrients are additive in diets containing a mixture of feed ingredients and are independent of animal factors (Kil *et al*., 2013). The Danish system uses in vitro digestibility techniques to evaluate the digestibility of CP, amino acids, OM, lipids, and carbohydrates to avoid the effects of animals on nutrient digestibility. Also, an estimate of enzyme-undigested DM at the distal ileum is obtained from the in vitro procedure to correct the energy value for the compounds originating from endogenous synthesis of protein and lipids throughout the GIT. The energy values for feeds in this system are expressed as Feed Units (FU), which are calculated from the PPE values of each nutrient in the diet. The system is based on the following equation:

$$FU_{gp} \text{ per kg DM} = [9.9 \times RDCP + 31.7 \times RDCF + \text{factor} \times IDC + 7.0 \times FC - 28 \times EUDM_i]/7375 \quad \ldots\ldots \quad (2.4)$$

$$FU_{gs} \text{ per kg DM} = [9.9 \times RDCP + 26.1 \times RDCF + \text{factor} \times IDC + 9.0 \times FC - 28 \times EUDM_i]/7540 \quad \ldots\ldots \quad (2.5)$$

where FU_{gp} is feed unit for growing pig; FU_{gs} is feed unit for gestating sow, RDCP is in vitro ileal digestible CP, RDCF is calculated ileal digestible fat,, IDC is ileal digestible carbohydrate, FC is fermentable carbohydrate and $EUDM_i$ is enzyme undigested ileal DM, where FU is expressed on a DM basis and other components are based on g/kg DM.

2.10 EVALUATION OF NET ENERGY SYSTEMS

The French system proposed by Noblet *et al*. (1994a) is based on a large set of measurements and the results have been validated in some later trials (Le Bellego *et al*., 2001;

van Milgen *et al*., 2001). Noblet and van Milgen (2004) when comparing other NE systems to the French system indicated that the NE Schiemann, NE Just, and NE Dutch are approximately 94, 83, and 96 % of the NE French, respectively, for several diets. These average differences are owing to variation in estimates of fasting heat production and diet composition (Noblet, 2000). Net energy Schiemann and NE Dutch system underestimated diets with higher starch content, while the NE Just system underestimated diets with higher starch content and overestimated diets with higher levels of CP and dietary fiber (Noblet and van Milgen, 2004). Kil (2008), on comparing the predicted NE values from the French and the Dutch system using 16 mixed diets containing various feed ingredients found higher values from the Dutch system than those predicted from the French system. Due to the fact that the values in the French and the Dutch system were determined in standardized conditions, their application in practical conditions could result inconsistent response (Boisen and Verstegen, 1998). That was one of the reasons why the Danish system was developed. In the Danish system, there is difficulty in implementing the in vitro digestion methods and also, this approach assumes that energy is used exclusively for ATP production – which is not the case for growing pigs (Noblet, 2000).

2.11 COMPARISON OF DE, ME AND NE SYSTEMS

One of the characteristic of an energy system is its capability to rank ingredients. Energy systems have a major influence on the hierarchy between feed ingredients (Noblet et al., 1994; Noblet, 2000; Rijnen et al., 2004). This is shown in Table 2.2. The energy value of protein or fibrous feeds is overestimated when expressed on a DE or ME basis. On the other hand, fat or starch rich ingredients are underestimated in a DE system (Noblet *et al*., 1994). These conclusions are more clearly demonstrated in studies by Noblet *et al*. (1993). For instance, DE values for wheat and soybean meals (3.86 and 3.91 Mcal/kg DM, respectively) were reported to be similar in studies by Noblet *et al*. (1993), but for NE, wheat had 34% more when compared to that with soybean meal (2.90 and 1.92 Mcal/kg DM for wheat and soybean meal, respectively). Similarly wheat and tapioca contained quite similar DE concentration (3.86 and 3.79, respectively), whereas the NE value of tapioca was 6% higher than that of wheat (3.09 and 2.90 Mcal/kg DM, for tapioca and wheat, respectively). Likewise, the NE value of canola meal was 53% of its DE value (1.64 and 3.11 Mcal/kg DM for NE and DE, respectively) in comparison with wheat which was 75% of its DE (2.90 and 3.86 Mcal/kg DM for NE and DE, respectively).

The ratio between NE and ME (k_g for NE in growing pigs) corresponds to the efficiency of utilization of ME for NE. This ratio varies according to the chemical characteristics of the feed because nutrients are not used with similar efficiencies. In studies conducted with growing pigs, k_g was increased when fat and starch contents were higher and reduced when protein or fiber contents were enhanced (Noblet *et al*., 1994a).

The variation in energy losses while moving from DE to ME can be associated with the utilization of digestible crude protein (DCP) in relation with the excretion of nitrogen as urea (Noblet *et al*., 1994a). Similarly, energy losses from ME to NE can be attributed to losses as heat increment and for growing pigs it concern all nutrients which is about 2.0, 1.0, 0.75 and 1.2 kcal per g of DCP, digestible ether extract, starch and digestible fiber, respectively (Noblet *et al*., 1994a).

A number of growth trials conducted with variable dietary fat or crude protein levels has shown that the energy cost of growth or daily energy requirement are independent of diet composition when expressed on a NE basis (Noblet, 2007). On the other hand, on a DE or ME basis, the energy cost is reduced when CP content is lowered or fat content is increased (Sauvant *et al.*, 2004). This shows that DE and ME overestimate the energy value of protein and underestimates the energy value of fat. As a result, unlike the NE system, the DE and ME systems are unable to predict the performance of pigs.

Table 2.2. Relative DE, ME, and NE values of selected feed ingredients[1]

Ingredient	DE	ME	NE
Reference diet	100	100	100
Animal fat	243	252	300
Soybean meal	107	102	82
Corn	103	105	112
Wheat	101	102	106
Pea	101	100	98
Barley	94	94	96
Canola meal	84	81	64
Distiller's dried grain	82	80	71
Wheat bran	68	67	63

[1] Data from Sauvant et al. (2004). Within each system, values are expressed as percentages of the energy value of the reference diet containing 68% wheat, 16% soybean meal, 2.5% fat, 5% wheat bran, 5% peas and 4% vitamins and minerals.

2.12 DRY EXTRUDED EXPELLED SOYBEAN MEAL IN SWINE DIETS

Soybeans are the gold standard of high quality protein for pigs because their amino acid profile complements the amino acid profiles of several cereal grains. This crop was introduced to North America in 1804. It is known that soybeans contain heat labile antinutritional factors including protease inhibitors, lectins, goitrogens, and antivitamins (Liener, 2000) which can cause inhibition of growth, decreased feed efficiency, goitrogenic responses, pancreatic hypertrophy, hypoglycemia, and liver damage in nonruminant animals depending on species, age, size, sex, state of health, and plane of nutrition (Palacios *et al*., 2004). Such antinutritional factors in soybean are reduced significantly during meal processing (Perilla *et al*., 1997). Soybeans are generally processed by solvent extraction which removes the maximum oil contend leaving less than 1.5% of oil in the meal (Baker and Stein, 2009). Even though solvent extraction is an efficient method to extract oil from soybeans, it requires substantial capital investment, a sophisticated technology and large scale operation. In addition hexane solvent is highly flammable, has carcinogenic property and represents an environmental hazard. These limitations opened the way to develop an alternative process that is less harmful.

Extrusion processing followed by expelling is a relatively recent technology which has been taken up as an alternative means of producing soybean meal and other oilseed meal and oils for human consumption or for the livestock industry, or both (Webster et al., 2003). The combination of dry extrusion with expelling of soybean meal produces a product called dry extruded-expelled soybean meal (DESBM) with a higher fat content compared with solvent extracted soybean meal (Webster *et al*., 2003; Opapeju *et al*., 2006a). Dry extrusion process results in the deactivation of the anti-nutritional factors and total rupturing of cells within seconds and as the product exits the barrel of the extruder, the oil is reabsorbed into the meal. Combining the dry extrusion with a horizontal press will instantly eliminate most of the reabsorbed oil, producing high quality soybean meal that contains up to 8% oil (Zhang *et al*., 1993; Wang and Johnson, 2001). Presence of higher dietary fat content has been shown to increase nutrient digestibility by prolonging the transit time and thereby allowing more time for enzymatic digestion (Li and Sauer, 1994). Extrusion has been shown to boost the denaturation of proteins by the use of the shear force and dry heat applied during passage through the extruder, exposing more peptide bonds to enzymatic hydrolysis (Oryschak *et al*., 2010). Previous research has shown that extruding raw soybeans increases the dry matter and nitrogen digestibility in comparison to the solvent-extracted soybean meal (Reese and Bitney, 2000). Also the digestible and metabolizable energy level in DESBM is significantly higher than that in the solvent-extracted soybean meal for growing pigs (Woodworth *et al*., 2001); the reason being the higher fat content of the meal. In addition the extrusion process itself has been shown to increase the DE and ME values of soybean meal fed to pigs (Rodhouse *et al*., 1992). Pigs fed DESBM or solvent extracted soybean meal had similar effect on growth performance when diets were formulated on the basis of equal apparent ileal digestible lysine and ME (Woodworth *et al*., 2001). Studies have shown significant variation in the feeding value of DESBM for pigs depending on the soybean source and also the processing temperature. However, published data pertaining to the energy values of DESBM for grower pigs are limited and the net energy values for the ingredient has not been determined.

2.13 EXOGENOUS CARBOHYDRASE ENZYMES IN SWINE DIET

In general, carbohydrases are those enzymes which catalyze a reduction in the molecular weight of polymeric carbohydrate. The most commonly used carbohydrase which account for more than 80% of the global carbohydrase market for livestock include 2 dominant proteins, xylanase and glucanase (Adeola and Cowieson, 2011). In addition, the other commercially available carbohydrases include α-amylase, β-mannanase, α-galactosidase, and pectinase.

Bedford and Schulze (1998) reported the benefits of carbohydrase enzymes supplementation to poultry diets. Enzymes have shown to improve performance and nutrient digestibility when added to cereal-based poultry diets (Bedford and Schulze, 1998). There are generally no consistent effects of carbohydrase supplementation on the growth performance of swine. There are reports of positive response to carbohydrase supplementation especially in diets containing high amount of non starch polysaccharide (NSP) (Cadogan *et al*., 2003; Barrerra *et al*., 2004; Kiarie *et al*., 2007), whereas others reported no improvement in weight gain in response to the enzymes (Mavromichalis et al., 2000; Olukosi *et al*., 2007a,b; Woyengo *et al*., 2008). The effect of such enzymes supplementation in pigs varies depending upon the differences in the type and quantity of cereal grains used, the age of the animal, the extent of deficiency of limiting nutrient, and the extent to which the enzyme increase digestible nutrient content (Adeola and Cowieson, 2011). Moreover the use of various carbohydrase enzyme complexes in poultry diets has been shown to produce added benefit than each of the enzyme acting individually (Olukosi *et al*., 2007c). Yet in another study with multi carbohydrase supplementation has reported the carbohydrase itself being digested in the presence of protease (Saleh *et al*., 2004). In view of this, it is essential to understand the optimum combination of enzymes to use in animal diets. The beneficial effect of enzyme combination may be dependent on diet composition (Meng and Slominski, 2005).

Energy digestibility in swine normally decreases with higher level of dietary fiber (Nortey et al., 2004) wherein NSP may reduce the capacity for absorption by reducing enzyme accessibility to substrate. Meng and Slominski (2005) reported that disruption of cell matrix by supplemental enzymes resulting in release of structural protein may be responsible for improved energy utilization in a soybean meal-based diet.

2.14 CONCLUSION

Escalating feed cost is one of the major concerns for livestock producers worldwide. Considering that feed is the major part of the total cost of production, it is no wonder that pork producers are looking for cheaper ingredients to lower diet costs. Dry extruded expelled soybean meal is one such ingredient with better digestible amino acids and energy when compared with the conventional solvent extracted soybean meal. The NE system is believed to be more accurate in expressing the energy value of a feedstuff than the DE and ME systems, because DE and ME systems tend to overestimate the energy value of protein and fiber-rich feedstuffs and underestimate the energy value of fat (Noblet *et al.*, 1994a). Studies have also shown that diets formulated on NE basis with lower protein but balanced for amino acids, resulted in higher energy retention, lower surplus of dietary N and thus a lower N excretion (Noblet and van Milgen, 2004). The NE of DESBM is yet to be determined and it is necessary to measure its NE content to more accurately express its energy value. Even though published prediction equations (Noblet *et al*., 1994a, b) for estimating NE content in swine diets have been supported by some recent studies (Ayoade *et al*., 2012), others have shown that predicted values may not always agree with empirical data (Kil *et al*., 2011). As a result there is a need to confirm the techniques involved in estimating the NE contends.

Despite the demonstrated dominance of the NE system, its utility in swine diet formulation has not gained extensive application in some regions, including North America. Hence it is also necessary to validate the NE system of feed formulation in swine for diets rich in fibre typical to the area.

3.0 MANUSCRIPT I

NET ENERGY CONTENT OF DRY EXTRUDED-EXPELLED SOYBEAN MEAL FED TO GROWING PIGS USING INDIRECT CALORIMETRY

3.1 ABSTRACT: The aim of this study was to determine the net energy (NE) content of dry extruded-expelled soybean (DESBM) in growing pigs using either an indirect calorimetry (IC; direct determination technique) or published prediction equations and determine the effect of diet design on estimated NE values. The study was conducted as a completely randomized design (n = 6), with 4 dietary treatments; a basal diet based on corn-soybean meal, 80:20 ratio of basal diet and DESBM with a constant crude protein content compared with the basal diet, 80:20 ratio of basal diet and DESBM with a constant corn:soybean meal ratio (2.4 : 1) compared with the basal diet and simple substitution with 80:20 ratio of basal diet and DESBM. Twenty four growing barrows (initial BW = 19.6 ± 0.51 kg) were allotted to 1 of 4 treatments. Pigs were fed for 16 d at 550 kcal of metabolizable energy (ME)/kg $BW^{0.60}$/d (high ME intake) for determination of digestible energy (DE) and ME in metabolism crates. Thereafter, pigs were transferred to the IC to measure O_2 consumption and CO_2 production for a 36-h period to determine heat production (HP) over a 24-h period of high ME intake and fasting heat production (FHP) over a 12-h period of fasting. Energy contents obtained using IC were 3,481, 3,462, 3,472 and 3,473 kcal/kg DM for DE, 3,392, 3,378, 3,386 and 3,386 kcal/kg DM for ME and 2,932, 2,872, 2,855 and 2,853 kcal/kg DM for NE, respectively, for treatments A, B, C and D. Corresponding contents obtained with published prediction equations were 3,457, 3,516, 3,520 and 3,507 kcal/kg DM for DE, 3,378, 3,473, 3,416 and 3,398 kcal/kg DM for ME and 2,632, 2,631, 2,612 and 2,593 kcal/kg DM for NE, respectively. The HP values obtained using IC among treatments (i.e., A, B, C and D) were

2,007, 1,930, 2,038 and 2,000 kcal/kg DM and the values for FHP were 1,547, 1,424, 1,508 and 1,468 kcal/kg DM, respectively. Thus, the NE content of DESBM was calculated to be 2,652, 2,548 and 2,540 kcal/kg DM in treatment B, C and D, respectively, using IC. Respective values obtained with published equations were 2,624, 2,530 and 2,436 kcal/kg DM in treatments B, C and D, respectively. This study demonstrated that the values obtained with the IC method were higher than the values obtained with published prediction equations. The discrepancy between the determination technique used was about 1% when diets were formulated with a constant protein content or corn:soybean meal ratio (1.0% and 0.7%, respectively), however, this was 4.1% when the experimental diet was formulated with simple substitution.

Key Words: Dry extruded expelled soybean, indirect calorimetry, net energy, pigs.

3.2 INTRODUCTION

Feed is the single most expensive input in commercial pork production and at least 50% of this cost can be attributed in supplying energy to the animal thus making energy financially the most vital component. Swine diets can be formulated on a variety of energy systems such as the digestible energy (DE), the metabolizable energy (ME) and the net energy (NE) systems of which the NE system provides more accurate estimates of the energy available to the animal (Noblet, 2007). Energy values of protein-rich feeds are often overestimated when expressed on a DE or ME system (Noblet *et al*., 1994a). These discrepancies in measurement of available dietary energy have a drastic effect on the economics of pig production and there is, therefore, an ongoing interest in adopting the NE system.

The most commonly used protein source in livestock diets is soybean meal (SBM), but it also contains certain antinutritional factors which depress animal growth performance. Studies show that such antinutritional factors are reduced significantly due to heat treatment during meal processing (Perilla *et al*., 1997). One such process is the combination of extrusion with expelling which produces a SBM product called dry extruded-expelled SBM (DESBM). However, published data pertaining to the energy values of DESBM for grower pigs are limited. The aim of this study was to determine the NE content of DESBM in growing pigs using either an indirect calorimetry (IC) or published prediction equations.

3.3 MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Protocol Management and Review Committee and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

Dry extruded expelled soybean was obtained from Jordan Mills, Winkler, MB, Canada. Dietary treatments were; a corn-soybean meal basal diet (Diet A), a diet containing Diet A and DESBM in a 80:20 ratio with a constant crude protein content compared with the basal diet (Diet B), a diet with 80:20 ratio of basal diet and DESBM with a constant corn:soybean meal ratio (Diet C) and a diet with simple substitution of basal diet with DESBM in 80:20 ratio (Diet D). Diets were formulated to meet or exceed NRC (1998) nutrient specifications for pigs in the BW range 20 to 50 kg. All diets were fed as mash. The pigs used in the experiment were Genesus (Yorkshire-Landrace female × Duroc male) obtained from Glenlea Swine Research Unit, University of Manitoba.

Table 3.1. Ingredient, calculated, and analysed compositions of the experimental diets[1]

Item	Diet A	Diet B	Diet C	Diet D
	Basal	Constant CP	Constant corn:soybean	Substitution
Ingredient, % of diet				
Corn	67.40	67.66	53.31	53.92
Soybean meal (44%)	28.20	8.57	22.30	22.56
DESBM[2]	0.00	20.00	20.00	19.94
Soybean oil	0.84	0.00	0.84	0.67
Limestone	1.00	1.00	1.00	0.80
Biofos	0.70	0.70	0.70	0.56
Iodized salt	0.50	0.50	0.50	0.40
Vitamin-Mineral Premix[3]	1.00	1.00	1.00	0.80
Lys-HCl	0.06	0.11	0.00	0.05
DL-Methionine	0.00	0.07	0.00	0.00
Threonine	0.00	0.00	0.00	0.00
Tryptophan	0.00	0.09	0.05	0.00
Titanium dioxide	0.30	0.30	0.30	0.30
Calculated provisions				
DE, kcal/kg	3,436	3,510	3,557	3,571
ME, kcal/kg	3,276	3,365	3,382	3,394
CP, %	18.00	18.00	22.85	22.99
Analysed composition				
DM, %	90.0	90.0	90.0	90.0
CP, %	18.2	18.0	21.8	22.7
GE, kcal/kg	3,940	4,028	4,078	4,086
Ash, %	4.7	4.3	5.1	5.0
NDF, %	9.7	10.4	10.4	11
ADF, %	3.2	4.4	4.3	4.4
Starch, %	42.5	33.1	35.2	33.5
EE, %	4.2	5.1	5.1	5.2

[1] as fed basis

[2] DESBM =Dry extruded expelled soybean meal.

[3] Supplied the following per kg of finished feed: vitamin A, 2,000 IU; vitamin D, 200 IU; vitamin E, 40 IU; vitamin K, 2 mg; choline, 350 mg; pantothenic acid, 14 mg; riboflavin, 7 mg; folic acid, 1 mg; niacin, 21 mg; thiamin, 1.5 mg; vitamin B6, 2.5 mg; biotin, 70 mg; vitamin B12, 20 mg, Cu, 25 mg; Zn, 150 mg; Fe, 100 mg; Mn, 50 mg; I, 0.4 mg; Se, 0.3 mg.

Table 3.2 Analysed composition of the DESBM on DM basis

Item	
a. CP, %	40.0
b. GE, kcal/kg	4,703
c. Ash, %	5.7
d. NDF, %	14.9
e. ADF, %	9.2
f. Starch, %	1.7
g. Ether extract, %	9.9

3.3.1 ANIMALS

A total of 24 growing male pigs (Large White × Landrace × Duroc) with an average initial BW of 19.6 ± 0.51 kg (mean ± SD) were acquired from the Gleanlea Swine Research Unit, University of Manitoba. Pigs were individually housed for 15 days in adjustable metabolism crates (1.80 × 0.60 m) with smooth transparent plastic sides and plastic-covered expanded metal sheet flooring in a temperature-controlled room (22 ± 2°C).

3.3.2 DIETS

The diets were formulated to meet NRC (1998) requirements for growing pigs. Four test diets were; corn soybean meal basal diet (Diet A), a diet containing Diet A and DESBM in a 80:20 ratio with a constant crude protein content compared with the basal diet (Diet B), a diet with 80:20 ratio of basal diet and DESBM with a constant corn:soybean meal ratio (Diet C) and a diet with simple substitution of basal diet with DESBM in 80:20 ratio (Diet D).

3.3.3 EXPERIMENTAL DESIGN AND PROCEDURES

The study was conducted in two consecutive periods (12 pigs per each period) using the same facility and similar experimental conditions and procedures because only 2 calorimetric chamber were available at the time of the current study. Pigs were assigned to one of four experimental diets in a completely randomised design to give 6 replicates per diet. Pigs were fed experimental diets for 15 days, including 10 days for adaptation to feed and environmental conditions and 5 days for total collection of faeces and urine to measure DE and ME contents.

Pigs were fed their respective diets at at 550 kcal ME/kg $BW^{0.60}$per day based on BW on days 1, 5 and 10 which was close to ad libitum intake. During the study, pigs were fed at 0830 hours and were trained to consume their daily feed allowance within 1 hour. Pigs had unlimited access to water via low pressure nipple throughout the study. During the last 5 days of each feeding period, total faecal and urine collection were carried out for estimation of DE and ME as described previously by Woyengo *et al*. (2010). From d 11 to d 16, feces was collected once daily in the morning and stored at -20°C. Collection of urine commenced on the morning of d 11 and ended on the morning of d 15. Urine was also collected once daily in the morning (in jugs containing 10 mL of 6N HCl to minimise N losses) and weighed. A sample (10% of the total weight) was obtained, strained through cotton gauze and glass wool, and stored at -20°C.

On d 16, two pigs each were transferred to the calorimetric chambers (1.22 × 0.61 × 0.91 m; Columbus Instruments, OH, USA) for 36 h of heat production (HP) and fasting heat production (FHP) measurement based on O_2 consumption, CO_2 production and urine output. Pigs were brought into the calorimetric chambers within 1 h of consuming their daily ration and HP was measured continuously for 24 hours (fed state) followed by 12 hours (fasting state) of FHP measurement. Fresh water was available in the chambers at all times and urine voided during the 24 hours and 12 hours period were collected separately, weighed, sub-sampled and stored at -20°C until required for N analysis. Temperature within the chamber was maintained at 22 ± 1°C and personnel movement in the chamber room was limited to avoid distressing pigs during HP and FHP measurements.

3.3.4 SAMPLE PREPARATION AND CHEMICAL ANALYSES

Fecal samples were oven dried at 50°C over a 5-d period, and finely ground before chemical analysis. Urine samples from metabolism crates and calorimetry chambers were thawed and pooled separately for each pig, sieved through cotton gauze and filtered with glass wool.

Diet and fecal DM was determined according to the AOAC (1990; method 925.09) by oven drying 5 g of sample at 102°C overnight. The GE content of DESBM, diets, feces and urine was measured using an adiabatic bomb calorimeter (model 6400, Parr Instrument, Moline, IL) which had been calibrated using benzoic acid as a standard. Nitrogen content in diets, feces and urine was determined using the combustion method (method 990.03; AOAC, 1990) using the LECO N analyser (model CNS-2000; LECO Corp., St. Joseph, MI) and CP was calculated as N x 6.25. Crude fat in diet and ingredient samples was determined after hexane extraction (method 920.39; AOAC, 1990) in an extraction apparatus. Starch content in the diets was measured using an assay kit (Megazyme Total Starch assay kit; Megazyme International Ltd., Wicklow, Ireland). Diets and fecal samples were analysed for titanium dioxide using inductively coupled plasma mass spectrometry. The ADF and NDF contents in diets were determined according to the method of Goering and Van Soest (1970) and ash content was determined according to AOAC (1990; method 942.05).

To determine the GE of urine, 0.5 g of cellulose was dried at 100°C for 24 h and 2 mL of urine sample was added over it and the weight of the resulting mixture was recorded. The urine-cellulose mixture along with a sample of pure cellulose was again dried in an oven at 50°C for 24 h and then weighed for estimation of urine DM. The GE of the dried urine-cellulose mixture and pure cellulose were determined using an adiabatic bomb calorimeter as described above and from which the GE of urine samples were calculated by the difference method (Fleisher *et al*., 1981).

3.3.5 CALCULATIONS

Heat production, fasting HP, retained energy, DM intake and NE values were calculated using the following equations:

$HP = 3.87 \times O_2 + 1.20 \times CO_2 - 1.43 \times \text{urinary N}$ (Brouwer, 1965) .... (3.1)

Where HP = heat production (kcal), O_2 = oxygen consumption (L), CO_2 = carbon dioxide production (L).

$RE = ME - HP$ …………………. (3.2)

Where RE = retained energy (kcal/d), ME = metabolizable energy intake, (kcal/d), HP = heat production (kcal/d).

$DMI = \text{feed intake} \times \text{feed DM}$ …………………. (3.3)

Where DMI = dry matter intake (kg), feed intake is in kg, and feed DM is in %.

$NE = (RE + FHP)/DMI$ …………………. (3.4)

Where NE = net energy (kcal/kg DM), RE = retained energy (kcal/d), FHP = fasting heat production (kcal/d), DMI = dry matter intake (kg).

Net energy was also calculated according to the following equations (Noblet *et al*. 1994a):

$NE = 0.843 \times DE - 463$ ………………………....…………………. (3.5)

$NE = 0.700 \times DE + 1.61 \times EE + 0.48 \times ST - 0.91 \times CP - 0.87 \times ADF$ ……. (3.6)

$NE = 0.870 \times ME - 442$ …………………. (3.7)

$NE = 0.726 \times ME + 1.33 \times EE + 0.39 \times ST - 0.62 \times CP - 0.83 \times ADF$ ……. (3.8)

$NE = 2{,}790 + 4.12 \times EE + 0.81 \times ST - 6.65 \times Ash - 4.72 \times ADF$ ………. (3.9)

$NE = 2{,}875 + 4.38 \times EE + 0.67 \times ST - 5.50 \times Ash - 2.01 \times (NDF - ADF) -$
$4.02 \times ADF$ ……….………. (3.10)

Where NE = net energy (kcal/kg DM), ME = metabolisable energy (kcal/kg DM), DE = digestible energy (kcal/kg DM), EE = ether extract (% DM), ST = starch (% DM), CF = crude fiber (% DM) and ADF = acid detergent fibre (% DM)

The energy content of DESBM was calculated using the difference method (Woyengo *et al*., 2010) by subtracting the NE contribution of the basal diet from the NE of the diets containing 20% DESBM.

The NE of test DESBM was calculated as follow:

$NE_{DESBM}\ (kcal/kg\ DM) = NE_{Basal\ diet} - [(NE_{Basal\ diet} - NE_{test\ diet})/0.2]$ ………. (3.11)

3.3.6 STATISTICAL ANALYSES

All data were subjected to the Mixed procedures of SAS (SAS Inst. Inc., Cary, NC). Effects of diet and period were included in the model for statistical analysis. The effect of period was not statistically significant; therefore, it was not included in the final model. The individual pig was considered as the experimental unit and probability of $P < 0.05$ was considered significant.

3.4 RESULTS

All animals adapted well to their respective diets and environmental conditions, remained healthy and readily consumed their daily feed allowance during the experimental period.

Table 3.3 details the energy, heat production and nitrogen utilisation values for the 4 dietary treatments as determined using the IC method. Energy contents of diets obtained using IC were 3,481, 3,462, 3,472 and 3,473 kcal/kg DM for DE, 3,392, 3,378, 3,386 and 3,386 kcal/kg DM for ME and 2,932, 2,872, 2,855 and 2,853 kcal/kg DM for NE, for treatments A, B, C and D, respectively. Corresponding contents obtained with published prediction equations were 3,457, 3,516, 3,520 and 3,507 kcal/kg DM for DE, 3,378, 3,473, 3,416 and 3,398 kcal/kg DM for ME and 2,632, 2,631, 2,612 and 2,593 kcal/kg DM for NE, respectively. The HP values obtained using IC among treatments (i.e., A, B, C and D) were 2,007, 1,930, 2,038 and 2,000 kcal/kg DM and the values for FHP were 1,547, 1,424, 1,508 and 1,468 kcal/kg DM, respectively. Thus, the NE content of DESBM was calculated to be 2,652, 2,548 and 2,540 kcal/kg DM for diets B, C and D, respectively using IC ($P < 0.0001$). Respective values obtained with published equations (Noblet *et al*., 1994a) were 2,624, 2,530 and 2,436 kcal/kg DM. The study demonstrated that the values obtained with the IC method were higher than those obtained with prediction equations. The discrepancy between the determination technique used was about 1% when diets were formulated with a constant protein content or corn:soybean meal ratio (1.0% and 0.7%, respectively), however, this was 4.1% when diet was formulated with simple substitution technique. As the values obtained from the two diet formulation techniques namely, the constant protein content or the constant corn:soybean meal ratio were not significantly different, the average NE value of DESBM evaluated was 2,544 kcal/kg DM.

Table 3.3 Energy balance in growing pigs and energy values of diets and DESBM determined by the indirect calorimetry method

Item	Dietary treatments				SEM	P-value
	A	B	C	D		
Energy value of diets, kcal/kg of DM:						
DE	3,481	3,462	3,472	3,473	9.40	0.586
ME	3,392	3,378	3,386	3,386	6.16	0.431
HP[1]	2,007	1,930	2,038	2,000	53.01	0.637
FHP[2]	1,547	1,424	1,508	1,468	53.99	0.513
RE[3]	1,385	1,448	1,348	1,386	52.85	0.686
NE[4]	$2{,}932^{a}$	$2{,}872^{b}$	$2{,}855^{b}$	$2{,}853^{b}$	5.66	< 0.001
Net protein Utilization, %	56.5^{ab}	59.3^{a}	52.6^{b}	53.6^{b}	2.21	0.032
Efficiencies of NE:						
NE/ME	0.87^{a}	0.85^{b}	0.84^{b}	0.84^{b}	0.003	0.002
NE/DE	0.84^{a}	0.83^{ac}	0.82^{bc}	0.82^{bc}	0.004	0.002
Energy value of DESBM, kcal/kg of DM:						
DE	-	$3{,}384^{b}$	$3{,}438^{a}$	$3{,}443^{a}$	10.58	0.011
ME	-	3,324	3,363	3,361	12.67	0.097
NE[5]	-	$2{,}652^{a}$	$2{,}548^{b}$	$2{,}540^{b}$	7.17	< 0.001

[a,b, c] Means not sharing a common superscript are significantly different

[1] Heat production = [3.87 x O_2 + 1.20 x CO_2 – 1.43 x urinary N]/DMI

[2] Fasting heat production = [3.87 x O_2 + 1.20 x CO_2 – 1.43 x urinary N]/DMI

[3] Retained energy = (ME intake – HP)/DMI

[4] Net energy = (RE + FHP)/DMI

[5] NE of DESBM was calculated using the difference method by subtracting the NE contribution of the basal diet from the NE of the diets containing 20% DESBM (Woyengo *et al.*, 2010).

3.5 DISCUSSION

Nutrient content of feed ingredients can differ from one season to other and from one supplier to the other. For this reason, it is important to determine the nutrient content of an ingredient. The DESBM sample used for the current study was locally obtained from Jordan Mills, Manitoba, Canada. A comparison of the analyzed nutrient composition of DESBM used in the current experiment with studies by Woodworth *et al*. (2001) in which the dry extruded-expelled soybean meal used were produced and provided by Insta-Pro International, Des Moines, IA showed similar values for DM, GE and ash, but with a high CP and low EE in the latter. This variation could be attributed to the various factors involved during the meal processing, namely the temperature, time or moisture contend. Similar variation for CP and EE, but with comparable concentrations of NDF and ADF were seen in studies by Baker and Stein. (2009). In studies by Opapeju *et al*. (2006a) where two batches of DESBM from the same source were used; DESBM showed similar EE contend but slightly higher CP. This higher CP (40 vs. 43%) content may be due to lower moisture content when compared with the present study (93 vs. 97 % DM).

Energy contents of diets determined using total collection method were 3,481, 3,462, 3,472 and 3,473 kcal/kg DM for DE, 3,392, 3,378, 3,386 and 3,386 kcal/kg DM for ME, respectively, for treatments A, B, C and D. The ME:DE ratio in the current study (0.98; average value for the 4 diets) is in accordance with the previous studies. Noblet and van Milgen, (2004) reported that in most circumstances for complete feed, the ME:DE ratio would be approximately 0.96.

Digestible energy value of DESBM obtained in the present study were 3,384, 3,438 and 3,443 kcal/kg DM for treatments B, C and D, respectively. Respective ME values were

3,324, 3,363 and 3,361 kcal/kg DM. Baker and Stein (2009) reported a higher DE value of 3,827 kcal/kg DM for extruded-expelled SBM from conventional soybeans. The probable reason for this could be the variation in body weight of pigs used for the studies and also lower CP content for DESBM used in the present study when compared with the latter. In the current study, pigs with an initial BW = 19.6 ± 0.51 kg were used and whereas pigs with an initial BW = 38.6 ± 3.46 kg were used in the study by Baker and Stein (2009). In growing pigs, digestibility coefficient of energy or DE:gross energy ratio increases with increasing BW (Noblet and Shi, 1994). Also Woodworth *et al*. (2001) reported similar results wherein the DE content for DESBM in growing pigs (initial BW 41 kg) were 4,120 and 4,210 kcal/kg (as-fed basis) for hulled and de hulled meals, respectively.

Heat production values among treatments (i.e., A, B, C and D) obtained in the present study were 2,007, 1,930, 2,038 and 2,000 kcal/kg DM. Noblet *et al*. (1994) reported a similar HP value of 2,062 kcal/kg DM for growing pigs. Values of FHP obtained in this present study were 1,547, 1,424, 1,508 and 1,468 kcal/kg DM for diets A, B, C and D, respectively. These values are comparable to that obtained by Noblet *et al*. (1994); 179 kcal/kg $BW^{0.6}$ for 35 kg pigs (equivalent to 1,517 kcal/kg DM of feed).

The NE content of DESBM was calculated to be 2,652, 2,548 and 2,540 kcal/kg DM in treatment B, C and D, respectively, using IC. Respective values obtained with published equations were 2,624, 2,530 and 2,436 kcal/kg DM in treatments B, C and D, respectively. This discrepancy for higher values for IC method when compared to the prediction equations could be due to not taking into account the physical activity of the animals while measuring fasting heat production. Physical activity could be defined as standing up, standing, eating, walking, lying down and sitting (Rijnen *et al*., 2003). About 8 % of ME intake may be dissipated for physical activity in growing pigs (van Milgen and Noblet, 2003).

The results from the present study shows that the NE values of DESBM obtained with the IC method were higher than those obtained with prediction equations for all the three dietary designs; the disparity being least when formulated with a constant corn:soybean meal ratio. So for routine NE determination where difference method is used to obtain the NE value for ingredients, diets should be formulated to contain a constant ration of other energy yielding components.

4.0 MANUSCRIPT II

EFFECT OF ENZYME SUPPLEMENTATION ON THE NET ENERGY CONTENT OF DRY EXTRUDED-EXPELLED SOYBEAN MEAL FED TO GROWING PIGS

4.1 ABSTRACT: The aim of this study was to determine the effect of a multi-enzyme complex (MC) on the net energy (NE) content of dry extruded-expelled soybean meal (DESBM) fed to growing pigs. Twenty four barrows (16.9 ± 0.76 kg) were allotted in a completely randomized design to 4 dietary treatments to give 6 replicates per treatment. Dietary treatments were; a corn soybean meal basal diet (Diet A), a diet containing Diet A and DESBM in a 80:20 ratio with a constant corn:soybean meal ratio (Diet B), Diet B + 0.05% MC (Diet C) and Diet B + 0.1% MC (Diet D). The MC used was a mixture of carbohydrases (Superzyme OM). Pigs were fed in metabolism crates for a period of 16 d at 550 kcal of metabolisable energy (ME)/kg $BW^{0.60}$/d to determine digestible energy (DE) and ME contents using the total collection method. Thereafter, pigs were moved into an indirect calorimeter (IC) where heat production was measured over a 36-h period based on O_2 consumption and CO_2 production. The energy content of DESBM was calculated using the difference method. The DE and ME contents obtained were 3,365, 3,361, 3,401 and 3,381 kcal/kg DM and 3,260, 3,245, 3,295 and 3,283 kcal/kg DM for Diet A, B, C, and D, respectively. Corresponding values for NE were 2,897, 2,823, 2,848 and 2,842 kcal/kg DM. The heat production values among treatments (i.e., A, B, C and D) were 1,595, 1,606, 1,602 and 1,595 kcal/kg DM and those for fasting heat production were 1,231, 1,184, 1,173 and 1,154 kcal/kg DM, respectively. Thus, the NE content of DESBM was determined to be 2,527, 2,652 and 2,621 kcal/kg DM in treatment B, C and D, respectively. Respective values obtained with published equations were 2,305, 2,435 and 2,362 kcal/kg DM. Data was analysed using Mixed procedure of SAS 9.2. The results demonstrated that enzyme supplementation improved ($P < 0.0001$) the energy content of both diet and test ingredient. In conclusion, supplementation with MC at 0.05% and 0.1% of the diet improved NE values of DESBM by 4.9 % and 3.7%, respectively.

Key Words: Dry extruded expelled soybean, enzymes, net energy, pig.

4.2 INTRODUCTION

Pig diets are mostly based on cereals, and they contribute the major part of the energy. The nutritive value differs between the cereals due to their chemical composition. They differ in total content of sugars, starch, ß-glucans, non-starch polysaccharides (NSP) and dietary fibre. Non-starch polysaccharides are complex carbohydrates that form the main component of dietary fiber sources. Whereas pigs lack the enzymes to digest such compounds, consequently NSP remain undigested through the small intestine. Also their presence in the digestive tract increases viscosity of digesta and can encapsulate other nutrients, preventing their absorption.

In vitro studies have shown that a combination of carbohydrase enzymes was more effective in NSP depolymerization of soybean meal, canola meal, and peas than when the individual carbohydrases were used (Meng *et al.,* 2002). Multienzyme preparations have been extensively used in chicken to enhance nutrient utilization, with comparatively less studies been conducted to determine their effects on pig performance. Woyengo *et al*. (2010) reported that supplementing a corn-soybean meal-based diet with a combination of multicarbohydrase, a preparation containing non-starch polysaccharide degrading enzymes in broiler chickens improved nutrient utilization and growth performance. Also Cowieson and Ravindran,(2008) reported that a combination of multicarbohydrase enzyme fed in broiler diets with both adequate and reduced energy and amino acid content showed an increase in apparent metabolizable energy. Yet in another recent study by Cozannet *et al*. (2012) carbohydrase enzyme addition increased the DE value of ingredients in 60-kg male growing pigs. But to our knowledge only limited information is available on the effect of exogenous enzyme on net energy aspect in growing pigs. The aim of this study was to determine the effect of a multi-enzyme complex (MC) on the net energy (NE) content of dry extruded-expelled soybean meal (DESBM) fed to growing pigs.

4.3 MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

4.3.1 ANIMALS

A total of 24 growing male pigs (Large White × Landrace × Duroc) with an average initial BW of 16.9 ± 0.76 kg (mean ± SD) were acquired from Gleanlea Swine Research Unit, University of Manitoba.

4.3.2 DIETS

As concluded in manuscript 1, the experimental diets in this study were formulated to contain a constant ratio between corn and soybean meal (Diet B, C and D). The diets were formulated to meet NRC (1998) requirements for growing pigs. Four test diets were; a corn soybean meal basal diet (Diet A), a diet containing Diet A and DESBM in a 80:20 ratio with a constant corn:soybean meal ratio (Diet B), Diet B + 0.05% MC (Diet C) and Diet B + 0.1%

MC (Diet D). The MC used was a mixture of carbohydrases (Superzyme OM, Canadian Bio-System Inc., Calgary, Alberta, Canada).

Table 4.1 Ingredients and analysed compositions of the experimental diets[1]

Item	Basal	Constant Corn:SBM without MC[2]	Constant Corn:SBM with 0.05% MC	Constant Corn:SBM with 0.1% MC
Ingredients, % of diet				
Corn	67.40	53.31	53.31	53.31
Soybean meal (44%)	28.20	23.30	23.30	23.30
DESBM[3]	0.00	20.00	20.00	20.00
Vegetable oil	0.84	0.84	0.84	0.84
Limestone	1.00	1.00	1.00	1.00
Biofos	0.70	0.70	0.70	0.70
Iodized salt	0.50	0.50	0.50	0.50
Vitamin-mineral premix[4]	1.00	1.00	1.00	1.00
Lys-HCl	0.06	0.00	0.00	0.00
Tryptophan	0.00	0.05	0.05	0.05
Titanium oxide	0.30	0.30	0.30	0.30
Analysed composition				
DM, %	89.0	90.0	90.0	90.0
CP, %	17.3	22.0	22.5	22.4
GE, kcal/kg	3,899	4,027	4,037	4,027
Ash, %	4.5	5.3	5.2	5.1
NDF, %	10.9	10.8	9.9	10.7
ADF, %	3.2	4.4	4.3	4.4
Starch, %	42.1	35.7	35.5	33.6
EE, %	4.3	5.1	5.1	5.2
Total NSP, %	9.5	10.8	10.5	10.2

[1] as fed basis

[2] Enzyme complex supplied 1,700 units of cellulose, 1,100 units of pectinase , 240 units of mannanase, 30 units of galactanase, 1,200 units of xylanase, 360 units of glucanase, 1,500 units of amylase, 120 units of protease.

[3] DESBM =Dry extruded expelled soybean meal. Analysed composition of DESBM: CP = 40.0%, GE = 4,703kcal/kg, DM = 93.0%, Ash = 5.7%, NDF = 14.9%, ADF = 9.2%, Starch = 1.7% and EE = 9.9%.

[4] Supplied the following per kg of finished feed: vitamin A, 2,000 IU; vitamin D, 200 IU; vitamin E, 40 IU; vitamin K, 2 mg; choline, 350 mg; pantothenic acid, 14 mg; riboflavin, 7 mg; folic acid, 1 mg; niacin, 21 mg; thiamin, 1.5 mg; vitamin B6, 2.5 mg; biotin, 70 mg; vitamin B12, 20 mg, Cu, 25 mg; Zn, 150 mg; Fe, 100 mg; Mn, 50 mg; I, 0.4 mg; Se, 0.3 mg.

4.3.3 EXPERIMENTAL DESIGN AND PROCEDURES

The study was conducted in two consecutive periods (12 pigs per period) using the same facility and similar experimental conditions and procedures. Three calorimetric chambers were used for the current study. Pigs were assigned to one of four experimental diets in a completely randomised design to give 6 replicates per diet. Pigs were fed experimental diets for 15 days in metabolism cages and thereafter, pigs were moved into the indirect calorimeter for HP measurements. The experimental procedures were similar to those described in manuscript I.

4.3.4 SAMPLE PREPARATION AND CHEMICAL ANALYSIS

Sample preparation and chemical analysis for the ingredient and diet samples were carried out using the same procedures as described in manuscript I.

Non-starch polysaccharides were analyzed using gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids). The procedure for neutral sugars was performed as described by Englyst and Cummings (1988) with some modifications (Slominski and Campbell, 1990). Uronic acids were determined using the procedure described by Scott (1979).

4.3.5 CALCULATIONS

Heat production, fasting heat production, retained energy, and NE were calculated using similar equations as those described in manuscript I.

4.3.6 STATISTICAL ANALYSES

All data were subjected to the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Effects of diet and period were included in the model for statistical analysis. The effect of period was not statistically significant; therefore, it was not included in the final model. The individual pig was considered as the experimental unit and probability of $P < 0.05$ was considered significant.

4.4 RESULTS

All animals adapted well to their respective diets and environmental conditions, remained healthy and readily consumed their daily feed allowance during the experimental period.

Table 4.2 details the energy and heat production values for the 4 dietary treatments as determined using the IC method. The DE and ME contents obtained were 3,365, 3,361, 3,401 and 3,381 kcal/kg DM and 3,260, 3,245, 3,295 and 3,283 kcal/kg DM for Diet A, B, C, and D, respectively. Corresponding values for NE were 2,897, 2,823, 2,848 and 2,842 kcal/kg DM. The HP values among treatments (i.e., A, B, C and D) were 1,595, 1,606, 1,602 and 1,595 kcal/kg DM and those for FHP were 1,231, 1,184, 1,173 and 1,154 kcal/kg DM, respectively. The NE content of DESBM was determined to be 2,527, 2,652 and 2,621 kcal/kg DM in treatment B, C and D, respectively. Respective values obtained with published equations were 2,305, 2,435 and 2,362 kcal/kg DM. The results demonstrated that enzyme supplementation improved ($P < 0.0001$) the energy content of both diet and test ingredient.

Table 4.2 Energy balance of pigs and energy values of diets and DESBM determined by the indirect calorimetry method

Item	Dietary treatments				SEM	P-value
	Basal	Constant Corn:SBM without MC	Constant Corn:SBM with 0.05% MC	Constant Corn:SBM with 0.1% MC		
Energy value of diets, kcal/kg of DM:						
DE	3,365^{b}	3,361^{b}	3,401^{a}	3,381^{ab}	11.03	0.011
ME	3,260^{bc}	3,245^{c}	3,295^{a}	3,283^{ab}	5.38	<0.001
HP[1]	1,595	1,606	1,620	1,594	352.85	0.990
FHP[2]	1,231	1,184	1,173	1,154	349.08	0.820
RE[3]	1,665	1,639	1,675	1,688	353.49	0.951
NE[4]	2,897^{a}	2,823^{b}	2,848^{b}	2,842^{b}	8.07	<0.001
Efficiencies of NE:						
NE/ME	0.89^{a}	0.87^{b}	0.87^{b}	0.87^{b}	0.003	<0.001
NE/DE	0.86^{a}	0.84^{b}	0.84^{b}	0.84^{b}	0.002	<0.001
Energy value of DESBM, kcal/kg of DM:						
DE	-	3,345^{c}	3,548^{a}	3,445^{b}	14.88	<0.001
ME	-	3,184^{c}	3,434^{a}	3,375^{b}	12.69	<0.001
NE[5]	-	2,527^{c}	2,652^{a}	2,621^{b}	6.85	<0.001

[a,b, c] Means not sharing a common superscript are significantly different

[1] Heat production = [3.87 x O_2 + 1.20 x CO_2 – 1.43 x urinary N]/DMI

[2] Fasting heat production = [3.87 x O_2 + 1.20 x CO_2 – 1.43 x urinary N]/DMI

[3] Retained energy = (ME intake – HP)/DMI

[4] Net energy = (RE + FHP)/DMI

[5] NE of DESBM was calculated using the difference method by subtracting the NE contribution of the basal diet from the NE of the diets containing 20% DESBM (Woyengo *et al*., 2010).

4.5 DISCUSSION

Carbohydrases in general include all enzymes, which catalyze a reduction in the molecular weight of polymeric carbohydrate. Non-starch polysaccharides are partially hydrolyzed by supplementing NSP-degrading carbohydrases (Parkkonen *et al*., 1997; Nortey *et al*., 2007) and therefore the nutrients entrapped by fiber get released. The NSP-degrading enzymes act by degrading the cell walls, thereby increasing the cell wall permeability and releasing nutrient into digestive tract. Supplementation with a carbohydrase mixture has shown to improve energy and nutrient digestibility in various feedstuffs fed to pigs (Omogbenigun *et al*., 2004; Emiola *et al*., 2009). In an in vitro incubation study, Slominski *et al.* (2006) established that a carbohydrase mixture containing cellulase, pectinase, mannanase, xylanase, and glucanase is effective in degrading cell wall polysaccharides and improving energy digestibility in flaxseed. Non-starch polysaccharides degrading enzymes in swine diets help in dietary fiber degradation there by increasing the digestibility of nutrients (i.e., CP, crude fat, and/or starch) resulting in higher energy digestibility (Bedford and Schulze, 1998). In the present study, the carbohydrase complex used contained enzymes that can target several NSP in the diet including arabinoxylans, β-glucans, arabinogalactans, mannans, galactomannans, or pectic polysaccharides. The multicarbohydrase complex used in the current study has been shown to improve the nutrient digestibility in broilers (Meng and Slominski, 2005; Meng *et al*., 2006; Woyengo *et al.*, 2010). As for swine, reports of improvement in nutrient utilization following carbohydrase supplementation is variable (Adeola and Cowieson, 2011; Cozannet *et al*., 2012). In pigs, carbohydrases not only improves nutrient digestibility (Omogbenigun *et al*., 2004; Ji *et al*., 2008), but also alters the characteristics of the digestive content, which may indirectly affect the integrity of gut mucosa (Jakob *et al*., 2005).

The energy contents for diets B, C and D obtained in this study were 3,361, 3,401 and 3381 kcal/kg of DM for DE, 3,245, 3,295 and 3,283 kcal/kg of DM for ME and 2,823, 2,848 and 2,842 kcal/kg of DM for NE, respectively. Enzyme supplementation increased the DE content of the diet by 1.2 and 0.6%, ME by 1.5 and 1.2% and NE by 0.9 and 0.7% for 0.05 and 0.1% enzyme, respectively. Cozannet *et al*. (2012) reported comparable results wherein multi-enzyme preparation (xylanase and glucanase) improved the DE of diets even though it was quantitatively low (0.09 MJ/ kg DM). In the same study by Cozannet *et al*. (2012), the ME and NE value of diets were also improved (0.7 and 0.9 %, respectively); though not statistically analyzed. The enzyme effect is also consistent with the observations of Coweison and Ravindran (2008) wherein supplementation with an enzyme cocktail of xylanase, amylase and protease in broiler diets improved AME by an average of 3%. Similar results were reported in broiler chicken fed corn soybean diets where a multicarbohydrase enzyme cocktail significantly improved the AME_n content (Meng and Slominski, 2005). The FHP values obtained in the present trials were not affected by dietary treatment and were close to those obtained by Noblet et al. (1994) (0.750 MJ. d^{-1}. $kg^{-0.60}$)

An effect of enzyme on the NE contend of DESBM was also observed in the current study. Enzyme addition increased the NE value of the test ingredient by 110 kcal/kg DM, on an average. Similar NE value for DESBM was obtained in the previous experiment (Diet B; manuscript I). It should, however, be noted that the CP contend for Diet B (manuscript I) was about 18% lower when compared to the Diets C and D in the current experiment. Lower dietary CP reduces deamination of excess AA and the consecutive production and excretion of urea in urine and lowers body protein turnover and heat production of the animals (Le Bellego *et al*., 2001). In addition, the synthesis, excretion and more commonly metabolism of urea associated with the excretion of N in excess, represents a non negligible energy cost to the animal. Consequently, at a given DE or ME intake, NE supply and therefore energy gain are higher for low CP diets (Noblet *et al*., 2001). To conclude, the NE content of DESBM was determined to be 2,527, 2,652 and 2,621 kcal/kg DM in treatment B, C and D, respectively, and supplementation with MC at 0.05% and 0.1% of the diet improved NE values of DESBM by 4.9 and 3.7%, respectively.

5.0 MANUSCRIPT III

VALIDATION OF NET ENERGY SYSTEM OF FEED FORMULATION IN GROWING-FINISHING PIGS FED A TYPICAL WESTERN CANADIAN DIET

5.1 ABSTRACT: The aim of this study was to determine the growth performance and carcass characteristics of growing-finishing pigs fed diets formulated on the NE basis thereby validating the NE system of feed formulation. Twenty four pigs (12 barrows and 12 gilts) with an initial BW of 25 ± 1.3 kg were blocked by sex and allotted 1 of the 3 treatments, resulting in 8 replicates, 4 barrows and 4 gilts per treatment. Dietary treatments were; a barley-based control diet formulated on the DE basis, control diet formulated on NE basis with or without multi-enzyme complex. Pigs were fed on a 3-phase dietary program for BW 25 to 50 kg (phase 1), 50 to 75 kg (phase 2), and 75 to 110 kg (phase 3). Diet A was formulated to contain 3,402 kcal/kg of DE and Diet B and diet C to contain 2,475 kcal/kg of NE with 0.98, 0.85, and 0.73% SID Lys for phase 1, 2 and 3, respectively. Individual pig BW and feed disappearance were monitored biweekly during each phase to determine average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G: F) ratio. Pigs on attaining a BW of 100 kg were slaughtered to determine carcass characteristics. Significant dietary effect was observed for ADFI ($P = 0.024$) in phase 1 and for ADG (P = 0.011) in phase 3. For overall performance, significant dietary effect was observed for ADG ($P = 0.017$) and G: F ($P = 0.05$). Carcass length, dressing percentage, loin eye area, loin depth, backfat thickness and belly firmness were not different ($P > 0.05$) across dietary treatments. The results indicate a better growth performance when diets were formulated on NE basis when compared to the DE system of diet formulation. Also addition of enzyme improved the performance for the NE system of diet formulation for high fiber diets.

Key Words: Digestible energy, high fiber, net energy, pig.

5.2 INTROCUCTION

The aim of commercial pig production is to provide the adequate, but not more nutrient to reach the optimum performance of the animals, thereby achieving profitable production. To accomplish this, the nutritional needs of the animals and the nutritive value of the feed ingredients should be expressed in accurate units. There are various energy systems such as the digestible energy (DE), the metabolizable energy (ME) and the net energy (NE) systems for formulating swine diets. Though it is an established fact that the NE system provides more accurate estimates of the energy available to the animal, still Canadian nutritionists continue to formulate diets using digestible or metabolizable energy systems. The major reasons as reported from previous studies being the complexity in estimating the NE from various sources in the feed and lack of data pertaining to NE values of many ingredients and co-products.

The recent increase in grain cost in North America has made pork producers to seek for alternatives to traditional ingredients in swine diets. These include co-products like distillers grains and opportunity ingredients like the canola being produced in surplus. Studies by Jha *et al*. 2010 have shown the use of co-products in swine diets as alternative feedstuffs is effective in reducing the feed costs and thereby improving economic sustainability of the industry. Inclusion of high fibre ingredients like the distillers dried grains with soluble (DDGS) into diets for grower-finisher pig that were formulated on DE or ME basis resulted in reduced performance (Whitney *et al*., 2006; Widyaratne and Zijlstra, 2007). Energy values of protein rich and high fibre diets are often overestimated when expressed on a DE or ME system (Noblet, 2005) resulting in a less dietary NE content because the DE and ME systems does not take into account the energy losses due to heat increment. Yet another setback of using higher amount of co-products is the feeding of diets with a higher content of non-starch polysaccharides (NSP).

Increasing the fermentable carbohydrate content in the diet reduces the ME utilization due to the increased gas production and fermentation heat lost (Szabo and Halas, 2013). Consequently NE system more precisely predict the amount of energy used and retained in the pig for fibrous feedstuffs when compared to the ME system (Payne and Zijlstra, 2007). Such diets open up the prospects for using NSP-degrading enzyme to improve nutrient digestibility (*Zijlstra et al*., 2010). So for a cost-efficient swine production, the emphasis is being shifted to the NE system. Net energy system along with standardised ileal nutrient digestibility concept will better serve to formulate diets that are economical without compromising animal performance and with fewer nutrients being excreted unutilized.

Most experiments evaluating the relationship between dietary ingredients and growth performance relied on diets formulated either on DE or ME basis. Therefore, the objective of this study was to validate the NE system for diet formulation in growing-finishing pigs fed a typical Western Canadian.

5.3 MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

5.3.1 ANIMALS

A total of 24 growing pigs, 12 barrows and 12 gilts (Large White × Landrace × Duroc) with an average initial BW of 25 ± 1.3 kg kg (mean ± SD) were obtained from the Gleanlea Swine Research Unit, University of Manitoba.

5.3.2 DIETS

The diets were formulated to meet NRC (2012) requirements for growing pigs. Three test diets were; a barley-based control diet formulated on DE basis (Diet A), control diet formulated on NE basis (Diet B) and Diet B + Multi-enzyme complex (Diet C). Pigs were fed on a 3-phase dietary program for BW 25 to 50 kg (phase 1), 50 to 75 kg (phase 2), and 75 to 110 kg (phase 3). Diets A was formulated to contain 3,402 kcal/kg of DE and Diet B and diet C to contain 2475 kcal/kg of NE with 0.98, 0.85, and 0.73% SID Lys for phase 1, 2 and 3, respectively.

Table 5.1. Ingredient and analysed composition of the experimental diets[1]

	Phase 1		Phase 2		Phase 3	
Item	Diet A	Diet B	Diet A	Diet B	Diet A	Diet B
Ingredient, %						
Barley	29.59	33.30	35.99	34.40	43.11	43.80
Wheat	10.30	10.30	10.30	10.30	10.30	10.30
Canola meal	13.28	9.00	10.31	10.04	7.70	6.51
DESBM[2]	8.00	12.00	4.95	7.25	2.48	4.30
Corn DDGS	13.88	10.00	11.95	9.73	11.40	9.80
Millrun	6.00	6.00	6.00	6.00	6.00	6.00
Peas	12.00	10.30	12.98	13.00	11.60	11.00
Soybean Oil	4.20	6.30	4.93	6.75	4.95	5.85
Limestone	1.00	1.05	0.89	0.88	0.78	0.78
Iodized salt	0.50	0.50	0.50	0.50	0.50	0.50
Vit-Min premix[3]	1.00	1.00	1.00	1.00	1.00	1.00
Lys-HCl	0.25	0.25	0.20	0.15	0.18	0.16
Calculated composition						
DE, kcal/kg	3,403	3,491	3,402	3,516	3,402	3,461
NE, kcal/kg	2,348	2,468	2,404	2,475	2,430	2,476
CP, %	21.37	20.09	19.22	19.12	17.44	17.12
NDF, %	19.51	18.37	19.15	18.41	19.22	18.76
ADF, %	8.07	7.20	7.38	7.16	6.84	6.56
Ca, %	0.66	0.66	0.59	0.59	0.52	0.52
P, %	0.63	0.58	0.58	0.57	0.55	0.54
SID Lys, %	0.99	1.00	0.85	0.85	0.73	0.73
SID Met, %	0.30	0.29	0.27	0.27	0.24	0.24
SID Cys, %	0.32	0.31	0.29	0.29	0.27	0.27
SID Thr, %	0.61	0.59	0.53	0.55	0.47	0.47
SID Trp, %	0.18	0.18	0.15	0.16	0.13	0.14

[1] as fed basis; Diet C was obtained by supplementing Diet B with a MC.

[2] DESBM =Dry extruded expelled soybean meal. Analysed composition of DESBM: CP = 40.0%, GE = 4,703kcal/kg, DM = 93.0%, Ash = 5.7%, NDF = 14.9%, ADF = 9.2%, Starch = 1.7% and EE = 9.9%.

[3] Supplied the following per kg of finished feed: vitamin A, 2,000 IU; vitamin D, 200 IU; vitamin E, 40 IU; vitamin K, 2 mg; choline, 350 mg; pantothenic acid, 14 mg; riboflavin, 7 mg; folic acid, 1 mg; niacin, 21 mg; thiamin, 1.5 mg; vitamin B6, 2.5 mg; biotin, 70 mg; vitamin B12, 20 mg, Cu, 25 mg; Zn, 150 mg; Fe, 100 mg; Mn, 50 mg; I, 0.4 mg; Se, 0.3 mg.

5.3.3 EXPERIMENTAL DESIGN AND PROCEDURES

Pigs were blocked on the basis of sex and randomly allotted 1 of the 3 treatments, resulting in 8 replicates (each with 4 barrows and 4 gilts) per treatment. Pigs were housed individually in floor pens [172" (length) x 48" (breadth) x 36” (height)] with plastic-covered expended metal sheet flooring in a temperature controlled room (19^0C to $2l^0$C). Each pen was equipped with a feeder and a nipple drinker to allow unrestricted access to feed and water at all times. Diets were provided to the animals as a mash. Individual pig weight and feed disappearance was measured bi-weekly during each phase to determine average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio.

5.3.4 SAMPLE PREPARATION AND ANALYSIS

5.3.4.1 CARCASS EVALUATION

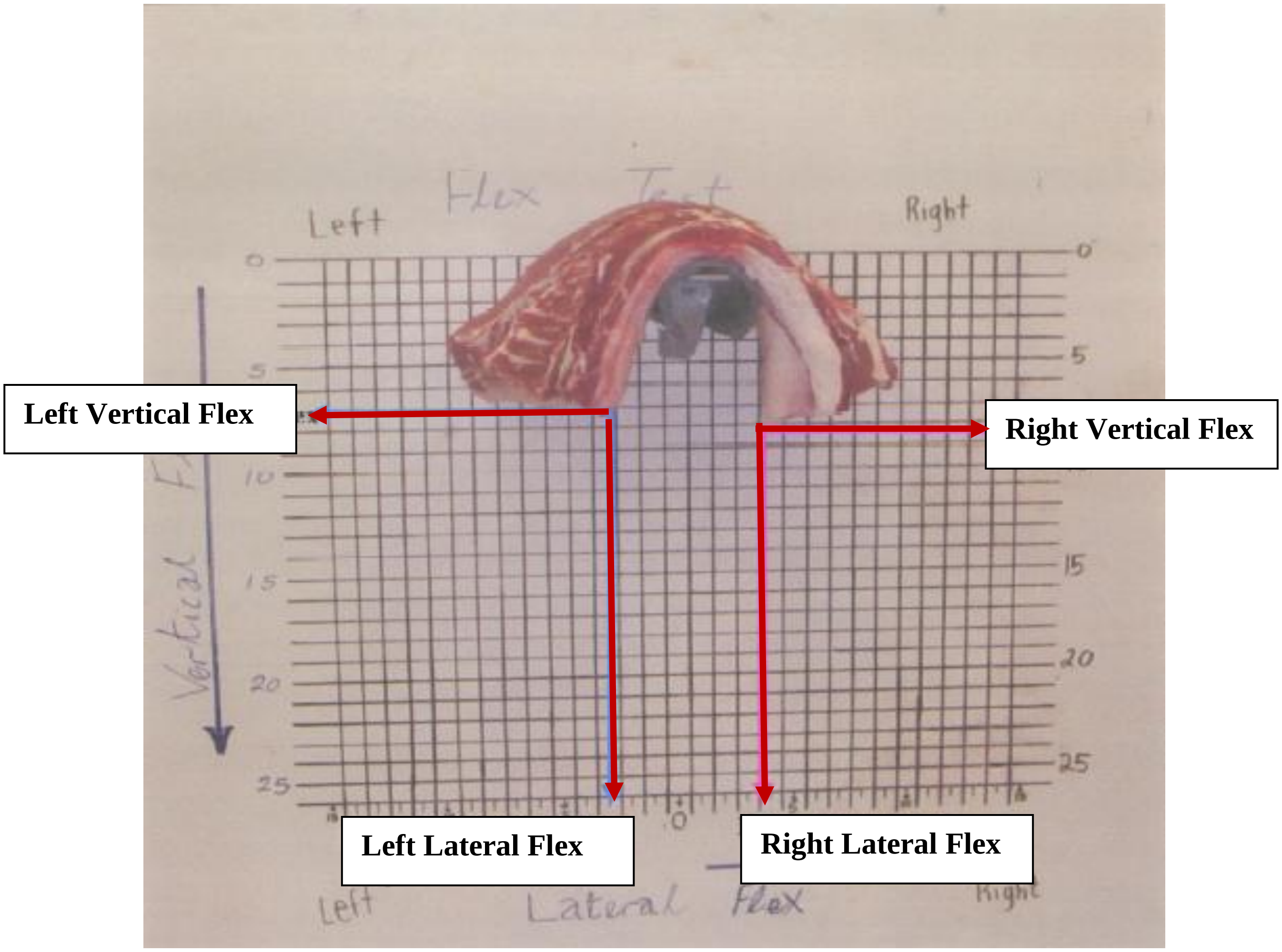
After reaching a minimum live BW of 100 kg, all pigs were slaughtered at the T. K. Cheung Centre for Animal Science Research, University of Manitoba after 18 h of feed deprivation. Prior to slaughter, pigs were weighed and sodium pentobarbital (110mg/kg BW) was administered intravenously through the jugular vein to provide for a plane of surgical anesthesia. Pigs were then exsanguinated, the viscera were removed and carcass weight was taken. The dressing percentage was determined as the ratio of hot carcass weight (weight of carcass after removal of internal organs and digestive tract) to live weight before slaughter. The carcasses were then split into two by cutting through the backbone and then stored in a cooler at 4^oC (Figure 5.1)

After 24 h in the cooler, carcass length was measured on the right half of the carcass as a distance between the cranial face of the first rib and the tip of the aitch bone. The belly was fabricated from the right half of the carcass according to the procedure of UN/ECE standard for porcine carcasses and cuts (ECE 1997) and the leaf fat and the attached ribs and cartilages were removed. Approximately 14" x 19" belly was cut out and subjected to the belly flex test according to the procedure of Rentfrow *et al*. (2003). Belly flex measurements indicate the softness and flexibility of the belly. Briefly, the fabricated belly was centered on a polyvinyl chloride pipe (3.5 diameter) mounted perpendicularly on a board marked with a 1 inch grid matrix with the skin side down and the chine side against the board (Figure 5.2). Lateral and vertical flexes were calculated as the average of the lateral and vertical left and right flexes, determined relative to the grid matrix on the left and right side of the board, respectively. The left half of the carcass was evaluated for backfat thickness, fat depth and loin eye area (LEA) according to the Canadian Hog Carcass Grading Settlement System (Anonymous, 1986). Midline backfat thickness was measured perpendicular to the skin at the first and last rib and at the last lumbar. The carcass was cut using the Hobart meat cutting machine (The Hobart Mfg. Co. Ltd., Toronto, Canada) between the 10th and 11th ribs to determine fat depth and loin eye area. The outline of the loin eye was traced on an acetate paper and the loin eye area was later determined using a 0.5 cm^2 grid (Opapeju *et al*., 2006b).

Diet samples were analysed for DM, ash, GE, CP, NDF, and ADF. Dry matter and ash were determined according to AOAC (1990; 942.05) and GE was determined using a Parr adiabatic O_2 bomb calorimeter. Crude protein (N x 6.25) was determined by the combustion method (AOAC, 1990; method 990.03) as described in manuscript 1. The ADF and NDF contents were determined according to the method of Goering and Van Soest (1970).

Figure 5.1 Carcass preparations for evaluation

Figure 5.2 Measuring lateral and vertical belly flex. Adapted from Opapeju *et al*, 2006b



A vertical belly flex of zero means the belly is parallel to the floor and completely stiff.

A lateral belly flex of 10 cm means that the belly will flex to a point where there is 10 cm between the end of the squared belly and a vertical line directly below the center of the supporting polyvinyl chloride pipe.

5.3.5 STATISTICAL ANALYSES

All data was analysed as a completely randomized design using the Mixed procedures of SAS 9.2 (SAS Inst. Inc., Cary, NC). The individual pig was considered as the experimental unit. Differences were considered to be significant at $P < 0.05$ and tendencies were observed at $0.05 < P < 0.10$.

5.4 RESULTS AND DISCUSSION

5.4.1 GROWTH PERFORMANCE

There was no significant gender and treatment vs. gender interactions for any of the response criteria measured for growth performance, therefore only the effects of treatment are presented. ADG, ADFI, and G:F for the three phases and also overall effect are presented in Table 5.2. There were no differences ($P > 0.05$) in ADG and G:F of pigs fed diets formulated on DE and NE basis during phase 1, but significant improvement in ADFI ($P = 0.024$) was observed between diets formulated on NE basis with or without exogenous enzyme supplementation. High fiber content in swine diets has been shown to reduce feed intake, growth performance, and nutrient digestion (Kyriazakis and Emmans, 1995; Owusu-Asiedu *et al*., 2006; Weber *et al*., 2010). On enzyme addition, digestion of fiber reduces the water holding capacity of feedstuffs, which in turn reduces the bulkiness of digesta, and thereby improve feed intake of pigs. In phase 2, no diet effect was observed for ADG, ADFI and G:F.

In phase 3, a significant difference ($P = 0.011$) was observed between treatments for ADG. The diets formulated on NE basis showed a daily BW gain of 0.89 kg when compared to 0.79 kg of diets formulated on DE basis. However, the addition of a multi enzyme complex did not improve the ADG between Diets B and C. Also a trend for an increase in G:F ratio ($P = 0.072$) was observed between diets formulated on DE and NE basis. Meanwhile for overall performance, diets formulated on NE basis had significant improvement in ADG (*P* = 0.017) and G:F (*P* = 0.05) when compared to the diet formulated on DE basis. As shown in Table 5.1, Diet B (formulated on NE basis) is typically lower in crude protein content than Diet A (formulated on DE basis) for all the three phases. For low-protein diets, the energy loss for metabolizing excess dietary protein is low (Kerr *et al*., 1995). It is well known that low-protein diets decrease the energy needed for deamination of excess amino acids and excretion of nitrogen in urine. Moreover, lowering the CP level can reduce body-protein turnover and heat production of animals (Kerr *et al*., 1995). Therefore, reducing CP increases the energy available for tissue deposition producing fatter carcasses (Tuitoek *et al*., 1997). Similar results were reported by Quiniou and Noblet (2012), where an improvement in ADG and G:F ratio was observed between diets formulated on increasing NE concentration. Multi-enzyme complex addition had no effect for overall performance in diets formulated on NE basis.

In the present study, it is evident from Table 5.1 (diet formulation) that diets formulated on DE basis had NE content (2,348, 2,404 and 2,430 kcal/kg as fed basis for phase 1, 2 and 3, respectively) below the NRC (2012) requirement for pigs (2,475 kcal/kg) when compared to Diet B formulated on NE basis. Though not statistically significant, a numerical reduction in overall ADFI by 2.8% was observed in the current study for Diets B when compared to Diet A. Pigs try to maintain energy intake by eating more when the dietary available energy content is reduced (Nyachoti et al., 2004). Similar variation was reported by Quiniou and Noblet (2012), where increased dietary NE concentration was associated with a linear reduction in ADFI for the entire experiment. This confirms the ability of pigs to adjust their voluntary feed intake to the dietary energy concentration. However, this phenomenon is also influenced by other nutrients such as amino acids (Henry et al., 1992; Quiniou et al., 1995).

The overall performance had no enzyme effect in the current study between diets formulated on NE basis with and without enzymes. In pigs, the ability to digest dietary fiber (DF) varies with their age or live weight. Studies have shown that digestibility of DF increases with live weight in growing pigs (Fernandez and Jorgensen, 1986 and Noblet and Shi, 1994). Moreover results on feed efficiency and growth performance of pigs fed with enzyme supplemented diet are not always consistent. Studies by Thacker *et al*. (1988) has shown no improvement in ADFI, ADG, and G:F when growing-finishing pigs were fed with hullless barley diets supplemented with a multi-enzyme preparation containing β-glucanase, pentosanase, cellulase, amylase and pectinase.

5.4.2 CARCASS CHARACTERISTICS

Carcass characteristics of pigs fed diets formulated on DE and NE basis are shown in Table 5.3. There were no differences ($P > 0.05$) in dressing percentage, carcass length, backfat thickness, loin depth and loin eye area among dietary treatments. In addition the belly firmness measured with the belly flex test was also not affected by dietary treatment. However, the days to attain target weight showed a trend ($P = 0.099$) towards pigs fed diets formulated on NE system gaining weight at a faster rate than those fed diets formulated on DE basis. Also, enzyme supplementation showed a tendency ($P = 0.099$) to increase the rate of BW gain.

A trend towards increased backfat thickness as a result of increased dietary NE concentration was observed by Campbell and Taverner (1988), Bikker *et al*. (1995) and Quiniou *et al*. (1996). However, in the present study, a trend towards increased backfat thickness was only observed for the parameter measured at the level of the first rib (*P* = 0.072). Such variation among studies may be an outcome of differences in the specific linear-plateau relationships (Hermesch *et al*., 1998) between protein deposition and energy intake (Quiniou and Noblet, 2012). Moreover, this relationship is affected by intrinsic factors such as BW (Bikker, 1994; Quiniou *et al*., 1995) and gender and genotype (Campbell and Taverner, 1988; Quiniou *et al*., 1996). The probable reason for no treatment effect (DE vs. NE) on carcass quality in the current study may be due to increase in both protein and lipid deposition rates simultaneous with increase in NE intake; the contrary is achieved if only lipid deposition rate is affected by energy restriction (Quiniou and Noblet, 2012).

Table 5.2 Effect of diets formulated on the DE basis and NE basis without or with multi-enzyme complex supplementation on growth performance of growing-finishing pigs

Item	Diet A	Diet B	Diet C	SEM	*P*- value
Phase I, 25-50 kg					
ADG, kg/d	0.93	0.96	0.99	0.028	0.308
ADFI, kg/d	1.54 ^{b}	1.57 ^{b}	1.78 ^{a}	0.061	0.024
G:F	0.60	0.62	0.55	0.020	0.107
Phase II, 50-75 kg					
ADG, kg/d	1.06	1.04	1.12	0.032	0.261
ADFI, kg/d	2.53	2.31	2.54	0.091	0.146
G:F	0.42	0.45	0.44	0.015	0.265
Phase III, 75-100 kg					
ADG, kg/d	0.76 ^{b}	0.89 ^{a}	0.90 ^{a}	0.033	0.011
ADFI, kg/d	2.41	2.42	2.47	0.091	0.875
G:F	0.32	0.37	0.37	0.017	0.072
Overall, 25-100 kg					
ADG, kg/d	0.90 ^{b}	0.96 ^{a}	0.99 ^{a}	0.020	0.017
ADFI, kg/d	2.12	2.06	2.21	0.053	0.154
G:F	0.43 ^{b}	0.47 ^{a}	0.45 ^{a}	0.011	0.050

^{a,b} Values within a row bearing different letters differ ($P < 0.05$).

Table 5.3 Effects of diets formulated on the DE basis and NE basis without or with multi-enzyme complex supplementation on carcass characteristics of growing-finishing pigs.

Item	Diet A	Diet B	Diet C	SEM	*P*- value
Days to 100 kg	83.88	80.00	77.50	1.978	0.099
Dressing percent	79.27	80.24	80.31	0.645	0.458
Carcass length, cm	80.41	80.37	80.49	0.520	0.987
Midline backfat					
First rib, cm	2.94	3.30	3.37	0.133	0.072
Last rib, cm	1.47	1.61	1.57	0.123	0.719
Last lumbar, cm	1.47	1.41	1.54	0.120	0.766
Fat depth, cm	1.77	1.98	1.79	0.112	0.363
Longissimus muscle area, cm^2	41.34	43.38	41.75	1.589	0.640
Belly flex					
Lateral, cm	6.99	7.30	7.14	0.452	0.885
Vertical, cm	28.26	30.80	28.26	1.105	0.200

6.0 GENERAL DISCUSSION

Feed is the single most cost governing factor in commercial swine production and about half of this cost accounts for supplying energy to the animal. In pig diets, energy accounts for the largest proportion of the cost. So it is reasonable to explore an energy system that best meets the energy needs of the animal. There are several systems available for the characterization of dietary energy; the most common ones being the digestible and metabolizable energy systems. Recently NE has been proposed as an advanced system that depicts the energy a pig actually does use. In addition, NE is the only system in which energy requirements and diet energy values are expressed on the same basis which should theoretically be independent of feed characteristics (Noblet and van Milgen, 2004). But still in North America nutritionists continue to formulate diets using digestible or metabolizable energy systems; the reasons as mentioned by Patience *et al*. (2004) and Patience and Beaulieu (2005) include lack of data about the energy contents of specific feed ingredients available in the area, lack of proper research to back up such an advanced energy system and lastly the simplicity in using the DE or ME system when compared to the complex NE system. But foreseeing the rapid increase in feed prices, there is an ongoing interest to adapt the NE system; as this system predicts the true energy requirements thus evading over or under feeding of nutrients which can not only significantly improve feed conversion but also decrease the cost of production.

The rapid expansion of the ethanol industry in North America has caused a substantial increase in the utilisation of cereal grains for ethanol production rather than for livestock feed. This scenario together with a limited annual yield has lead to an increase in cereal grain prices. These limitations lead to the incorporation of co-products like DDGS from the ethanol industry and wheat millrun from the wheat flour industries into livestock feed (Shrestha, 2012). Studies have reported that the nutrient concentrations of such co-products are higher than that of the parent grain (Slominski *et al*., 2004; Robinson *et al*., 2008). Consequently, co-products could be alternative feedstuffs in cost-effective swine diet formulation. But DDGS and wheat millrun contain more fiber than cereal grains. In swine diets formulated with high fiber ingredients, more energy is liberated as heat and thereby lost, and the NE system can account for the reduction in energy efficiency whereas other energy systems do not. In addition high fiber diets in growing pigs reduce growth performance (Hedemann *et al*., 2006). The detrimental effect of dietary fiber on growth performance of pig could be minimized by supplementation of exogenous enzymes as discussed previously.

The NE system was developed to provide more accurate estimates of the actual energy in an ingredient (and subsequent diet) that is going to be available for a pig to use for maintenance and production (Payne and Zijlstra, 2007). However, unlike DE or ME, NE is often estimated by prediction equations (Noblet et al., 1994), being more complex and the determination techniques being more time-consuming and expensive. Hence the objectives of the current study were to determine the NE content of DESBM; a locally available feed ingredient for growing pigs using a direct determination technique, to evaluate the indirect calorimetry technique for determination of NE and to validate the NE system of feed formulation in swine with ingredients typical to Western Canada.

In North America the prediction equations have not been used extensively due to the lack of confidence in the numbers incorporated in these equations (Pettigrew, 2009). As a step to rectify this situation, a study was conducted (Manuscript 1) to determine the NE contend of a recently introduced feed ingredient for swine by indirect calorimetry and thereby compare the value with the published prediction equations. In this study 24 growing barrows were fed 4 dietary treatments; a corn soybean meal basal diet (Diet A), a diet containing Diet A and DESBM in a 80:20 ratio with a constant crude protein content compared with the basal diet (Diet B), a diet with 80:20 ratio of basal diet and DESBM with a constant corn:soybean meal ratio (Diet C) and a diet with simple substitution of basal diet with DESBM in 80:20 ratio (Diet D). Pigs were fed in metabolism crates for a period of 16 d and thereafter moved into an indirect calorimeter to determine HP and FHP. The analyzed nutrient composition of DESBM used in the current study showed similar values for DM, GE and ash, but with a higher CP and low EE when compared to the studies by Woodworth *et al*. (2001). Similar variation for CP and EE, but with comparable concentrations of NDF and ADF were seen in studies by Baker and Stein. (2009) and such discrepancy could be attributed to various factors like temperature, duration or moisture content during the meal processing.

Energy contents of diets for Exp.1 as reported in manuscript 1 were 3,481, 3,462, 3,472 and 3,473 kcal/kg DM for DE, 3,392, 3,378, 3,386 and 3,386 kcal/kg DM for ME, respectively, for treatments A, B, C and D. The ME:DE ratio (0.98; average value for the 4 diets) is in accordance with the studies by Noblet and van Milgen, (2004). Values of FHP obtained in this study (1,547, 1,424, 1,508 and 1,468 kcal/kg DM for diets A, B, C and D, respectively) were comparable to that obtained by Noblet *et al*. (1994). Digestible energy of DESBM obtained in the present study (3,384, 3,438 and 3,443 kcal/kg DM for treatments B, C and D, respectively) were less than the DE values reports for extruded-expelled SBM (Baker and Stein, 2009). The probable explanation for this could be the variation in body weight of pigs used for the studies. In growing pigs, digestibility coefficient of energy or DE:gross energy ratio increases with increasing BW (Noblet and Shi, 1994). The initial body weight of the pigs used in the current study was nearly half when compared to the pigs used in the study by Baker and Stein (2009). Similar results were reported by Woodworth *et al*. (2001) wherein the DE content for DESBM in growing pigs (initial BW 41 kg) were 4,120 and 4,210 kcal/kg (as-fed basis) for hulled and de hulled meals, respectively. The NE content of DESBM determined in this study (2,652, 2,548 and 2,540 kcal/kg DM in treatment B, C and D, respectively) was higher compared with those obtained using published prediction equations which could be due to not taking into account the physical activity of the animals while measuring fasting heat production. Studies have shown that about 8 % of ME intake may be dissipated for physical activity in growing pigs (van Milgen and Noblet, 2003). In addition, significant variation in NE values of the test ingredient (DESBM) was observed depending on the technique adopted for diet formulation. This discrepancy could be due to the variation in dietary CP when diets were formulated using different techniques (Table 3.1). Lower dietary CP content leads to lesser deamination of excess AA and the consecutive production and excretion of urea in urine and lowers body protein turnover and heat production of the animals (Le Bellego *et al*., 2001) resulting in higher energy gain. The discrepancy between the determination technique used was about 1% when diets were formulated with a constant protein content or corn:soybean meal ratio (1.0% and 0.7%, respectively), however, this was 4.1% when diet was formulated with simple substitution technique. As the values obtained from the two diet formulation techniques namely, the constant protein content or the constant corn:soybean meal ratio were not different, the average NE value of DESBM evaluated was 2,544 kcal/kg DM.

As a follow up to Exp 1, a second study was conducted (Manuscript 2) where the effects of multi-carbohydrase enzyme supplementation on the NE content of DESBM was determined. As per the results of Exp 1, the diets for Exp 2 were formulated by maintaining a constant ratio between the energy yielding components. The 4 dietary treatments used in this study were; a corn soybean meal basal diet (Diet A), a diet containing Diet A and DESBM in a 80:20 ratio with a constant corn:soybean meal ratio (Diet B), Diet B + 0.05% MC (Diet C) and Diet B + 0.1% MC (Diet D). The results of the current study (Exp 2) indicated that NSP degrading enzyme improved the energy digestibility. Enzyme supplementation increased the DE content of the diet by 1.2 and 0.6%, ME by 1.5 and 1.2% and NE by 0.9 and 0.7% for 0.05 and 0.1% enzyme, respectively. Comparable results were reported wherein multi-carbohydrase preparation improved the energy contents of the diets in growing pigs (Cozannet *et al*., 2012) and in broiler chicken (Meng and Slominski, 2005). An effect of enzyme on the NE content of DESBM was also observed in the current study with an improvement of 4.9 and 3.7% in NE value of the test ingredient with MC at 0.05% and 0.1% of the diet.

It is an established fact that the NE system provides more accurate estimates of the energy available to the animal. Still the North-American swine producers rely on the DE and ME system, which do not provide sufficient accuracy with fibrous or high-protein feedstuffs (Pettigrew *et al*., 2009). Therefore, a third study (Manuscript 3) was conducted to validate the NE system for diet formulation in growing-finishing pigs fed a typical Western Canadian. Twenty four pigs with an initial BW of 25 kg were allotted 1 of the 3 treatments to evaluate the effects of diet on growth performance and carcass characteristics. Dietary treatments were; a barley-based control diet formulated on the DE basis (Diet A), control diet formulated on NE basis (Diet B) and Diet B + Multi-enzyme complex (Diet C). Pigs were fed on a 3-phase dietary program based on BW. The results from the study showed no differences in ADG, ADFI and G:F of pigs fed diets formulated on DE and NE basis during phase 1 and 2. However, an improvement for ADG and a trend for an increase in G:F ratio was observed between diets formulated on DE and NE basis during phase 3. Meanwhile for overall performance, diets formulated on NE basis had significant improvement in ADG and G:F when compared to the diet formulated on DE basis. As shown in Table 5.1, Diet B (formulated on NE basis) is typically lower in crude protein content than Diet A (formulated on DE basis) for all the three phases. However, the standardized ileal digestible amino acid levels of the essential amino acids (Lys, Thr, Trp and Met) were maintained in the diets so that the negative effects of low CP on animal performance could be avoided as reported in previous research (Canh *et al*., 1998; Dourmad *et al*., 1993; Le Bellego *et al*., 2001; Kerr *et al*., 2003; Patience *et al*., 2003). For low protein diets, the energy loss for metabolizing excess dietary protein is low (Kerr *et al*., 1995). Therefore, reducing CP increases the energy available for tissue deposition producing fatter carcasses (Tuitoek *et al.*, 1997). Similar results were reported by Quiniou and Noblet (2012), where an improvement in ADG and G:F ratio was observed between diets formulated on increasing NE concentration. An added benefit of lower CP in diets formulated on NE basis is that the nitrogen excretion is minimized (Le Bellego *et al*., 2001). Canh *et al*. (1998) reported a 10% reduction in nitrogen excretion for every percentage reduction in dietary CP in pigs. Reduced nitrogen excretion results in drop in ammonia emissions and odor in the barns, which leads to better animal performance. In addition one more advantage of having low CP in diets formulated with NE system is that the feed cost is often reduced both on a per ton basis and a per pig basis (Patience, 2005). For enzyme effect, a significant improvement in ADFI during phase 1 was observed between diets formulated on NE basis with or without exogenous enzyme supplementation. High dietary fiber has shown to reduce feed intake, growth performance, and nutrient digestion in growing pigs (Kyriazakis and Emmans, 1995; Owusu-Asiedu *et al*., 2006; Weber *et al*., 2010). Digestion of fiber by supplemental enzymes causes reduction in the water holding capacity of feedstuffs, which in turn reduces the bulkiness of digesta, and thereby improve feed intake of pigs. This ability to digest dietary fiber (DF) improves with the animal's age or live weight (Fernandez and Jorgensen, 1986 and Noblet and Shi, 1994). That could be the reason for no enzyme effect during phase 2 and 3 between diets formulated on NE basis with and without enzymes.

No significant dietary effect was observed for carcass quality, except for a trend towards improved backfat thickness for the NE system. However, the days to attain target weight showed a trend towards pigs fed diets formulated on NE system gaining weight at a faster rate than those fed diets formulated on DE basis. Also, enzyme supplementation showed a tendency to increase the rate of BW gain. The probable reason for no treatment effect (DE vs. NE) on carcass quality in the current study may be due to increase in both protein and lipid deposition rates simultaneous with increase in dietary energy intake; the contrary is achieved if only lipid deposition rate is affected by energy restriction (Quiniou and Noblet, 2012).

7.0 SUMMARY AND CONCLUSION

NE content of DESBM was determined to be 2,652, 2,548 and 2,540 kcal/kg DM for diets B, C and D, respectively, using IC. Respective values obtained with published equations (Noblet *et al.*, 1994a) were 2,624, 2,530 and 2,436 kcal/kg DM. The discrepancy between the determination technique used was about 1% when diets were formulated with a constant protein content or with a constant corn:soybean meal ratio; however, this was 4.1% when diet was formulated with simple substitution technique. As the values obtained from the two diet formulation techniques namely, the constant protein content or the constant corn:soybean meal ratio were not different, the average NE value of the DESBM evaluated was 2,544 kcal/kg DM. Supplementation of diets with enzyme improved the energy content of both diet and DESBM. According to the results supplementation with MC at 0.05% and 0.1% of the diet improved NE values of DESBM by 4.9 % and 3.7%, respectively. Results for the NE validation study indicated better growth performance when diets were formulated on NE basis compared to the DE system. Also addition of enzyme improved the animal's performance for diets formulated on NE basis. Further research is suggested to:

1) Validate the NE value of DESBM obtained from this study with growth performance trials where pigs will be fed with graded levels of the tested feed ingredient.
2) Validate other ingredients and co-products used in swine feed formulation with the IC technique
3) Extend the application of IC technique for NE determination to poultry, so as to create a NE database for ingredients used in poultry diets.

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