USE OF DIATOMITE FILTER AID RESIDUE IN CATTLE FEED

Doctoral Dissertation
Submitted for the degree of Doctor of Agricultural Sciences
of the Faculty of Agricultural Sciences

Georg-August-University Göttingen (Germany)

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Göttingen, July 1999

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Date of oral examination:	08.07.1999

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Δ pH	pH gradient
°C	degrees Celsius
µ	micron
µg	microgram
µm	micrometer
a	water soluble fraction = the rapidly soluble fraction
a+b	the potential degradability = the total potential ruminal degradation fraction
ADG	average daily gain
ADP	adenosine diphosphate
ANOVA	analysis of variance
ARC	Agricultural Research Council
ATP	adenosine triphosphate
b	the potential degradability of the component of dry matter which will, in time, be degraded
B (Baht)	Thai monetary unit
BUN	blood urea nitrogen
BW	body weight
$BW^{0.75}$	metabolic body weight
c	the rate constant for the degradation of 'b'
C2	acetic acid
C3	propionic acid
C4	butyric acid
CCK	cholecytokinin
C.I.S.	Commonwealth of Independent States, an alliance of former Soviet republics formed in December 1991, including: Armenia, Azerbaijan, Belarus, Kazakhstan, Kyrgyzstan, Moldova, Russian Federation, Tajikistan, Turkmenistan, Ukraine and Uzbekistan.
CNS	central nervous system
CP	crude protein
CRD	Completely Randomised Design

D_3	cholecalciferol
DFR	diatomite filter aid residue
DM	dry matter
DMRT	Duncan's new multiple range test
ed1	effective degradation in the rumen at 0.02/h passage rate
ed2	effective degradation in the rumen at 0.05/h passage rate
ed3	effective degradation in the rumen at 0.08/h passage rate
FCR	feed conversion ratio
FMD	Foot and Mouth Disease
FW-20	Celatom diatomite
g	gram
GS-GOGAT	glutamine synthetase-glutamate synthase couple reaction
h	hour
i.e.	that is
iu	international unit
kg	kilogram
K_m	Michaelis constant
km^3	cubic kilometre
lb	pound
ME	metabolisable energy
mg	milligram
MJ	megajoule
MJ/kg	megajoule per kilogram
ml	millilitre
mM	millimolar
mmol/l	millimoles per litre
molar %	molar percent
N	Nitrogen
NADH	dihydronicotinamide adenine dinucleotide
NADPH	dihydronicotinamide adenine dinucleotide phosphate
NEL	net energy for lactation
NFE	nitrogen free extract
NPN	non protein nitrogen

NRC	National Research Council
OMD	organic matter digestibility
P	degradability
PCF	Pre coat cutting filter
PDV	portal-drained viscera
pg	picogram
	$=10^{-12}$gram
pH	hydrogen ion concentration
Pi	inorganic phosphate
RCBD	Randomised Completed Block Design
SEM	standard error of mean
Si	Silicon
t	incubation time in hours
TDM	total dry matter
TDN	total digestible nutrient
U.S.A.	The United States of America
VDK	Vereinigte Deutsche Kieselguhrwerke
VFA	volatile fatty acid
VFA's	volatile fatty acids

1. INTRODUCTION

Two interesting and interrelated problems which our world are currently facing are the shortage of food supply and the pollution of the environment due to disposal of waste. To over come these problems, relevant research and techniques must be developed to raise the level of food production for human beings in an environmentally sustainable way.

In the area of livestock production, the ruminants are the most predominant animals, because they are able to utilise plant fibre efficiently as their main energy source and they do not compete for food with human beings. Moreover, the ruminant animals such as cattle, sheep and goats have a high potential for converting the biomass from waste materials into meat, milk and leather and they have provided draught to the mankind throughout history (GILLIES, 1978).

The diatomite filter aid residue (DFR) is an industrial waste product from filter aid material used in the filtration step during the production of monosodium glutamate. About 2.200 tonnes of DFR are produced annually in Thailand. This waste product has a molasses-like aroma which makes it attractive for use as cattle feed. It contains crude protein, crude fibre, ether extract, ash and nitrogen free extract (NFE) in the proportions of 5.10, 3.14, 2.03, 47.04 and 42.69 % of DM respectively (FEED ANALYSIS DIVISION, 1991). Since one of the major constituents of the DFR is nitrogen free extract (NFE), easily fermentable carbohydrate, disposal of this waste product is of major environmental concern. Disposal of the DFR as feed for the ruminants is one of the most feasible means because of it's price which make it a very cheap alternative feed resource and it is available throughout the year. Because of the nutritional profile, the physical property on the molasses-like aroma and the low cost, the DFR have attracted the interest of animal nutritionist who view it as a potential animal feed source, which need to be further investigated.

The objective of this research is to evaluate the use of the DFR as an alternative feed resource for cattle. To meet this objective, the following experiments were carried out:

1. A preliminary experiment on the effect of diatomite filter aid residue in feed on the growth performance of Wistar Albino rats.

2. An experiment on the effects of diatomite filter aid residue in feed on rumen fermentation and blood parameters in cattle.

3. An experiment on the effect of restricted feeding of a basal diet constituting different levels of diatomite filter aid residue on feedlot performance of male crossbred Holstein x Thai-indigenous cattle.

4. An experiment on the effect of *ad libitum* feeding of a basal diet constituting different levels of diatomite filter aid residue in feed on feedlot performance of male crossbred Holstein-Thai-indigenous cattle.

5. An experiment on the ruminal dry matter degradability of feed containing different levels of diatomite filter aid residue.

6. An experiment on the economic assessment of the use of diatomite filter aid residue in feed under feedlot conditions of male crossbred Holstein-Thai-indigenous cattle.

2. REVIEW OF LITERATURE

2.1 Diatomite

Diatomite also called diatomaceous earth or Kieselguhr (in German) is a siliceous, sedimentary rock consisting principally of the fossilised remains of unicellular aquatic plants related to algae known as diatoms (ANONYMOUS, 1987). This rock is a raw material for the manufacture of filter aids useful for industrial filtration (ROSKILL, 1994). On occasions, diatomite was called a miraculous mineral because of its lightness and porosity (EAGLE-PICHER, 1988).

2.1.1 Diatomite sources

Diatomite is found widely in the world either in the earth (PHILIPPI, 1925; EAGLE-PICHER, 1988; EDWARDS, 1991), or accumulated on the bottom of lakes (SIGURDSSON, 1992). The world's diatomite resources have been estimated at 2 billion tonnes (SIGURDSSON, 1992), i.e. approximately 1,000 km^3 of algal mats (ANONYMOUS, 1993; KEMP and BALDAUF, 1993). The largest part of world reserves of the diatomite is in Europe and in the United States of America (ANONYMOUS, 1987). Diatomaceous earth is also found in Asia, South America, Oceania and Africa (ROSKILL, 1994).

2.1.2 The world production of diatomite

Diatomaceous earth is used as a raw material for the production of commercial diatomite which has been used for different purposes for more than hundred years. In Germany, the "Vereinigte Deutsche Kieselguhrwerke (VDK)" at Lüneburger Heide produced diatomite, in German called Kieselguhr, since 1863 (ANONYMOUS, 1987). The diatomite industry is considerably stable and the production of the diatomite is correlated with the demand. The world production of diatomite as reported by the US Bureau of Mines, rose from approximately 2 million tonnes in the late 1970s to reach its peak level of almost 2.2 million tonnes in 1985 and 1986. Thereafter, it was scaled down to 2 million tonnes between 1988 and 1990 and has been just below 1.8 million tonnes since 1992 (ROSKILL, 1994).

Table 2.1 World production of diatomite (1,000 tonnes).

Countries	Production Years									
	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993
Algeria	2	3	3	4	3	4	4	4	4	4
Argentina	5	10	14	5	7	6	7	11	5	5
Australia	6	8	9	10	11	12	10	11	11	11
Brazil	16	13	20	16	14	16	13	13	13	13
Canada	4	4	4	4	4	4	4	8	10	10
Chile	2	2	3	3	3	3	4	4	4	5
China	85	90	90	100	100	100	100	100	100	100
C.I.S.	240	245	250	255	260	260	250	220	190	150
Columbia	-	-	-	-	3	4	3	4	4	4
Costa Rica	1	-	-	-	5	5	4	12	12	10
Denmark	72	78	79	72	72	82	98	96	96	96
France	248	270	260	250	250	250	250	250	250	250
Germany	49	48	49	49	58	58	64	47	52	50
Hungary	25	20	15	15	10	10	10	8	8	8
Iceland	27	29	22	23	25	25	25	23	20	19
Italy	28	30	27	28	28	25	25	2	-	-
Japan	235	225	215	205	205	201	210	200	200	200
Kenya	2	3	1	1	1	1	1	1	1	1
South Korea	48	54	55	65	72	75	55	91	77	75
Macedonia	-	-	-	-	-	-	-	-	4	4
Mexico	45	46	36	35	37	44	51	46	46	47
Peru	7	15	9	21	30	20	20	26	25	25
Portugal	2	2	2	2	2	3	2	2	2	2
Romania	300	300	300	280	55	50	40	30	15	14
South Africa	-	1	2	-	-	-	3	2	1	1
Spain	73	101	133	78	99	104	108	60	36	38
Thailand	1	-	-	-	-	1	5	7	10	8
Turkey	3	3	3	3	1	-	-	-	-	-
U.S.A.	569	576	570	596	629	617	631	610	595	599
Yugoslavia	7	8	11	5	5	6	6	4	-	-
Total	2,102	2,184	2,182	2,125	1,989	1,995	2,003	1,894	1,794	1,750

Source : ROSKILL, 1994.

The United States of America remains the world's leader in diatomite production and consumption. Romania, France and Russia are second, third and fourth respectively (ANONYMOUS, 1987; ROSKILL, 1994). Other countries which also produce diatomite are shown in Table 2.1.

2.1.3 The formation of diatomite

The diatomite deposited in most commercial diatomite sources is the sedimentary rock or siltstone of fossils of the diatoms. When the diatom die, the siliceous shell or frustule of the diatom settle in the lake or sea where they accumulate at variable rates. Sediments of very active diatom colonies may accumulate several millimetres of thickness in one year (ANONYMOUS, 1987). The accumulation period of the siliceous shells of diatoms began during the cretaceous period (135 to 65 million years ago) but most of the commercial deposits exploited today are of Miocene age which was about 26-7 million year ago (ANONYMOUS, 1987). Today some diatom species are still abundant in lakes and seas (ROUND *et al.,* 1990).

2.1.3.1 The diatoms

Diatoms are unicellular, eukaryotic micro-organism (ROUND *et al.,* 1990). They were classified in the Bacillariophyta division (HUSTEDT, 1930; WERNER, 1977; ROUND *et al.,* 1990) whereas some other taxonomists classified them in the Chrysophyta division (HENDEY, 1964). More than 10,000 (MILLS, 1933) or 12,000 (HENDEY, 1964; ROSKILL, 1994) to about 15,000 (REUTHER, 1965) species of diatoms have been identified.

The diatom cell has a cell wall and a protoplast. The protoplast of the diatom cell have nothing particular, it contains the same organelles such as nucleus, cytosomes, mitochondria and plastid as other eukaryotic. The distinguishing feature of the diatom is it's cell wall. This wall is highly differentiated and almost always heavily impregnated with silica (SiO_2 n H_2O). The wall is multipartite, always consisting of two large, intricately sculptured units called valves (ROUND *et al.,* 1990). Because the wall component is often loosely, it was called frustules (WERNER, 1977, ANONYMOUS, 1987, ROUND *et al.,* 1990). Besides silica, the wall also contains organic material, which forms a thin coating, the so called gelled capsule (DANIEL *et al.,* 1987) or jelly which decomposes when the diatom dies (ROSKILL, 1994).

2.1.3.2 Silica shell formation in diatoms

The shell or frustule of the diatoms is made of silica which is extracted from surrounding water (REIMANN, 1964). The water from different sources, at different seasons (TESSENOW, 1966) and with different media culture contain different levels of silicic acid concentration (WERNER, 1977). The silicic acid concentration in the surrounding water range from 0.0 μg Si/litre (KREY, 1942) to 20-50 μg Si/litre (TESSENOW, 1966). The uptake of silicate from surrounding water into the diatom cell to be used for silicate shell formation requires a certain amount of energy (WERNER, 1977). The silica content of living diatom cells varies according to the intensity of light, temperature, pH, nutrients concentration and density of the culture. Some diatom species with a size of 7x5 μm contained 100 pg Si/cell (WERNER, 1977). The diatom shell are formed at the same time as the new cell walls. At this time, silicon is deposited within vesicles which are bound by a unit membrane, termed the silicalemma. The wall formation of the diatom may involve 5 sequences of biochemical events. The diatoms take up silicon from the medium, then the constituent is synthesised and the macromolecules are comprised to the silicalemma. After that the silicon from the cytoplasm is translocated into the silicalemma and then the polymerisation of the silicon within this membrane system takes place. Finally, the synthesised organic material is added to the wall (COOMBS *et al.*, 1967).

2.1.3.3 Silica in shell and fossil of diatom

When it is first recognised in the cell of the diatom, the silica already has the same electron density as in the mature cell walls. This silica is surrounded by an organic membrane (REIMANN, 1964) and pectin (JØRGENSEN, 1955). In most diatoms, the greatest part of the silica is formed in a new valve following cell division (REIMANN, 1964). The silica content in the dead cell wall consist of pure silicic acid whereas in the fossil of diatoms or, in the diatomaceous earth it occurred as silicates (amorphous silica) (FESSENDEN and FESSENDEN, 1967). This form of silica is dissolved very slowly (COOPER, 1952). The solubility of silicate in the diatom shell depends on the ratio of silica to alkali metal. When the ratio is around 2, the silicate is water soluble. However, if the ratio is higher, it is insoluble (FESSENDEN and FESSENDEN, 1967). The solubility of the diatomaceous earth is one of the most important properties used as criteria for grading the quality of raw material used for the production of

commercial diatomite. To qualify as a raw material for the commercial production of filter aids, the diatomite must be pure, inert and insoluble (EAGLE-PICHER, 1988).

2.1.4 Mining and processing of the diatomite

The raw diatomaceous earth with good economic features are investigated and evaluated before it is used for the production of commercial diatomite (EDWARDS, 1991). Most of the diatomite production factories are located near the raw diatomite sources because the raw material contains much water. Up to 60% of the unprocessed diatomite is water (ANONYMOUS, 1987; ROSKILL, 1994). The mining processes for production of commercial diatomite vary from place to place. While in the USA, the diatomite deposits are processed by quarrying or open pit methods, in other parts of the world, mining is by surface, underground and under water methods (ANONYMOUS, 1987). A typical diatomite flowsheet is illustrated in Figure 2.1 below.

2.1.5 The use of diatomite

The properties of diatomite make it useful for a wide range of activities. However, since their properties vary between and within individual deposit sources, the final product may only be applicable to a specific range of activities. Some diatomite products are suitable for absorbents but are not suitable for filtration (ANONYMOUS, 1987). The principal applications of diatomite are for filtration, fillers, insulation and absorbents. Together these applications account for almost 90% of all demand (ROSKILL, 1994). The diatomite can be used as uncalcined, calcined or flux calcined products (REUTER,1965; EAGLE-PICHER, 1988; ANONYMOUS, 1987; ROSKILL, 1994).

Accounting for 50% of all consumption, filters are the main product of the processed diatomite, (ROSKILL, 1994). Diatomite filters are used for filtration of beer, whisky, wine, raw sugar, liquors, swimming pool water, dry cleaning solvents, pharmaceuticals, fruit and vegetable juices, effluents, chemicals varnishes and lacquers (ANONYMOUS, 1987).

The second largest use of diatomite is as a functional filler in paints, plastics, rubbers, drugs, pharmaceuticals, toothpaste, polishes, chemicals, paper, agro chemicals, animal feed, fertiliser, catalyst carriers, abrasives, cement and concrete. More over, diatomite is also used as non

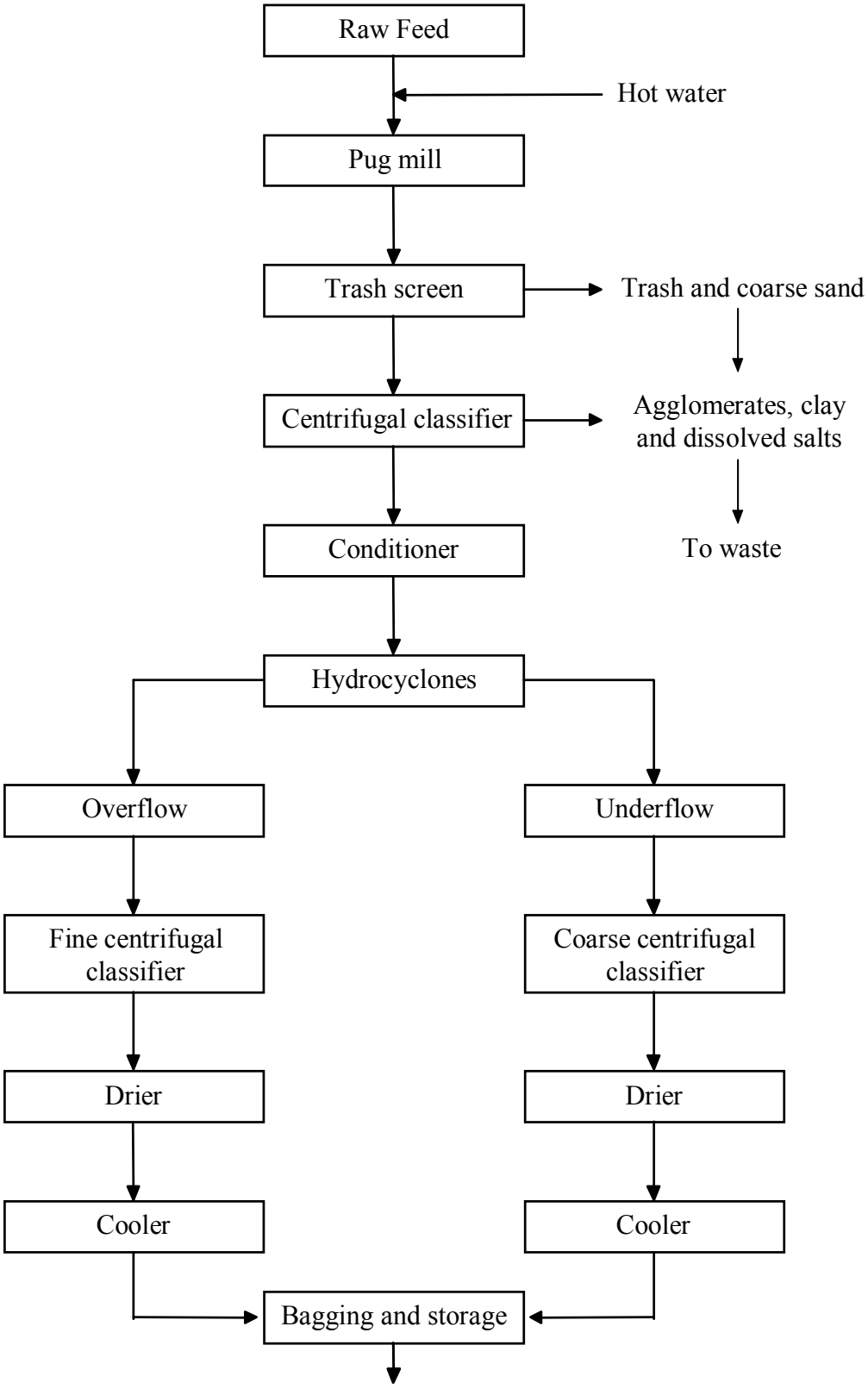

Figure 2.1 Flowsheet of diatomite processing plant (ROSKILL, 1994).

functional filler which means simply to bulk out or extend other ingredients, usually to reduce cost (ANONYMOUS, 1987; ROSKILL, 1994).

2.1.5.1 The use of diatomite as filter aid

Filtration is the removal of fine solid matter from a liquid by passage through a septum or layer having small openings (EAGLE-PICHER, 1988). The diatomite is used as filter aid because it posses outstanding physical properties, i.e., it has a porous structure which prevents the passage of material above a certain size (ROSKILL, 1994). The use of a septum as filter mash traps only large particles and soon becomes blocked, reducing through flow. The use of the diatomite as a filter aid, overcomes these shortcomings (ROSKILL, 1994).

Filter aids are finely divided materials which, when added to the liquid to be filtered, help to control flow and to remove solids. They do not interfere chemically with the liquid to be filtered so that the process of filtration is purely mechanical (ANONYMOUS, 1987). An effective filter aid will produce the purest possible filtrate, allow a high flow rate through the filler and function for the longest possible time before being replaced (ROSKILL, 1994).

The diatomite used for filter aids are applied either as precoat aids or body aids. Precoat aids are applied as a thin layer of the septum, preventing the particles from becoming enmeshed in the filter medium. Body aids are incorporated in the liquid before separation. Diatomite precoats are used for both vacuum and pressure filtration. Vacuum filters use a rotating horizontal drum which is partially submerged in a basin containing the precoat slurry. A vacuum is applied from within the drum and the precoat is drawn onto the septum, forming a uniform filtration layer. Additional diatomite is added as body aid to keep the particles apart in the effluent slurry and to prevent early blockage of the filler. Excess filter cake is removed by a blade as the drum rotates (ROSKILL, 1994).

2.1.5.2 Physical and chemical properties of the diatomite used for filter aids

The requirements of a good filter aid are suitable particle size and shape characteristics to achieve optimum cake permeability, chemical inertness, lightweight, availability in a number of grades, and relatively low cost (ANONYMOUS, 1987). Diatomite used as filter aids must be the best both in physical and chemical properties. It must be pure, inert, insoluble, and capable of

forming precoats and cakes which will consist mostly of pore linked together to form a very large number of tiny channels (EAGLE-PICHER, 1988). The typical properties of diatomite filter aids are shown in Table 2.2.

The physical and chemical properties of the commercial diatomite are variable depending on the kind of diatom fossil from which they are prepared (ANONYMOUS, 1987; EDWARDS, 1991; ROSKILL, 1994). Normally, fresh water diatoms contain more silica per unit of biovolume than marine diatoms (CONLEY *et al.*, 1989). The silica content in the diatomite ranges from 89.20 to 92.80 per cent of the total minerals (EAGLE-PICHER, 1988). The variance in chemical composition of the diatomite effects its use. If the processed diatomite contains more Fe_2O_3 in the composition, it will create complexes and thus reduce chemical inertness. The level of iron in the product is therefore of major consideration for some specific applications (ANONYMOUS, 1987).

Table 2.2 Typical physical and chemical properties of diatomite filter aids.

Items	Natural	Calcined	Flux-calcined
Colour	Off- White	Pink	white
Free moisture (%)	4.0	-	-
Specific gravity	2.0	2.2	2.3
pH	8	7	10
Refractive index	1.46	1.46	1.46
Chemical analysis (%)			
SiO_2	89.2	92.8	89.5
Al_2O_3	4.0	4.2	4.1
Fe_2O_3	1.5	1.6	1.5
CaO	0.5	0.6	0.6
MgO	0.3	0.3	0.3
Na_2O	-	-	3.5
Other oxides	0.5	0.5	0.5
Ignition loss	4.0	-	-

Sources: EAGLE-PICHER, 1988.

2.1.5.3 The use of diatomite as filter aids in the monosodium glutamate production industry in Thailand

Although Thailand produces about 10,000 tonnes of diatomite per year and some of this is exported to other countries such as South Korea and Malaysia, annually about 4,300 tonnes of

diatomite is imported to Thailand (ROSKILL, 1994). In Thailand, the monosodium glutamate production industry is one of the biggest users of diatomite filter aids (AJINOMOTO, personal communication).

The celatom diatomite FW-20 is usually used as filter aid in the filter step of the monosodium glutamate production industry. This step of the production procedure, releases a waste product called diatomite filter cake (ROSKILL, 1994) or with a more relevant name, it is called diatomite filter aid residue (DFR). A flowchart which illustrates the procedure of the monosodium glutamate production is shown in figure 2.2.

2.2 Diatomite filter aid residue

The term "diatomite filter aid residue" (DFR) as used throughout this thesis refers to the residue of the diatomite used for filter aid in the filtration step of the production of monosodium glutamate. The use of diatomite as filter aid in other industries gives different types of waste-products. The breweries industry in Germany for instance, produces a waste product called waste-kieselguhr or kieselguhr sludge (SCHILDBACH, *et al*., 1992) whereas in some other industries, it is called diatomite filter cake (ROSKILL, 1994) or spent filter cake (EAGLE-PICHER, 1988).

2.2.1 Production of diatomite filter aid residue from the monosodium glutamate production industry in Thailand

In the filtration procedure of the monosodium glutamate production process (figure 2.2), the diatomite is used as a pre-coat for filter aids. The diatomite is applied as a thin layer to the septum, preventing the particles from becoming emmeshed in the filter medium. This process uses the vacuum filter method in which rotating horizontal drums are applied. A vacuum is applied from within the drum and the precoat is drawn on to the septum, forming a uniform filtration layer. Excess filter cake is removed every half an hour by a blade as the drum rotates. Annually, about 2,200 tonnes of DFR as waste-products is produced from this factory (AJINOMOTO, personal communication).

2.2.2 Chemical and physical properties of diatomite filter aid residue produced in Thailand

The chemical composition of the DFR is shown in Table 2.3. The composition of the DFR varies depending on the sample used for analysis. A more fresh sample gives higher sugar content than an old one because some of the residual sugar is fermented after the DFR is released. The physical appearance of the DFR is like fine sand. It has an aroma flavour like molasses.

This aroma flavour as well as the content of the nitrogen free extract (NFE) have attracted the interest of animal nutritionists who view DFR as a potential animal feed source (AJINOMOTO, personal communication).

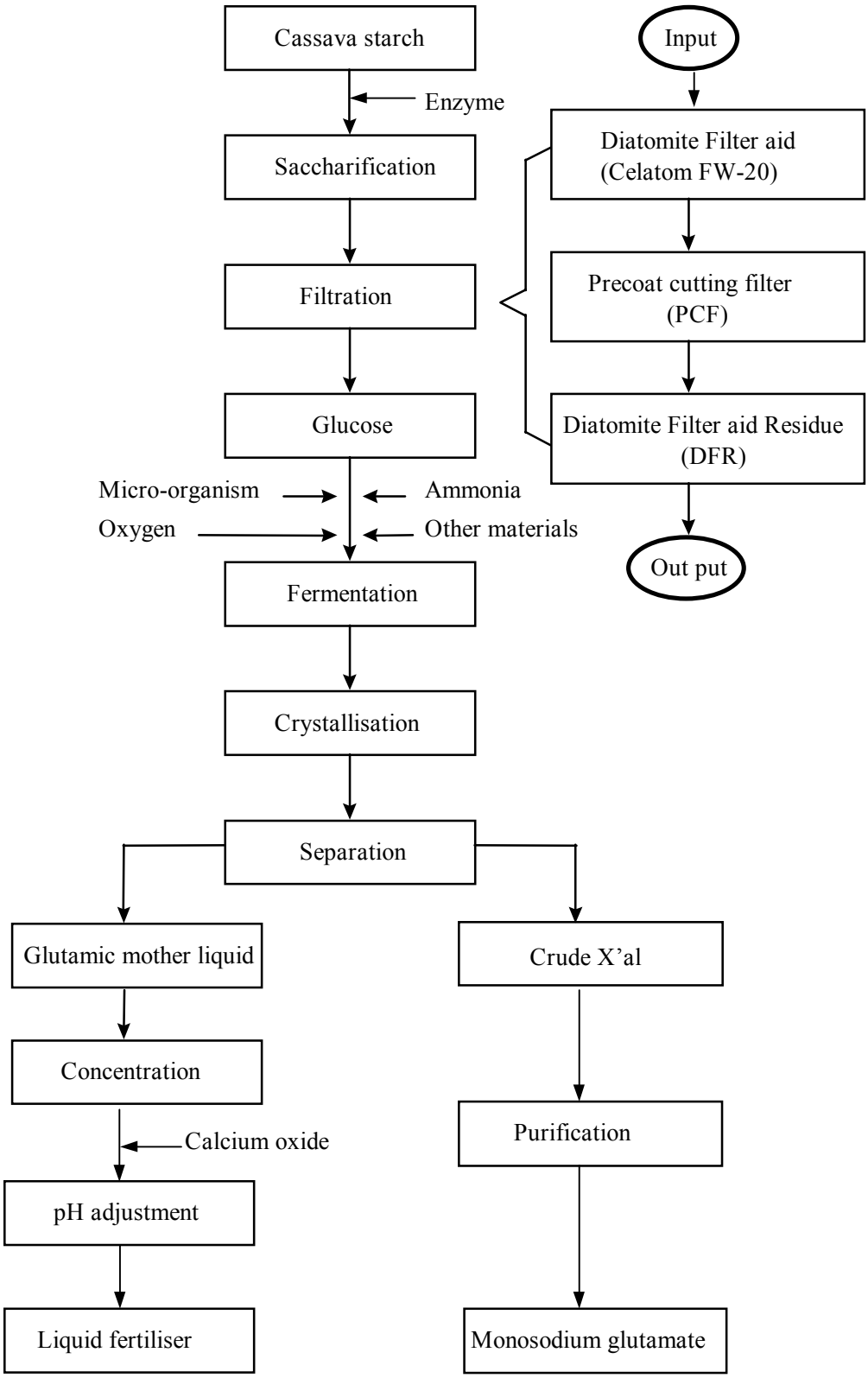

Figure 2.2 Flowchart of the monosodium glutamate production process (Adapted from Ajinomoto, personal communication).

Table 2.3 Chemical composition and properties of the DFR (%DM).

	Analysis sources	
Item	1/	2/
Dry matter	93.93	73.27
Crude protein	5.10	1.81
Crude fibre	3.14	-
Ether extracted	2.03	-
Total sugar	-	30.13
Nitrogen free extracted (NFE)	42.69	-
Ash	47.04	-
P_2O_5	-	0.14
K_2O	-	0.03
Ca^{++}	-	0.91
Mg^{++}	-	0.01
$SO_4^{=}$	-	0.38
Na^{+}	-	0.06
pH	-	3.85

1/: Feed analysis division, 1991.
2/: AJINOMOTO, personal communication.

2.2.3 Environmental aspects of the diatomite filter aid residue

The disposal of the DFR from the monosodium glutamate production industry is rather problematical since the DFR contains a great amount of organic matter in terms of nitrogen free extract i.e., readily fermentable carbohydrate. The organic decomposition results within a short time in troublesome odour emission and leaching of nitrogenous compounds such as nitrate into the ground water (SCHILDBACH, 1988, SCHILDBACH *et al.,* 1992). In Germany, investigations concerning development of environmentally safe ways to dispose waste-kieselguhr from breweries have been undertaken (HODENBERG *et al.,* 1987; SOMMER, 1988; SCHILDBACH, 1988; FINIS and GALASKE, 1988; RUß, 1992; SCHILDBACH *et al.,* 1992).

2.2.4 Alternative use of diatomite filter aid residue

Besides looking for means and ways of disposal, research has been carried out to seek for alternative uses of DFR. The waste-kieselguhr can, with the help of different methods be recycled and reused (HODENBERG *et al.,* 1987; SOMMER, 1988; SCHILDBACH, 1988; FINIS and GALASKE, 1988; Russ, 1992; SCHILDBACH *et al.,* 1992). The use of waste-kieselguhr as a nutrient substrate for the cultivation of edible mushrooms has been tried out by SCHILDBACH *et al.,* (1992). SCHILDBACH (1988) used waste-kieselguhr as a nitrogen source and compared it with the use of nitrogen fertiliser for cultivation of sugar beet, barley and corn both in the green house and in the field. The growth performance of the plants during the vegetative period was slightly different but the quality of the plants were not affected. Further more, in Germany, waste-kieselguhr is used not only for agricultural purposes but also in the cement industry. Burnt waste kieselguhr can be used as an additive in the production of cement (RUß, 1992).

2.2.4.1 The use of diatomite filter aid residue in agricultural production

Disposal of the waste-kieselguhr from the breweries factories on farm land is one of the reasonable and practicable alternatives in Germany (HODENBERG *et al.,* 1987). Research on how to use waste-kieselguhr in agricultural production has been undertaken both in green houses (SCHILDBACH, 1988) with temperature control (HODENBERG *et al.,* 1987) and in the field (SCHILDBACH, 1988; SCHILDBACH *et al.,* 1992). When waste kieselguhr was applied on the agricultural land, both soil and plant quality improved. The soil properties, such as the soil porosity, soil microbial activity, soil water and nutrient reabsorption capacity and phosphate absorption were improved with application of waste-kieselguhr whereas the soil pH value slightly decreased from 5.2 to 5.0 (HODENBERG *et al.,* 1987; RUß, 1992). In the production of the common field mushroom (*Agaricus bisporus*) and Oyster mushroom (*Pleurotus ostreatus*) as well as *Agrocybe aegerita, Flammulina velutipes* and *Lentinus edodes*, it was clearly indicated that when 2 per cent of waste kieselguhr sludge was added to the substrate, it led to the highest yields (SCHILDBACH *et al.,* 1992). When waste-kieselguhr mixed with sand was used as a growth medium, mixtures with higher kieselguhr level produced more stalk, higher yield and gave a better disease resistance (HODENBERG *et al.,* 1987). Maize and wheat grown in soil containing more waste-kieselguhr produced more hair cells in the leaf than the plants grown in soil containing a lower level of waste-kieselguhr (HODENBERG *et al.,* 1987).

2.2.4.2 Use of diatomite filter aid residue as fertiliser

The kieselguhr sludge can be used as an agricultural fertiliser, but due to its high moisture content, the distribution of kieselguhr sludge to the fields is problematic (SCHILDBACH, 1988; SCHILDBACH *et al.,* 1992). To overcome this obstruction and to achieve a complete utilisation of the waste-kieselguhr, the biofilter kieselguhr recycling system (Figure 2.3) was developed (SCHILDBACH, 1988; SCHILDBACH *et al.,* 1992).

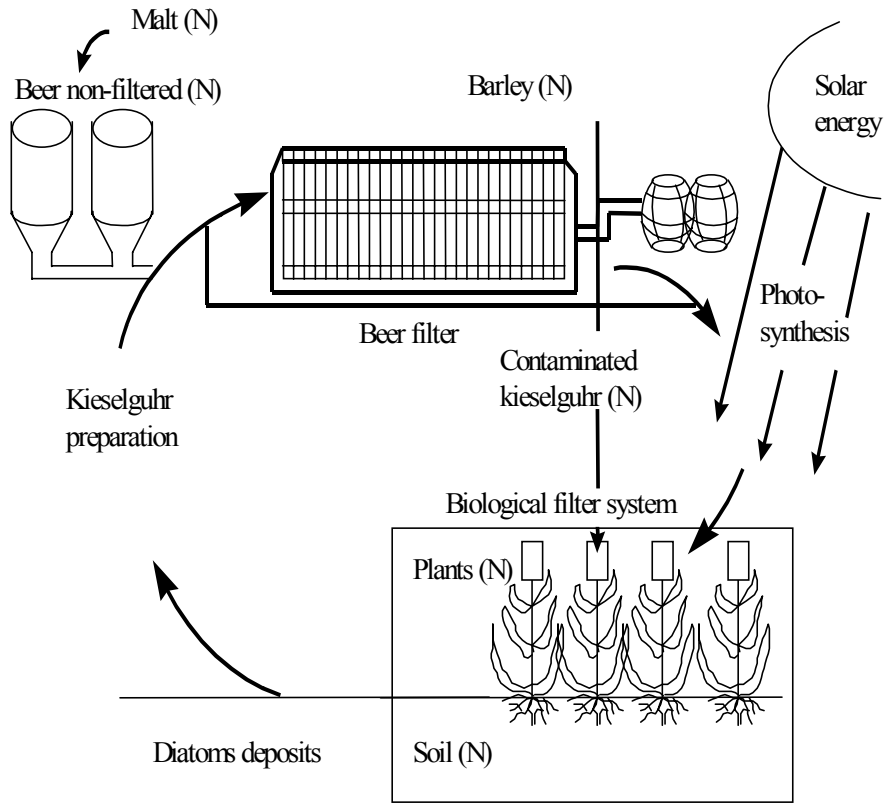

Figure 2.3 Biofilter kieselguhr recycling system (SCHILDBACH, 1988).

The diatomite deposits are prepared for the use as a filter aid by burning off any organic matter. In the brewing process, the kieselguhr is recontaminated with readily decomposable organic matter. The guhr is then returned to the soil where it is cleaned by the photosynthesis of cultivated plants (SCHILDBACH, 1988; SCHILDBACH *et al.,* 1992).

The experiments in green houses with maize, sugar beet and malting barley show that the nitrogen from the waste-kieselguhr can be assimilated by the plants in the same quantity as the mineral N-fertiliser. The response of plants to the waste-kieselguhr when it is used as source of nitrogen in field trials compared with the mineral nitrogen fertiliser depends on the plant species. Winter wheat offered crumbling kieselguhr cake as N-source gives the same yields as the control offered mineral nitrogen fertiliser, especially with high application of nitrogen fertiliser (SCHILDBACH *et al.*, 1992). Malt barley gave lower production when waste-kieselguhr was applied post germination instead of nitrogen fertiliser. When it was applied pre-germination, the yield was similar (SCHILDBACH *et al.,* 1992).

2.2.4.3 Use of diatomite filter aid residue for animal feeding

Formerly, waste-kieselguhr was used as feed stuff in Germany. However, the European Community changed the law. The acid soluble ash content in commercial animal feeds was limited at 2 per cent. The use of waste-kieselguhr as animal feed, therefore was limited (RUß, 1992). The waste kieselguhr can be used only for the production of mixed rations by mixing with the ”Treber”, a waste product from beer production factories (KRÜGER *et al.,* 1982). Mixed treber-waste-kieselguhr in Germany, nevertheless, has no consumers (RUß, 1992).

In the manufacture of animal feed, diatomite is used as an anti-caking agent, especially in low fibre rations. Some feed manufactures in Germany such as the Franz Bertram GmbH in Hamburg use a small amount of diatomite as filler in animal feed (ROSKILL, 1994).

In Thailand, research dealing with the use of diatomite filter aid residue in broiler and growing pigs have been undertaken by the research unit of the Ajinomoto research farm (AJINOMOTO, 1994; AJINOMOTO, 1995). The objectives of this research was to determine the possibility of using DFR in the feeds and to establish its influence on growth performance and feed efficiency. It was found that the use of an optimum level of DFR in animal feed gave a positive tendency on growth performance and feed efficiency of both animals (AJINOMOTO, 1994; AJINOMOTO, 1995).

2.2.4.3.1 Use of diatomite filter aid residue for poultry and swine

DFR from the monosodium glutamate production industry in Thailand has been used for poultry and swine in a research level pilot project. In the broiler production, DFR at 3 per cent level was added into the mixed rations and was fed to the broiler from 0 to 42 days. The chicken fed on a diet with 3 per cent DFR inclusion level had higher average daily gain and better feed conversion ratio than the group on the control diet (AJINOMOTO, 1995). In the trial with swine, it was found that a diet containing DFR had no adverse effect on daily gain and feed conversion ratio (AJINOMOTO, 1994).

2.2.4.3.2 Use of diatomite filter aid residue for ruminants

Research on the use of DFR in ruminant feed is not available. However, previously research on the effect of the use of commercial diatomite in feeds on rumen fermentation and blood parameters of crossbred Holstein-Thai-indigenous cattle compared with the use of zeolite was conducted (INSUNG *et al.*, 1997). Reviews on the diatomaceous earth as a conditioning agent for problematical components in the animal feed industry have been published (SCHULENBERG and RABELING, 1996).

2.3 Ruminant feed and feed intake

2.3.1 The Ruminants and their feed

Ruminants, cloven-hoofed mammals of the order *Artiodactyla,* obtain their food by browsing or grazing, subsisting on plant material. Sheep, cattle, goats, camels, llamas, buffalo, raindeer, caribou (HUNGATE, 1966) and some other wild animals such as Impala, Giraffe, Bali (Banteng) Gaur and Koprey (Mc DOWELL, 1985) as well as the very small Mausedeer *Hyemoschus* or *Tragulus* (CHURCH, 1988) are also classified as ruminants. Generally, both wild and domestic ruminants rely mainly on herbaceous materials including grass, legumes, browseable trees and other ingestible plant by-products. The ruminants, therefore, can be classified according to their natural feed types in to three categories, namely, the concentrate selectors, the grass or roughage types and the intermediate types (HOFMANN, 1988).

2.3.2 The feed for ruminants

Feeds for domestic ruminants, especially for the beef and dairy cattle can be classified as either roughage or concentrates (MATSUSHIMA, 1979; MILLER, 1979). In general, the concentrates, many of which are basal feeds such as grains, grain by-product and protein supplement feeds, have less fibre and more digestible energy (MILLER, 1979). Protein content of the concentrates varies tremendously, from 20 to 800 g/kg (MATSUSHIMA, 1979). The roughage, on the other hand, are mainly grasses, legumes and browseable trees which have a relative high carbohydrate content but are low in protein content which only account for 20 to 220 g/kg DM (MATSUSHIMA, 1979) or might be less than 30 to 270 g/kg DM with a mean of 142 g/kg DM (MINSON, 1990). The roughage for ruminants can be classified as dried forage such as hay prepared by different methods, plant stover and stalk, plant cobs and hulls and the green or succulent forages such as soilage and silage (MATSUSHIMA, 1979). Although the roughages contain less protein, they still play an important role as source of energy derived from fermentation of carbohydrate in the rumen (BLAXTER, 1962; ARMSTRONG, 1964; HUNGATE, 1966; ØRSKOV, 1975; VAN SOEST, 1982; FAHEY and BERGER, 1988; Ø RSKOV, 1990). Moreover, roughages as sources of active fibre are required for normal rumen function, lactational performance and health.

Feed for the ruminants which differ in types, breed, size, sex, age, and productive status must be different in chemical compositions and physical properties. Nutrient and energy content in ruminant feed, moreover must be evaluated and calculated to meet the requirement for those particular conditions. The systems used for evaluation both nutrient and energy content and availability are different between the countries.

In Germany, for instance, the hay equivalent system was proposed by Albrecht Daniel Thaer in the year 1809 (THAER, 1809). Although he did not suggest a feeding standard, he reported that a large milking cow should be fed with 30 lb of hay per day. The valuable conclusion on nutrient requirement from Thaer is that the nutritional requirement are dependent upon the size of animal and level of production. Another outstanding German scientist who worked with nutrient composition of foodstuff and energy requirement of cattle was Wilhelm Henneberg, working at the Weende Research Station, at Göttingen. He and his colleague, Stohmann, (HENNEBERG and STOHMANN, 1860) developed the Weender System of Analysis or the Proximate Analysis that are still in world-wide use today (FLATT, 1988). Besides this, Henneberg also worked with

fattening cattle and he suggested that a 1000 lb oxen, required 0.7 lb digestible protein, 8.4 lb digestible carbohydrate, and 0.15 to 0.25 lb of digestible fat. The total digestible nutrient (TDN) system was later developed, using the principle based on Henneberg's suggestion. It is still used in the United State of America (FLATT, 1988).

Another feed evaluation system used for estimating the energy requirement in cattle in Germany was the starch equivalent system which was introduced by Oskar Kellner (KELLNER, 1905). He expressed the energy value in relation to starch as standard nutrient. The starch equivalent system has been widely used in Germany and throughout European countries, until 1979, when the new net energy system for lactation (NEL) replaced this system (ter MEULEN *et al.*, 1998) and in the year 1996 the starch equivalent, which previously were used for beef cattle, was replaced by the new system based on metabolisable energy (HERZ and ter MEULEN, 1997). Other European countries such as the Netherlands (VAN ES, 1978), France (VERMOREL, 1978), and Switzerland (BIKEL and LANDIS, 1978) have their own new energy system for ruminant since 1977. In the Netherlands the new energy system was used since May, 1977 (VAN ES, 1978).

In other parts of the world such as in North America (LOFGREEN and GARRETT, 1968) and in Great Britain (ARC, 1965), after the second world war, evaluation systems for nutrient and energy requirements for ruminants were developed. Although many countries in Europe and North America have their own either nutrient and energy evaluation system, the most frequent reference nutrient recommendation for ruminants is the recommendation from both the Agricultural Research Council (ARC, 1980; ARC, 1984) from the United Kingdom and from the National Research Council (NRC, 1976; NRC, 1980) from the United State of America.

2.3.3. Factors affecting feed intake of the ruminants

Although the concept of multiple controls over intake is fairly well established, some of the scientists have tried to segregate the signals of satiety into those which limit the intake of roughage and others which limit the intakes of high-concentrate diets (GROVUM, 1988). For the roughage, distension of the reticulorumen, a physical factor, is thought to be the main factor limiting consumption. The animal may feel satiety due to this factor before the energy requirements of ruminants have been satisfied (GROVUM, 1984). CONRAD *et al.* (1964) concluded based on 114 trials conducted with lactating dairy cows on the voluntary feed intake

and dry matter digestibility that both physical and physiological factors regulating feed intake change in importance with increasing digestibility. Forbes (1986) classified the factors controlling intake as the animal physiological factors, dietary factors and environmental factors. The dietary factors include the energy density of offered feed whereas the physical factor depend on animal gut capacity. The environmental factors include high temperature and humidity. Housing such as slippery floors can also decrease daily intake of dairy cattle (GRANT, 1996). Other scientist classified the factors affecting animal feed intake into a dietary factor, animal physiological condition and grazing condition factors (FREER, 1981).

2.3.4 Control mechanisms of feed intake in the ruminants

Earlier, numerous experiments merely demonstrated that certain areas of the diencephalon are able to regulate feed intake (LARSSON, 1965). Although up to now, the specific mechanisms involved are not well understood, the central nervous system (CNS) presumably is responsible as the primary site for the control of feed intake in the ruminants (NRC, 1987). This physiological control is probably achieved by the negative feedback controls which inform the CNS, particularly in the ventromedial and ventrolateral hypothalamus (DE JONG, 1986). The model of the control mechanism is that the activity of the lateral areas is regulated by feed back from the ventromedial hypothalamus. Its function is to meter signals that indicate the level of nutrient stores in the body and adjust the satiety control in the lateral area so as to maintain a set-point for the stores and, thereby, for the long-term energy balance of the animal (FREER, 1981). However, there are some situations which show that the control of intake is extra-hypothalamic and it seems that these centres represent a junction in a hierarchy of control systems stretching from peripheral control in the gut to higher centres in the brain (MORRISON, 1977). In the ruminant, for instance, it has been hypothesised, the amount of forage intake might be limited by the capacity of the rumen (CAMPLING, 1970). Moreover, some metabolites in the rumen, such as the volatile fatty acids (BAILE and FORBES, 1974) or some amino acids such as lysine and glycine in plasma of sheep (BAILE and MARTIN, 1971), as well as some pancreatic hormones such as Insulin which is effective as a satiety hormone and glucagon and some peptides such as cholecytokinin (CCK) including some brain peptides such as neurohormone or neuro transmitters may play a role in the control of feed intake by ruminants (NRC, 1987). Therefore, both the brain and the gastro intestinal tract are involved in the regulation mechanism.

2.4 Rumen metabolism

2.4.1 The rumen as the intimate functional mutualism sort

The intimate functional mutualism between the ruminant and its microbes has evolved by selection of adaptations in many elements of the association. The micro-organisms depend on the ruminant for the intake of feed, its mixing and propulsion, secretion of saliva and removal whereas the host animal will be supplied with substances derived from the microbial fermentation through the rumen wall (HUNGATE, 1966). Because of the mutual action between the host and the microbes, the ruminants have greater digestive capacity to convert cellulose and other fibrous materials into useful products than non ruminant herbivores (McDOWELL, 1985).

2.4.2 Rumen fermentation

Fermentation in the reticulorumen is the result of physical and microbiological activities which convert components of feedstuffs to products such as volatile fatty acids (VFA), microbial cell or microbial protein, carbondioxide (CO_2), methane (CH_4), ammonia (NH_4^+) (HUNGATE, 1966; LENG, 1970; RUSSELL, 1984; OWEN and GOETSCH, 1988; RUSSELL and BRUCKNER, 1991), nitrate (OWEN and GOETSCH, 1988), or occasionally lactic acid (RUSSELL and BRUCKNER, 1991). Whereas the volatile fatty acids are waste products of the micro-organisms, they are of value to the host (HUNGATE, 1966). Other fermentation products such as ammonia and nitrate are harmful to the host whereas methane and carbondioxide are useless (OWEN and GOETSCH, 1988).

The quality and quantity of the products from the fermentation is dependent on types and activities of the micro-organism (RUSSELL, 1984). Among the microbes, bacteria are most diverse in species and activities. About 200 species of bacteria (RUSSELL, 1984; CZERKAWSKI, 1986; RUSSELL and BRUCKNER, 1991) and more than 20 species of protozoa (HUNGATE, 1966; CZERKAWSKY, 1986) have been identified.

The diversity of the ruminal bacteria within the ruminal ecosystem is very great. More than 15 (RUSSELL and BRUCKNER, 1991) to 20 species are able to achieve number greater than 10^7 g^{-1} (CZERKAWSKI, 1973; RUSSELL, 1984; RUSSELL and BRUCKER, 1991) and there can

be found about 10^{10} to 10^{11} cell/g of rumen content (YOKOYAMA and JOHNSON, 1988). The total number of protozoa ranged from 0 (RUSSELL and BRUCKNER, 1991) to 106 g^{-1} (CZERKAWSKI, 1986; RUSSELL and BRUCKNER, 1991). However, the rumen protozoa, because of their size which ranges from 20-200 μm, account for about 50% of total microbial mass in the rumen (RUSSELL and BRUCKNER, 1991). The diversity of ruminal fungi was not as much as bacteria and protozoa. The density of the zoospore population measured in rumen fluid of sheep ranged from 10^3-10^5 ml^{-1} (ORPIN, 1974).

The activities of the microbes in the rumen ecosystem are not dependent only on the density of the population of the microbes but also on the interaction between the microbes in the whole rumen ecosystem (HUNGATE, 1966; WOLIN and MILLER, 1988; JOBLIN, 1997). Different types of competition occur in the rumen, either within the bacterial species (HUNGATE, 1966; RUSSELL and BRUCKNER, 1991), or between protozoa and bacteria (HUNGATE, 1966; RUSSELL and BRUCKNER, 1991). Some species of bacteria such as cellulolytic bacteria have been shown to affect fungal activities (JOBLIN, 1997). It has been estimated that about 50% of all bacteria produced in the rumen were engulfed by protozoa (RUSSELL and BRUCKNER, 1991). The interaction between various kinds and species of the microbes is, therefore, important to know if one wants to manipulate the microbial fermentation in the rumen (HUNGATE, 1970; COLEMAN, 1975; WOLIN, 1975; OWENS *et al.,* 1984; VAN NEVEL and DEMEYER, 1988).

2.4.3 Rumen fermentation of feed components

Different species of microbes have different affinity to different substances. The *Bacteriodes ruminicala* grew poorly when amino acids were provided as nitrogen source whereas the growth was much better when casein hydrolyzate was substituted for amino acids (PITTMAN and BRYANT, 1964). Similar relationships also exist for the degradation of the polysaccharides. The cellulolytic species do not hydrolyze starch, and vice versa (WOLIN and MILLER, 1988). The fermentation of substrate by the microbes within the rumen is, therefore, considered based on kinds of substrates (HUNGATE, 1966; BEEVER, 1993).

2.4.3.1 Digestion of carbohydrate

Carbohydrates are the predominant component of plant tissue eaten by ruminants and therefore are the main substrates of the rumen fermentation (PRINS *et al.,* 1984). The carbohydrates in plants may be divided into two main groups namely non structural carbohydrate and structural polysaccharide. Cellulose and hemicellulose, so called holocellulose, are two major components of the fibre or the structural fraction (BEEVER, 1993), whereas starch, the storage polysaccharides, and sugar are the major parts of the non structural carbohydrate (CHESSON and FORSBERG, 1988). Both the structural and non structure carbohydrate will be degraded in the rumen by the activities of the rumen microbes.

The digestion of cellulose is undertaken by the cellulolytic bacteria. The most outstanding two species of the ruminococci related to the digestion of cellulose are *Ruminococcus albus* and *R. flavefaciens* (WALKER, 1965; HUNGATE, 1966; HOBSON, 1976; CHESSON and FORSBERG, 1988; FAHEY and BERGER, 1988). Other cellulolytic bacteria are *Bacteriodes succinogines* (HUNGATE, 1966; HOBSON, 1976; FAHEY and BERGER, 1988), *Butyrivibrio fibrisolvens*, *Clostridium lochheaddii* (HUNGATE, 1966) and *Cillobacterium cellulosovens* (HUNGATE, 1966; HOBSON, 1976).

The most predominant starch digesting rumen microbe is *Bacteriodes amylophilus*. Some other importance bacteria are *Streptococcus bovis*, *Saccinimonas amylotica* and *Bacteriodes ruminicola* (HUNGATE, 1966; HOBSON, 1976; FAHEY and BERGER, 1988).

Besides rumen bacteria, protozoa and fungi are also involved in carbohydrate fermentation in the rumen. All protozoa ingest solid particle and will build up starch-type polysaccharide reserves when exogenous carbohydrate is in excess and, indeed, some protozoa such as the holotrich protozoa will burst with reserve polysaccharide granules if sufficient sugars are present externally. The polysaccharide reserves can be used as reserve material and is fermented when external polysaccharides are lacking (HOBSON, 1976). The rumen protozoa are involved in the digestion of both structural and non structural carbohydrate (JOUANY and MARTIN, 1997). The amount of VFA and ammonia as well as digestibility of substrate in the rumen therefore are greater when protozoa are present (YOKOYAMA and JOHNSON, 1988).

The rumen fungi involved in carbohydrate digestion are C*allimastix frontalis* (ORPIN, 1974; ORPIN and JOBLIN, 1988), *Neocallimastix frontalis* (ORPIN, 1975; ORPIN and JOBLIN, 1988), *Spaeromonas communis* and *Piromonas communis* (ORPIN, 1977; ORPIN and JOBLIN, 1988). These zoospore fungi produce a wide range of enzymes which can digest the major structural carbohydrates of plant cell walls (PEARCE and BAUCHOP, 1985; WILLIAMS and ORPIN, 1987a; LOWE *et al.,* 1987) and hydrolyse a range of glycosidic linkages (WILLIAMS and ORPIN, 1987b).

The main products derived from fermentation of carbohydrates in the rumen are acids, mostly referred to as volatile fatty acids and microbes or the microbial cells. The proportions of the principal volatile fatty acids (VFA) in the rumen are : about 63% acetic, 21% propionic and 16% butyric and higher acids (HUNGATE, 1988). The ratio of the VFA, however, vary depending on properties and qualities of the carbohydrate sources. The VFA derived from fermentation of the structural carbohydrate include a high proportion of acetic acid (BLAXTER, 1962; ØRSKOV, 1975; ØRSKOV, 1992). When the cattle are fed a diet containing a higher percentage of roughage, then the molar proportion of acetic acid (C2) was higher than that of cattle fed on a diet containing a lower roughage proportion (ANNISON and ARMSTRONG, 1970). On the other hand, when the cattle were fed with a diet containing a higher starch content, then the molar proportion of acetic acid (C2) was lower than by those fed with a diet containing a lower starch content (ISHIZAKI *et al.,*1997). The concentration of propionic (C3) and butyric (C4) acids, however, was vice versa.

The maturity of the roughage sources also had an influence on the proportion of VFA produced in the rumen. The fermentation of young Rye and Timothy grass produced lower proportion (molar%) of acetic acid (C2) than the mature grass whereas the proportion of propionic (C3) and butyric acid (C4) of the mature grass was greater (ARMSTRONG, 1964). The fermentation of the concentrate containing more soluble sugars tends to yield greater proportions of propionic and butyric acids (HUNGATE, 1966; ISHIZAKI *et al.,*1997). The concentration of acetic acid however, is still higher than that of propionic and butyric acids for every concentrate to roughage ratios (ANNISON and ARMSTRONG, 1970). The ruminal VFA proportional concentrations of acetic, propionic and butyric acids can vary from 62.1:23.5:11.2 mol% in the cattle fed on high starch containing diets to 63.7:21.3:11.8 mol% in the cattle fed on low starch diets. Although total VFA in the rumen were not affected by the starch level in the diets, the

molar proportion of acetic acid and the ratio of acetic: propionic acid (C2: C3) were higher in cattle fed on a diet with a low starch content (ISHIZAKI *et al.*,1997).

2.4.3.2 Digestion of protein and non protein nitrogen

It was recognised since 1938 that micro-organisms were responsible for proteolytic activity within the rumen (BLACKBURN, 1965). The bacterial species which are responsible for protein digestion in the rumen vary greatly depending on the animal and quality of substrate. The amount of these bacterial species range from 12-38% of the total bacterial population in the rumen (BRYANT and BURKEY, 1953) or from 30 to 50% of the bacteria isolated from the rumen fluid (FULGHUM and MOORE, 1963). The predominant proteolytic bacterial species include *Bateriodes, Butyrivibrio, Selenomonas, Eubacterium, Lachnospira,*and *Streptococcus* (BRYANT and SMALL, 1956; BRYANT *et al.,* 1958; BLACKBURN and HOBSON, 1962; FLUGHUM and MOORE, 1963; ALLISON, 1970; HAZLEWOOD and NUGENT, 1978; RUSSELL *et al.*, 1981; HAZLEWOOD *et. al.,* 1983). Three of these species are thought to be the most important concerning proteolytic activity under various feeding conditions namely *Bateriodes ruminicola, Bateriodes amylophilus* and *Butyrivibrio fibrisolvens* (COTTA and HESPELL, 1986; YOKOYAMA and JOHNSON, 1988). Another very active proteolytic rumen bacteria is *Clostridium lochheadii* (HUNGATE, 1966) and also *Prevotella ruminicola* has a significant dipeptidyl peptidase activity (WALLACE, 1997).

The degradation of ingested protein by bacteria within the rumen represents the sum of a number of microbial activities, including protein hydrolysis, peptide degradation, amino acid deamination and fermentation of resultant carbon skeletons. The initial step of the degradation process requires the action of periplasmic or extracellular proteolytic enzymes produced by ruminal micro-organism (COTTA and HESPELL, 1986). Ingested protein is largely degraded to peptides, amino acids (BLAXTER, 1962; HUNGATE,1966), ammonia and VFA's (BLAXTER, 1962; HUNGATE,1966; COTTA and HESPELL, 1986; WALLACE and COTTA, 1988).

Beside bacteria, both protozoa and fungi also take part in protein degradation and metabolism. Some protozoa species such as *Entodinium caudatum* can degrade casein resulting in increasing amounts of NPN (ABOU AKKADA, 1965), whereas the *Ophryoscolex caudatus* can utilise crude protein from cotton seed, soybean and linseed oil meal very well (WILLIAM *et at.,* 1961). The proteolytic activity of the ciliates protozoa, however, are not very important. Their specific

proteolytic activity can be as low as one-tenth of that of the bacteria (BROCK *et al.*, 1982). On the other hand, part of the large bacterial amino acid pool which they consume is degraded, rather than reassembled into protozoan protein (ØRSKOV,1990). For the proteolytic activity of the ruminal fungi, WALLACE and MUNRO (1986) found that the proteolytic activity associated with the solid fraction of digesta in the rumen is considerably increased in the presence of *Neocallimastix frontalis*.

2.5 Rumen parameters related to rumen fermentation of ingested feed

Due to the microbial waste products fermentation of ingested feed or feedstuff together with the physical function of the host animal will result in changes in the rumen ecology. Changes in feeding regime and feeds or feedstuffs offered to the ruminants will thus alter rumen fermentation. This leads to changes in intermediates or metabolites and different metabolism end products. Some rumen metabolites or end products such as the volatile fatty acids (ØRSKOV and OLTJEN, 1967), ammonia (NIKOLIC and FILIPOVIC, 1981) and pH (ØRSKOV *et al.,* 1974; ØRSKOV and FRASSER, 1975; RUSSELL *et al.,* 1979) have been used as indicators for monitoring the possible availability of the particular feed or feedstuff in the ruminants and subsequent positive or negative influence on the ruminal ecosystem.

2.5.1 Ruminal pH

It is generally recognised that changes in pH of the rumen fluid influences the ruminal ecosystem in which the efficiency of growth of predominant bacteria (HUNGATE, 1966; DIRKSEN, 1970; THERION *et al.,* 1982; RUSSELL, 1991), protozoa (DIRKSEN, 1970; FORBERG *et al.,* 1984; WILLIAMS and COLEMAN, 1988; ØRSKOV, 1992) and fungi (ORPIN, 1976; WALLACE and JOBLIN, 1985) vary considerably with varying pH. A diet containing large amounts of soluble carbohydrate will result in a decrease in pH since the metabolites of this substrate has free hydrogen ion (H^+) or proton donors (DIRKSEN, 1970; SLYTER, 1976; DAWNSON and ALLISION, 1988; ØRSKOV, 1992; ØRSKOV, 1995). As previous described the fermentation of feed ingredients in the rumen provides a great amount of volatile fatty acids (VFA), mostly acetic, propionic and butyric acids (HUNGATE, 1966, Ø RSKOV and OLTJEN, 1967; ANNISON and ARMSTRONG, 1970). Because volatile fatty acids are lipid-soluble compounds they can bind and release protons and influence the pH gradient (RUSSELL and STROBEL, 1993). The volatile fatty acids, therefore, act as

uncouplers. These are in general highly lipid soluble weak acids or bases that act in a cyclic fashion to translocate either H^+ or OH^- across the cell membrane (WALLACE *et al.,* 1989). In the normal rumen fermentation, the uncouplers maintain a relatively steady pH at about 6.5 (BAUCHOP, 1977). The action of uncouplers on translocation of the ion of acetate through a bacterial membrane is shown in figure 2.4 .

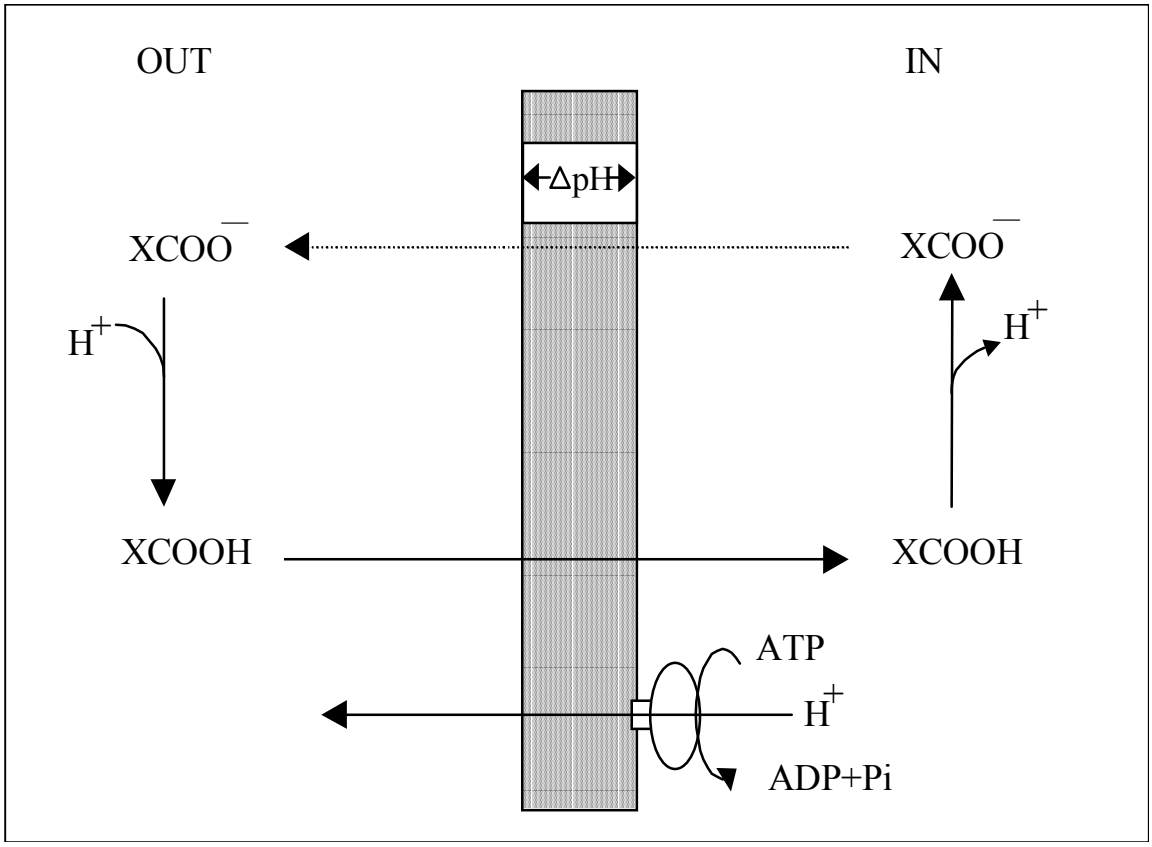

Figure 2.4 A schematic presentation of uncouplers action in a bacterial membrane. Acetate will move across the cell membrane in response to the transmembrane pH gradient whereas the membrane is impermeable for the anion (dash line) (Adapted from RUSSELL and STROBEL, 1993).

The proton donor from outside travels across the cell membrane and releases a proton in response to the pH gradient. The anion is then driven to the external surface of the membrane by the negative electrical potential inside the cell membrane. The anion, over there, is protonated and the cycle continuous (RUSSELL and STROBEL, 1993).

Normally the pH of the rumen contents fluctuates between 6-7. Only under extreme conditions and for short time does it drop to pH 5 (CZERKAWSKI, 1986). To maintain the pH at the optimum level for the microbial activity, a buffering capacity must be provided. This is achieved

by production of copious quantities of saliva containing bicarbonate and phosphate salt (HUNGATE, 1966; BAUCHOP, 1977; COUNOTTE *et al.,* 1979; OWENS *et al.*, 1984; CZERKAWSKI, 1986; YOKOYAMA and JOHNSON, 1988; ØRSKOV, 1992; THEODOROU and FRANCE, 1993). It appears that the amount of saliva secreted during the consumption of roughage and concentrate and the subsequent rumination, together with the carbondioxide produced during the fermentation, is essential geared to maintain a pH at an optimum level for the activity of the rumen microbes (ØRSKOV, 1992). Every day about 6-12 litres of saliva in sheep (KAY, 1966) and 110-170 litres in cattle is secreted from the salivary glands, particularly the parotid glands (BAUCHOP, 1977; CHURCH, 1988).

It is well documented that the rumen pH has great influence on the efficiency of the rumen fermentation. The efficiency on the production of microbial protein is greatly different between pH levels. The amount of microbial protein produced in the culture at pH 5.7 was only half the amount produced at pH 6.7 (STROBEL and RUSSELL, 1986). Although the proteolytic enzymes of the rumen microbes have their optimum pH in the neutral range, the optimum level, however, varies between types and species of microbes. The proteolytic rumen protozoa have their peak activity at pH 5.8 (FORBERG *et al.*, 1984) while the pH optimum for the activity of fungi is 7.5 (WALLACE and JOBLIN, 1985). The wide spectrum of rumen bacteria, on the other hand, have a broad pH optimum between 6 and 7 (BLACKBURN and HOBSON, 1960; YOKOYAMA and JOHNSON, 1988).

To observe the sensitivities of the microbes at different pH they are grown in glucose or cellobiose-limited continuous cultures in the pure culture with hydrochloric added in to the reservoir (RUSSELL and DOMBROWSKI, 1980). In the rumen, on the other hand, the supplementation with some amount of bicarbonate to the feed may have some beneficial effects (STOKE, 1983). In batch culture studies, a preponderance of rumen bacteria stop growing at pH values between 5.0 and 5.5 (PRINS and CLARKE, 1980).

The sensitivity of the rumen microbes to the pH changes measured in different studies are also different. Among the microbes, the rumen protozoa are the most sensitive to the pH change within the rumen (SCHWARTZ and GILCHRIST, 1975). Within the protozoa species, the Entodinia are more resistant to acidity than other genera. The ability of the rumen protozoa to survive, however, falls off at the pH below 6.0 (HINO *et al.,* 1973). If the rumen pH goes below

5.0, the protozoa die off rapidly and the animal may become defaunated (DIRKSEN, 1970; SCHWARTZ and GILCHRIST, 1975).

Because of the diversity of the rumen bacteria both in types and species, the critical pH level for them is wider than for other microbes. Whereas the maximum growth rate of some rumen bacteria are found to be at the pH value of 6.0 (RUSSELL *at al.,* 1979), other bacteria species such as the Lactobacillus have the optimum pH level lower than 5.0 (DIRKSEN, 1970). A majority of the rumen bacteria, however, stop growing at pH values between pH 5.0 and 5.5 (PRINS and CLARKE, 1980) since bicarbonate will become exhausted near pH 5.5 and many rumen organisms require HCO_3^- the active form of carbondioxide (PRINS and CLARKE, 1980). None of the bacteria were able to grow at pH values lower than 5.0 (RUSSELL, 1984).

The impact of lower pH level in the rumen to the host animal is observed when the animal has digestive disorders such as acidosis or lactic acidosis (DIRKSEN,1970; SCHWARTZ and GILCHRIST, 1975; DAWSON and ALLISON, 1988; HUNTINGTON, 1988; ENSMINGER, 1993; OWENS *et al.*, 1998). Etiology of this metabolic disorder described in figure 2.5 can be considered in to two phases (HUNTINGTON, 1988).

The first phase is based on the microbiological changes in the rumen fluid due to improper feeding management. The changes of microbiological activity occur when the ruminants are over fed on, or abruptly change to diets that contain large amount of starch or other rapidly fermented carbohydrates (DIRKSEN, 1970; SLYTER, 1976; DAWSON and ALLISON, 1988; HUNTINGTON, 1988; ØRSKOV,1992; ØRSKOV,1995; NOCEK, 1997). At the begin of indigestion, the acid producing cocci such as *streptococcus bovis* (DIRKSEN, 1970) and *selenomonas ruminantium* produce lactic acid in increasing amounts (DOWSON and ALLISON, 1988). Similarly several species of ciliated protozoa, when abundant of carbohydrate was supplied, produce more lactate but less acetate and butyrate (WILLIAMS, 1986). Under these circumstances, the lactic acid utilising bacteria can not metabolise lactate fast enough. The lactate, therefore, rapidly accumulates lowering the pH. As the pH falls, the streptococcus are very rapidly overgrown by lactobacillus, gram positive rod microbes, whose optimum pH is below 5 (DIRKSEN,1970).

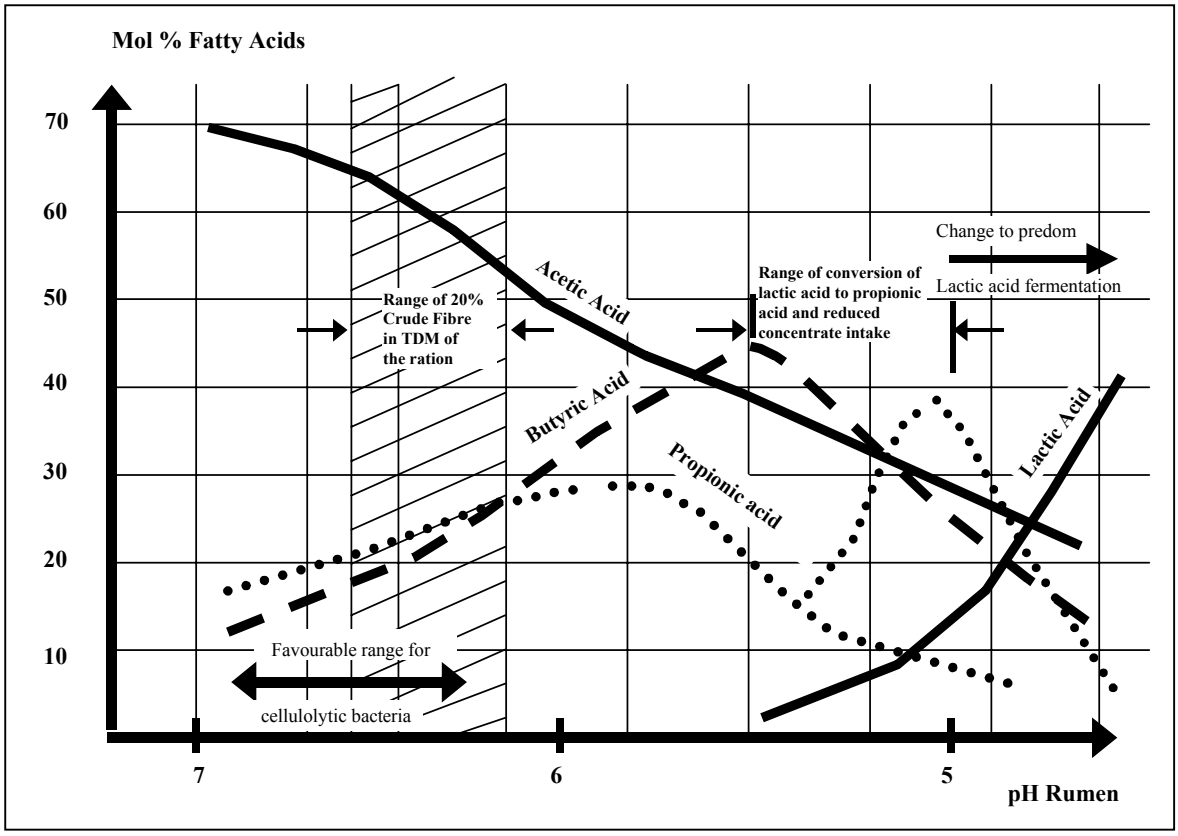

Figure 2.5 Scheme of carbohydrate fermentation in the rumen related to rumen acidosis (adapted from DIRKSEN, 1970).

In the second phase, lactic acid at high concentrations is absorbed into the animal blood circulation resulting in acidosis (DIRKSEN, 1970, HUNTINGTON, 1988). Raminitis, parakeratosi, ruminis and ruminitis chronica hyperplastica will later be observed in the animal (DIRKSEN, 1970). Injecting of slaframine, a parasympathemimetic substance, reduce the decrease of pH by increasing the saliva secretion (HIBBARD *et al.,* 1995).

ØRSKOV (1999) pointed out, that in the practice the negative effects of decrease in ruminal pH, mainly occur when excessively processed concentrate is given in a large quantities two times per day. This lowers rumen pH which in turn depress cellulose digestion and intake of cellulosic feed, and further leads to problems of acidosis and secondary ketosis due to off-feed conditions. The reduction in pH value due to higher concentrate level in the feeding regime was also reported by RUSSELL (1998), who found that the cows fed on higher (90%) concentrate level had lower ruminal pH and acetic and propionic acids ratio than those fed on roughage only. Lower pH, moreover, not only affect the C2:C3 ratio but also the methane production, deamination and ammonia concentration (LANA *et al.*, 1998).

2.5.2 Rumen ammonia

There is ample evidence in the literature both from *in vitro* and *in vivo* experiments that ammonia results from protein degradation within the rumen (ANNISON *et al.,* 1954; WARNER, 1956; LEWIS and McDONALD, 1958; MOORE and KING, 1958; BLACKBURN and HOBSON, 1960; SATTER and STYTER, 1974; BRODERICK, 1978; CRAIG and BRODERICK, 1981; BRODERICK and CLAYTON, 1992; KYRIAZAKIS and OLDHAM, 1997). Ammonia nitrogen in rumen content also derive from metabolism of some other nitrogenous compounds such as peptides, amino acids, amides, nitrates, urea (DINNING *et al.,* 1948; LEWIS, 1957; OLTJEN, 1969; TILLMAN and SIDHU, 1969; HELMER and BARTLEY, 1971; BARTLEY *et al.*, 1976; ROFFLER *et al.,* 1976; FALVEY, 1982; NIKOLIC' *et al.,* 1980; WALLACE and COTTA, 1988) and some other non protein nitrogen compounds (NPN) such as ammonium chloride (SONG and KENNELLY, 1989). Most of the results show that diets containing more soluble protein give rise to large concentrations of rumen ammonia.

During the protein degradability within the rumen, the rate of deamination is somewhat slower than proteolysis (BLACKBURN and HOBSON, 1960), therefore, the concentration of amino acid and peptides in the rumen increases immediately after feeding, but eventually virtually all amino acids are deaminated releasing great quantities of ammonia (BLACKBURN, 1965).

The concentration of ammonia in the rumen depends on four major factors: rate of formation within the rumen, rate of passage to omasum, rate of absorption from rumen and rate of uptake by bacteria (McDONALD, 1958). In the grazing animal the ruminal ammonia concentration also varies depending on the quality of roughage. Cattle grazing tropical pasture in the dry season had about 50% lower ammonia concentration than those in wet season (PLAYNE and KENNEDY, 1976). With meal-fed animals, ruminal ammonia concentrations change with time after feeding (OWENS and ZINN, 1988). Normally the peak concentration of ammonia occurs within first (NIKOLIC *et al.,* 1980) to three hours (BLACKBURN, 1965) after feeding. The concentration of rumen ammonia within the same animal, nevertheless, was different depending on location within the rumen, time and method of sampling of rumen fluid (WOHLT *et al.,* 1976). Although other products such as peptides, amino acids and volatile fatty acids are also produced from the fermentation, ammonia production and utilisation are the major process concerned with the utilisation of nitrogenous compounds in the rumen (figure 2.6) (NOLAN, 1975).

Ruminal ammonia plays a crucial role in nitrogen metabolism within the rumen. Numerous *in vitro* and *in vivo* studies indicate the importance of ammonia for the synthesis of microbial protein in the rumen (SATTER and SLYTER, 1974; RUSSELL and STROBEL, 1987; HRISTOV and BRODERICK, 1994; WALLACE, 1997). The processes of microbial protein synthesis within the rumen is well documented (HESPELL, 1984; LENG and NOLAN, 1984; CZERKAWSKY, 1986; WALLACE and COTTA, 1988). In the process of using rumen ammonia for microbial protein synthesis, the rumen ammonia, will first be transported into a cell and later be assimilated into amino acid glutamate (WALLACE and COTTA, 1988).

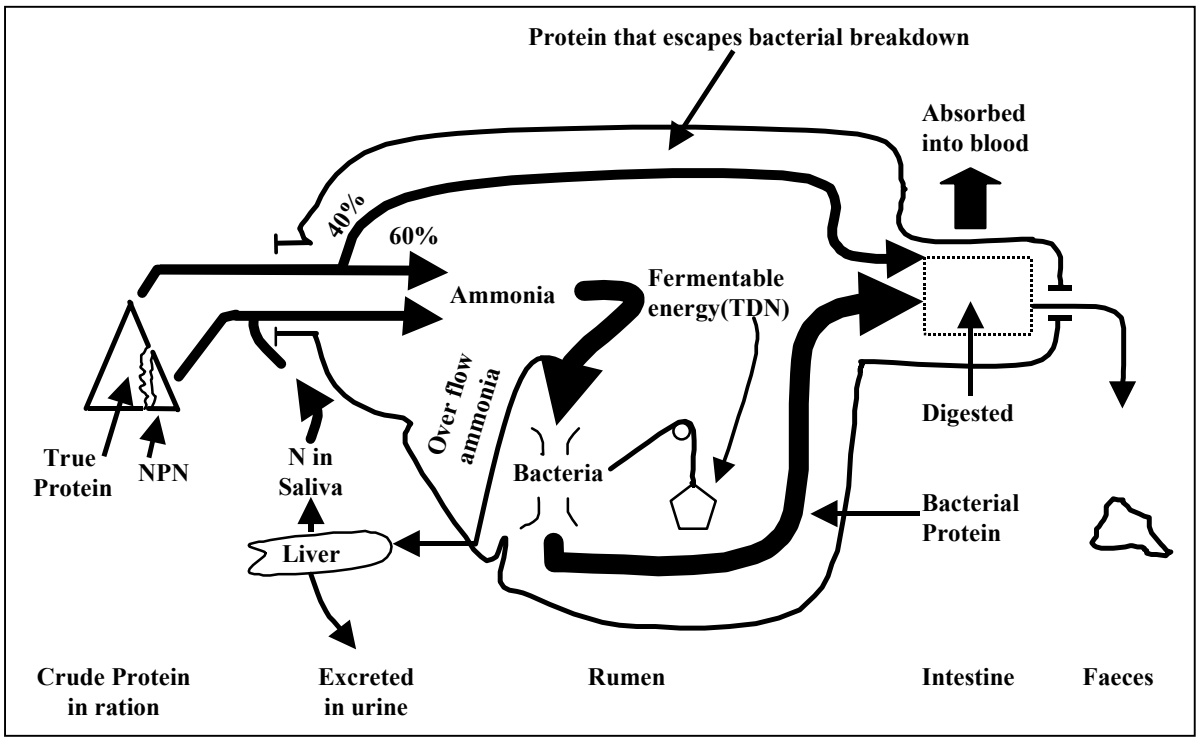

Figure 2.6 Schematic summary of nitrogen utilisation by the ruminant related to ammonia production and utilisation (Adapted from SATTER and ROFFLER, 1981).

Two main pathways are involved with the assimilation of ammonia into microbial cell (HESPELL, 1984). Under many ruminal growth conditions, assimilation of ammonia into glutamate via glutamate dehydrogenase (EC 1.4.1.4) constitutes an important route (ALLISON, 1969; ALLISON, 1970). In mixed rumen bacteria culture, glutamate dehydrogenase requires both NADH and NADPH as co-enzymes, whereas the NADPH-specific activity is required in some bacterial species. The study in pure culture showed that normally NADPH-specific activity is common but in some bacterial species such as *Ruminococcus albus* and *Megasphaera elsdenii* have a NADH-specific activity (HESPELL, 1984). Glutamate dehydrogenase is a

constitutive enzyme with a low affinity for ammonia (K_m=5 mmol/l)(BALDWIN and KOONG, 1980).

The second major pathway for ammonia assimilation in ruminal microbes consists of a set of two concerted enzyme reactions, glutamine synthetase and glutamate synthase (HESPELL, 1984), working as couple reaction of the enzyme (GS-GOGAT). They, therefore, have the highest affinity for ammonia (BROWN *et al.,* 1974) with K_m=0.2 mmol/l (HARRISON and McALLEN, 1980). In the glutamine synthetase - glutamate synthase couple reaction (GS-GOGAT), ammonia is first incorporated into amide group of glutamine, using glutamate as substrate and ATP is hydrolysed. The amide-NH_2 is then transferred to ∝-oxoglutarate to form 2 molecules of glutamate (Figure 2.7)(WALLACE and COTTA, 1988).

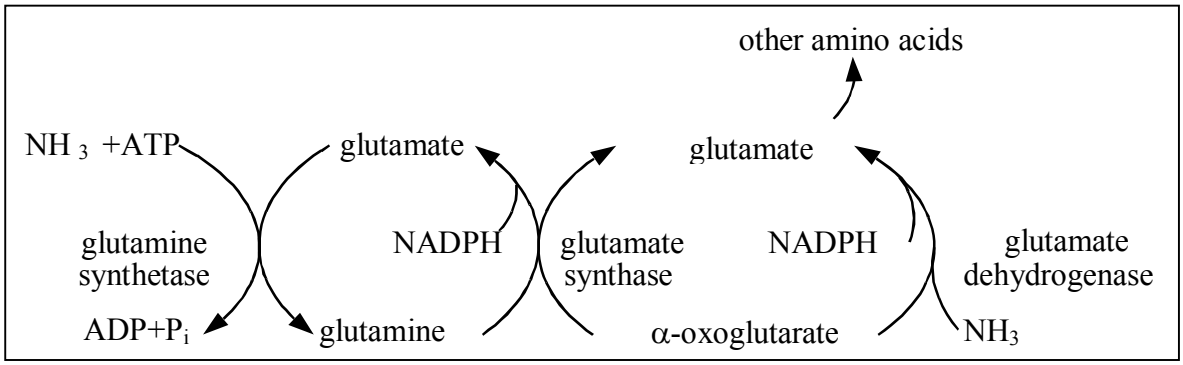

Figure 2.7 Pathways of N fixation in micro-organisms at high or low ammonia concentrations (Adapted from LENG and NOLAN, 1984).

The assimilation of ammonia for synthesis of microbial cells at high ammonia concentrations, therefore, occurs primarily via a process involving glutamate dehydrogenase (EC 1.4.1.4) (LENG and NOLAN, 1984; OWENS and ZINN, 1988). The assimilation, however, under low concentration of ammonia occurs via a two-step process involving glutamine synthetase (EC 6. 3.1.2) and glutamate synthase (EC 1.4.1.13). These reactions involve amidation of glutamate to glutamine and then a reductive transfer of the amine-N of glutamine to α-oxoglutarate that requires ATP (LENG and NOLAN, 1984) with couple glutamine synthetase- glutamate synthase reaction (GS-GOGAT).

The concentration of ruminal ammonia has been used as an index for the rate of microbial protein synthesis since most of the ruminal microbes require ammonia nitrogen for growth

(MAENG *et al.,* 1997). SATTER and SLYTER (1974) concluded that the concentration of ammonia up to about 5 mg/100 ml rumen fluid was a possible minimal concentration for optimising microbial protein yield. When the concentrations are lower than 3 to 4 mM, microbial growth decrease significantly (SATTER and SLYTER, 1974). HOOVER (1986) found that the level of ruminal ammonia for optimum growth of ruminal microbes and for organic fibre digestion ranged from 3.3 to 8.0 mg/100 ml. The optimum growth of rumen microbes, however, not only depends on the concentration of ammonia nitrogen but also depends on the rate of carbohydrate digestion supplying the energy sources which are necessary for microbial protein synthesis. Sinclair *et al.* (1993) formulated synchronous or asynchronous diets for *in situ* degradation and found that characteristics of ingredients can alter microbial growth and efficiency of utilisation of nutrients. The synchronisation between nitrogen and fermentable energy is important for microbial growth. The microbes in batch cultures reduce the efficiency of nutrient utilisation when they are supplied with asynchronous culture (NEWBOLD and RUST, 1992). The requirement of ammonia as nitrogen source for the synthesis of microbial protein, therefore, depends on the rate of digestion of fermentable energy sources (ODLE and SCHAEFER, 1987) and on the fermentation characteristics of carbohydrate sources (CHAMBERLAIN *et al.,* 1985).

High concentration levels of rumen ammonia might cause toxicity to the animal, particularly when the animals consume diets with a high percentage of rumen digestible protein or by overgenerous supply of non-protein nitrogen such as urea or other non protein nitrogen (NPN) sources (LEWIS, 1960; STILES *et al.,* 1970; HELMER and BARTLEY, 1971; BARTLEY *et al.,* 1976; MATSUSHIMA, 1979; BARTLEY and DEYOE, 1981; HIBBITT, 1988). Some NPN sources normally used in ruminant feed such as urea, biuret, isobutylidene diurea, ammonium salts and uric acid contain significant amounts of nitrogen (NIKOLIC *et al.,* 1980). The degradation of the NPN sources in the rumen course the ammonia concentration to increase to a very high level in a short period. The ammonia is absorbed and passes via the portal circulation to the liver, where, depending on the efficiency of liver function, it is converted to urea (HIBBITT, 1988). High concentration of rumen ammonia obviously do not necessarily indicate ammonia toxicity. High rumen ammonia concentration commensurate with high rumen pH, however, would indicate toxicity because the free NH_3 concentration would be much higher at high pH than at low pH (BARTLEY and DEYOE, 1981). Ammonia exists as free NH_3 at high pH but as ammonium ion (NH_4^+) at lower pH. Because tissue membranes are permeable to the lipid soluble NH_3 form but impermeable to the charged NH_4^+ form, more ammonia is, therefore,

absorbed at high pH than at low pH (HIBBITT, 1988). When the rate of ammonia absorption exceeds the capacity of the liver to convert it to urea, ammonia accumulates in the blood and toxicity may result (MATSUSHIMA, 1979).

It has been established since 1958 that the concentration of ruminal ammonia depends on 4 major factors: the rate of formation within the rumen; rate of passage to omasum; rate of absorption from the rumen and rate of uptake by ruminal bacteria (McDONALD, 1958). Further the influences of different feed ingredients on ruminal ammonia concentration, when mixed in feed (DINNING *et al.*, 1948; PRESTON *et al.*, 1961; ROFFER and SATTER, 1975; SLYTER *et al.*, 1979; GRUMMER *et al.*, 1984), directly orally administered (DINNING *et al.*, 1948) or infused into the rumen (ROFFER *et al.*, 1976; FALVEY, 1982; FIRKINS *et al.*, 1987) has also been investigated to confirm or extend the previous argument. Because the ruminal microbes are capable of capturing nitrogen from ammonia for their microbial protein synthesis, the concentration of ruminal ammonia had long been determined since it is necessary for microbial protein synthesis (HUNGATE, 1966).

The aim of a determination of the concentration of ruminal ammonia is to establish the optimum level of ammonia nitrogen concentration within the rumen. The optimum value found from different experiments, however, are different since there are difference in the objectives and the methods used for the determination and there are both *in vitro* and *in vivo* methods. BRYANT and ROBINSON (1961); HENDERSON *et al.* (1969); ALLISON (1970); SATTER and SLYTER (1974) and SLYTER *et al.* (1979) found that the optimum level of the ruminal ammonia vary from 2-6 mg/100ml, whereas the results reported from other *in vivo* methods gives a corresponding ammonia nitrogen concentration which range from 8.8-13.3 mg/100 ml (HUME *et al.*, 1970) to 28.9 mg/100 ml (MILLER, 1973). The optimum ruminal ammonia concentration, however, is correlated with other rumen parameters especially the rumen pH. It was found that when bacteria from the same cattle were incubated at different pH, the ammonia production was highly significantly different (LANA *et al.*, 1998).

Another reason to determine the ruminal ammonia concentration is ground on the fact that when the animals are offered a diet containing high soluble nitrogen or rapidly degradable protein it always causes toxicity to the animal (LEWIS, 1960; STILES *et al.*, 1970; HELMER and BARTLEY, 1971; BARTLEY *et al.,* 1976; MATSUSHIMA, 1979; BARTLEY and DEYOE, 1981; HIBBITT, 1988). To prevent metabolic disorder such as urea or ammonia toxicity, it is

necessary to feed the animals with diets optimising the released ruminal ammonia from the fermentation to manipulate the ruminal ammonia concentration to be within the optimum range for microbial requirement.

The ammonia toxicity can appear when the concentration of blood ammonia is elevated to 0.9 mg/100 ml in 1 hour whereas the normal level is only 0.5 mg/100 ml (BARTLEY *et al.*, 1976). Nevertheless, it is well established that high concentrations of rumen ammonia obviously do not necessarily indicate ammonia toxicity. In a condition where high ammonia concentration commensurate with high rumen pH, however, toxicity would be indicated. The reason is ground on the fact that at higher pH level the free ammonia (NH_3) concentration will be much higher than at lower pH. At low pH, the ammonia exists as ammonium ion (NH_4^+). The membrane of the rumen is permeable to the lipid soluble NH_3 form but impermeable to the ammonium ion (NH_4^+) form, more ammonia is therefore absorbed at the higher pH than at low pH. Toxicity, appears when a great amount of ammonia is absorbed into blood circulation (BARTLEY and DEYOE, 1981).

For the improvement of the efficiency of the use of nitrogen as source of ruminal ammonia and of carbohydrate as source of carbon required for ruminal microbial growth the optimum proportion in the degradation rate between both carbohydrate and protein sources must be considered to assure synchronisation in the degradation of carbohydrate and protein in the diets (HERRERA-SALDANA *et al.*, 1990; SINCLAIR *et al.*, 1993; SINCLAIR *et al.*, 1995). It is well established that microbial nitrogen production was more efficient when the dietary energy and nitrogen supply was synchronised (SINCLAIR *et al.*, 1995).

The relationship between the ruminal ammonia nitrogen and the blood urea nitrogen (BUN) is ground on the fact that when the diet contain a great amount of nitrogen, the ruminants will absorb substantial amounts of this dietary nitrogen as ammonia. The ammonia nitrogen which is absorbed by the portal-drained viscera (PDV) is subsequently removed by the liver and detoxified, mainly it is converted into urea, which is released into the vena cava (REYNOLDS, 1992). A high ammonia absorption due to a high nitrogen intake, therefore, will rise the level of blood urea nitrogen (BUN). A positive correlation have been found between both parameters (HIGGINBOTHAM *et al*, 1989; ROSELER *et al.,* 1993). The correlation coefficient between BUN and ammonia nitrogen concentration are reported from copious experiments (LEWIS,

1957; ABOU AKKADA and OSMAN, 1967; McINTYRE, 1970; HA and KENNELI, 1984). The correlation coefficient of BUN and ruminal ammonia concentration ranged from 0.51 (HA and KENNELI, 1984) to 0.88 (McINTYRE, 1970).

3. PRELIMINARY EXPERIMENT: EFFECT OF DIATOMITE FILTER AID RESIDUE IN FEED ON THE GROWTH PERFORMANCE OF WISTAR ALBINO RATS

3.1 Objectives

The objective of this study was to determine the effect of diatomite filter aid residue inclusion in feed on growth performance of rats. The DFR was stepwise introduced and palatability and acceptability were tested.

3.2 Materials and methods

3.2.1 Animals

Four male and four female Wistar Albino rats of the same age and averaging 164.38 (SEM=12.98) gram initial body weight were used. All the animals were obtained from the Experimental Animal Section of the Department of Animal Science, Faculty of Agriculture, Chiang Mai University, Thailand.

3.2.2 Animal housing

The rats were individually housed in stainless steel wire cages of 20 cm length, 30 cm width and 25 cm height. The cages were equipped with water bottles and feed trays and were placed on the shelf in a room under temperature control with a mean ambient temperature of 25±0.5 degrees Celsius. The relative humidity was kept at 50% throughout the experimental period.

3.2.3 Diets

Ground grower diet for swine mixed with three different levels of diatomite filter aid residue (DFR) and one ration of pure DFR as shown in Table 3.1 was used. The pelleted growing swine feed contained 87.0% dry matter and crude protein, crude fat and crude ash in the proportions of 16.0, 3.0 and 6.0% of DM respectively.

Table 3.1 Feed ingredients (g/kg DM) and the calculated contents of the rations containing either 25, 50, 75 or 100% diatomite filter aid residue (DFR).

	Diet			
	25%DFR	50%DFR	75%DFR	100%DFR
Ingredient				
Diatomite filter aid residue[1]	250.00	500.00	750.00	1000.00
Grower diet[2]	750.00	500.00	250.00	0
Total	1000.00	1000.00	1000.00	1000.00
Calculated value				
Crude protein	125.18	90.35	55.53	20.70
Metabolisable energy (MJ/kg)[3]	11.06	8.72	6.38	4.04

DFR: Diatomite filter aid residue.
[1] Contained approximate 2.07% CP and 4.04 MJ ME/kg DM.
[2] Contained approximate 16.0% CP and 13.4 MJ ME/kg DM.
[3] Calculated according to Close and Menke (1986).

3.2.4 Experimental procedure

3.2.4.1 Feeding and management

The animals had free access to feed in feed trays. Feed was given daily. Water was supplied in water bottle generally used for experimental rats. The light was turned on during 06.00 to 18.00 hours.

3.2.4.2 Data collection

The initial weight and weight change of animals at 6, 8 and 12 days were measured. Weight gain of each animal was calculated at the end of the experiment as the difference between the final and initial weights. Feed - and faecal samples were collected every day for determination of ash content.

3.2.5 Data analysis

Data on weight changes were use to determine the average body weight gain throughout the 12 days experimental period. The mean values on weight changes across treatments were compared.

3.3 Results

Data on weight gain and percentage of ash in feed and faeces are shown in Table 3.2. The weight gain of the rats fed on 25% DFR inclusion level was highest, whereas the rats fed on 100% DFR inclusion level lost weight. Weight gain of the animals decreased with increasing DFR inclusion level. Ash content both in feed and in faeces increased proportionally with increasing amounts of DFR in the feed.

Table 3.2 Weight gain, ash content in feed and in faeces of Wistar Albino rats offered diets with different levels of diatomite filter aid residue (DFR).

	Diet			
Parameter	25% DFR	50% DFR	75% DFR	100% DFR
Weight gain (g)	60.00	52.50	30.00	-45.00
Ash in feed (%)	16.26	26.52	36.78	47.04
Ash in faeces (%)	53.69	69.71	79.76	85.76

3.4 Discussion

The body weight of the experimental rats fed on the diet containing 100% DFR decreased with almost 28% of the initial weight through the 12 days experimental period, whereas the body weight of the rats in all other groups increased opposite proportionally with the rate of DFR inclusion levels. The calculated value of crude protein and metabolisable energy content of experimental feed varied from 125.18, 90.35, 55.53 and 20.70 g CP/kg and 11.06, 8.72, 6.38 and 4.04 MJ ME/kg, respectively (Table 3.1). Both crude protein and energy content in the diets, therefore, decrease proportionately with increasing DFR. The body weight gain of the rats in each group had the same tendency as crude protein and metabolisable energy content in their feed.

According to the recommendation of the NRC (1995) on nutrient requirement of the laboratory animals, especially of rats, the crude protein requirement for maintenance and growth are 50 and 150 g/kg of the diet, respectively, whereas the requirement for metabolisable energy for growth of the rats is 15 MJ/kg (NRC, 1995). This recommendation, however, base on the condition that the rats are allowed free access to feed and the diet is not deficient in other nutrients. When protein and energy content of all experimental diets are compared to the recommendation, it is obviously indicated that all the diets contain lower protein and energy content as recommended for growth. However, all diets cover the protein requirement for maintenance. The rats fed on the diet containing 100% DFR scratch and waste a lot of their feed from the feed tray in search of feed particles that contain enough of both protein and energy to cover their requirements. The animals in this group, therefore, loose body weight, in average about 45 g through the 12 days experimental period.

It is well documented that the energy level of the diet exerts an effect on the rate of body weight gain in rats (FORD and WARD, 1983). The body weight gain of the rats, moreover, depends on the rate of protein synthesis in the liver which is retarded by lower dietary intake or dietary restriction (BIRCHAENALL-SPARKS *et al.,* 1985). The results from other studies on the relationship between dietary protein content and feed intake in rats indicates that the weight loss of the rats was slightly affected by the diet quality and drastically affected by feed intake (SAINZ *et al.*, 1986). Considering both of crude protein and metabolisable energy content and the rats acceptability to the diet, it can be concluded that the pure DFR can not be used as animal feed.

To determine the level to which DFR can be included in animal feed, also the inorganic content in the mixed diet must be considered. About 47.04% (Table 2.3) of the DFR content (DM basis) is inorganic matter or ash (FEED ANALYSIS DIVISION, 1991). The calculated ash content in the rations are 162.60, 265.20, 367.80 and 470.40 g/kg, respectively.

The high amount of ash in the feed in terms of total ash and acid soluble ash might cause a legal obstruction to the possibility of using of DFR as animal feed in some countries. Since 8th April, 1981, the amount of acid soluble ash in the animal feed in the European Community Countries was limited to 2% of DM (KRÜGER *et al.,* 1982). In other countries, including Thailand, the level of acid soluble ash content in the animal feed, however, is not yet limited. Therefore, there is no legal obstruction to the use of DFR as animal feed.

In the countries where the level of acid soluble ash in the animal rations is not limited, the high level of total ash content seem to give a chemical obstruction to the use of DFR in animal feed due to a too high mineral content and too low organic matter digestibility (OMD). The diatomite itself, however, is completely inert, non-resorbable, non swelling and non toxic (SCHULENBERG and RABELING, 1996). The results in Table 3.2, which shows the ash content in faeces, are in line with this findings. The animals fed on a diet containing higher ash content have also a higher ash content in the faeces. This means that the unabsorbable fraction in the animal feed was excreted by the normal mechanism of nutrient digestion, utilisation and excretion. The use of DFR in animal feed, therefore, has no adverse effects in terms of digestion inhibition, interference with other feedstuff, toxicity or accumulation in the body.

3.5 Conclusion

The use of DFR in the feed fed to the Wistar-Albino rats at inclusion levels of 75% or less did not cause any adverse effects to the animals. The animal fed on diets containing lower DFR inclusion levels showed better growth performance. This was related to the protein and energy contents in the mixed rations in which the diet containing higher DFR inclusion levels had a lower content of both crude protein and metabolisable energy. Drying the DFR with an appropriate method to prevent organic matter fermentation, the cause of changes of the nutritional profiles and cause of lower metabolisable energy content, might be needed to maintain the quality of the DFR.

4. EXPERIMENT 1. EFFECTS OF DIATOMITE FILTER AID RESIDUE IN FEED ON RUMEN FERMENTATION AND BLOOD PARAMETERS IN CATTLE

4.1 Objectives

This experiment was conducted to evaluate the effect of diatomite filter aid residue in feed on ruminal ammonia concentration, ruminal pH and on blood urea nitrogen, blood calcium and blood phosphorus concentration in cattle.

4.2 Materials and methods

4.2.1 Animals

Four rumen fistulated Holstein-Thai-indigenous crossbred heifers averaging 250±3.16 Kg body weight were used in this experiment. The ruminal cannulae were made of silicone (Elastosil R401/50 Wacker GmbH Munich Germany) and were 10 cm in diameter.

4.2.2 Housing

The cattle were housed in conventional stanchions. Feed troughs and automatic drinking water cups were located at the front part of each stanchion. The pens had concrete floors and were roofed.

4.2.3 Diets

Four isonitrogenous diets were formulated according to NRC (1980) recommendations to contain 148 g CP/kg DM. Diatomite filter aid residue (DFR) was included at four levels varying from 0-60 per cent. Soybean meal was used as protein source. Premix (Ramical®) and normal salt were used as sources of vitamins and minerals and molasses added as a feed appetiser. Urea was used to adjust the level of crude protein in the rations. Palm kernel meal was used for the substitution of diatomite filter aid residue in the feed. For the control diet, broken rice was used as an energy source instead of DFR. Urea was ground and mixed with part of the palmkernel meal, soybean meal, Ramical® as well as normal salt. This was to prevent urea from aggregating

and also to facilitate the dispersion of vitamins and minerals within the diet. The diatomite filter aid residue was dried in a hot air oven at 105°C for 24 hours before being used in the rations. Palmkernel meal was ground with a hammer mill using a sieve with a diameter of 4 millimetres. All of the feed ingredients were mixed together using a horizontal feed mixture bin. Molasses was added during mixing. Feed was prepared every second day throughout the experiment. The ingredients and chemical composition of the experimental rations used is shown in Table 4.1.

4.2.4 Experimental procedure

4.2.4.1 Experimental design

The cattle were used in a 4x4 Latin square design with the four animals going through four periods of dietary treatments. The allocation of the diets to the animals is shown in Table 4.2.

4.2.4.2 Feeding and management

The cattle were offered feed two times a day at 07.30 and 16.30 hours. The amount of concentrate offered at each feeding time was calculated based on the animal's body weight. As roughage sources the animals were offered rice straw *ad libitum*.

4.2.4.3 Sample collection and analysis

Each period was 14 days long with 13 days for adjustment and 1 day for sample collection. Rumen fluid and blood samples were taken in the morning shortly before feeding (0 hr) and at 2, 4, and 8 hours post-feeding. The rumen fluid was obtained through the fistulae via a suction pump. The pH of the ruminal fluid was determined immediately using a glass sensitive electrode (Schott Gerate, Handylab 1). Rumen fluid was prepared for ammonia analysis by centrifuging at 5.000 g for 10 minutes. The supernatant fraction was decanted and kept frozen at -20°C until analysed for ammonia (CHANTHAI, 1990). Samples of blood were taken from the jugular vein immediately after the collection of rumen fluid. Heparin was used as an anticoagulant. Blood plasma was separated by centrifuging whole blood at 5.000 g for 10 minutes. Samples were kept frozen at -20°C until analysed (AUTTASART *et al.*, 1992).

Table 4.1 Feed ingredients (g/kg DM) and chemical composition of the experimental rations containing either 0, 20, 40 or 60% diatomite filter aid residue (DFR).

	Diet			
	0% DFR	20% DFR	40% DFR	60% DFR
Ingredient				
Diatomite filter aid residue	0.00	200.00	400.00	600.00
Broken rice	600.00	0.00	0.00	0
Palmkernel meal	333.30	750.80	542.60	326.30
Urea[1]	16.70	15.00	20.00	23.70
Soybean meal	20.00	4.20	7.40	20.00
Molasses	20.00	20.00	20.00	20.00
Vitamin-premix[2]	5.00	5.00	5.00	5.00
Normal salt	5.00	5.00	5.00	5.00
Total	1000.00	1000.00	1000.00	1000.00
Crude protein (calculated)	148.00	148.00	148.00	148.00
Chemical analysis				
Dry matter[3]	879.03	896.88	852.04	801.35
Organic matter	964.78	827.05	694.57	562.88
Crude protein	147.86	136.05	134.73	125.22
Crude fibre	92.12	219.23	162.13	122.06
Ether extract	22.74	49.07	40.23	34.44
Crude ash	35.22	172.95	305.43	437.12
Nitrogen free extract	702.06	422.70	357.48	281.16
Calcium	3.77	5.71	9.27	11.88
Phosphorus	3.05	4.23	3.67	2.76
Sodium	1.71	3.45	4.44	3.91
Potassium	4.43	2.87	0.67	0.43
Organic matter digestibility[4]	677.27	485.67	489.07	376.83
Gas production (ml/200 mg DM)	58.44	35.94	35.36	21.82
Gross energy (MJ/kg)	18.45	17.05	14.63	12.65
Metabolisable energy (MJ/kg)[5]	10.38	6.78	6.57	4.31

DFR: Diatomite filter aid residue.
[1] Contained 46% Nitrogen.
[2] Contained Vitamin A, 1.250.000 iu; Vitamin D_3, 250.000 iu; Vitamin E, 1.000 iu; Zinc, 5.200 mg; Iron, 2.250 mg; Manganese, 2.500 mg; Copper, 1.000 mg; Cobalt, 60 mg; Iodine, 80 mg; Selenium, 25 mg and Sodium, 20 mg.
[3] Expressed as g/kg at air dry basis.
[4, 5] Calculated according to Close and Menke (1986) on page 134.

Table 4.2 Allocation of diets to the fistulated heifers in the Latin square design.

Period	Animal number			
	1	2	3	4
1	T2	T1	T4	T3
2	T4	T3	T2	T1
3	T1	T4	T3	T2
4	T3	T2	T1	T4

Treatment T1 to T4 correspond to diets containing diatomite filter aid residue at 0, 20, 40, and 60 per cent respectively, in Table 4.1.

Ammonia concentration in the rumen fluid was determined using gas-sensing electrode (Ammonia electrode ORION® model 95-12 and ORION® pH/ISE meter, model 290A) (CHANTHAI, 1990). Blood urea nitrogen, calcium and phosphorus were determined using a Biochemical analyser (WAKO® Model 20R).

4.2.4.4 Chemical analysis

4.2.4.4.1 Feed samples preparation for analysis

Feed samples were mixed and ground first through a 4 mm screen to reduce the volume of the samples and afterwards through a 2 mm screen before chemical analysis.

4.2.4.4.2 Proximate analysis

The proximate analysis was carried out and calcium, phosphorus, sodium and potassium analysed as described by NAUMANN and BASSLER (1976). All sample were analysed in triplicates. Gross energy measurement and Hohenheim gas test as described by MENKE *et al.* (1979) were also undertaken.

4.2.5 Statistical analysis

Data was analysed by analysis of variance according to the general linear model procedures of the statistical analysis system (SAS, 1988) using a model suitable for a Latin square design. Duncan's new multiple range test was used to compare differences between treatment means.

4.3 Results

4.3.1 Ruminal pH

The rumen pH across treatments at different times after feeding were significantly different ($P < 0.05$) except at 0 hours which was not significantly different ($P > 0.05$)(See Appendix 2.1). The average pH value declined up to 4 hours post-feeding (Figure 4.1) and then it increased again. The cattle fed 40% DFR has highest average pH value through the 8 hours collecting period. Mean pH across treatments was 6.98±0.11.

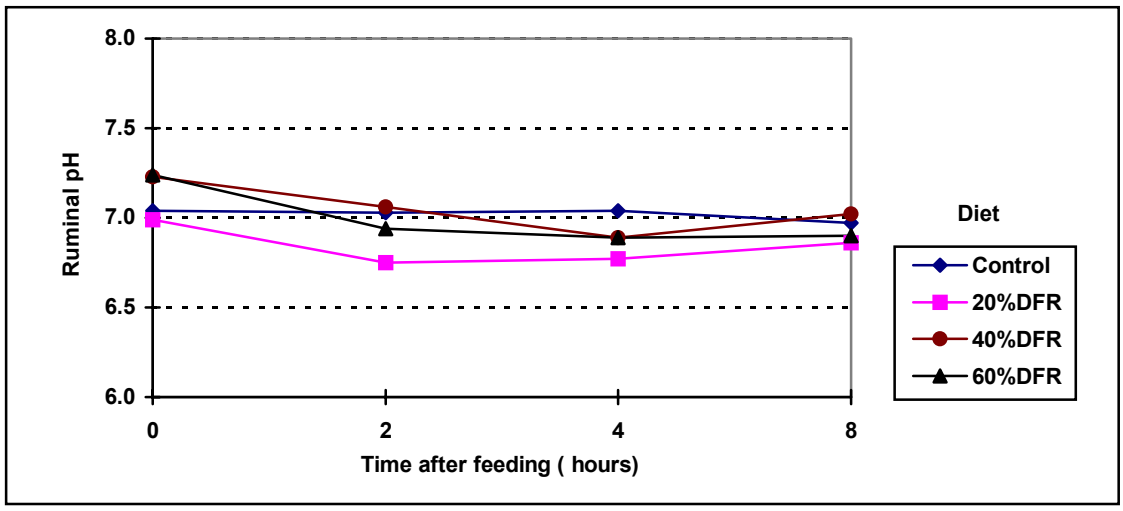

Figure 4.1 Ruminal pH at different times after feeding.

4.3.2 Ruminal ammonia nitrogen concentration

Ruminal ammonia nitrogen concentration at every time period after feeding was significantly different ($P < 0.05$) across treatments (See Appendix 2.1) except for at 8 hours post-feeding where the ammonia nitrogen concentration was not significantly different ($P > 0.05$) across treatments. The ruminal ammonia concentration raised and peaked at 2 hours post-feeding for all treatments and then it declined up to 8 hours after feeding (Figure 4.2). The cattle fed on 40% DFR diets had the highest ammonia nitrogen concentration at 2 hours after feeding. The ammonia nitrogen concentration of the cattle fed on every inclusion levels of DFR was higher than that of in the control group.

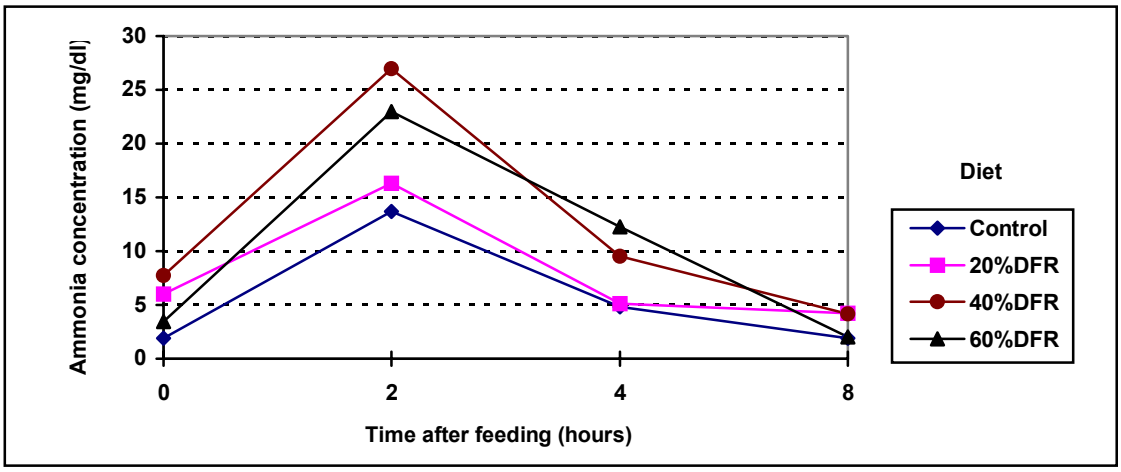

Figure 4.2 Ruminal ammonia concentration at different times after feeding.

4.3.3 Blood urea nitrogen

Blood urea nitrogen (BUN) concentration at every time period were significantly different ($P < 0.05$) across treatments (See Appendix 2.1). The concentration of blood urea nitrogen increased after feeding and peaked at 4 hours after feeding in every group of cattle except for the cattle fed on 20% DFR diets (See Figure 4.3). Their blood urea nitrogen concentration reached the peak at 2 hours after feeding. Cattle fed on 40% DFR has the highest BUN concentration at 4 hours post-feeding. The control diet has lower values of BUN at every time periods after feeding than the other treatment groups. Therefore, the period with high BUN concentration was prolonged by application of diatomite filter aid residue in the diets. The trends of ammonia-nitrogen in rumen fluid and BUN in blood circulation were similar.

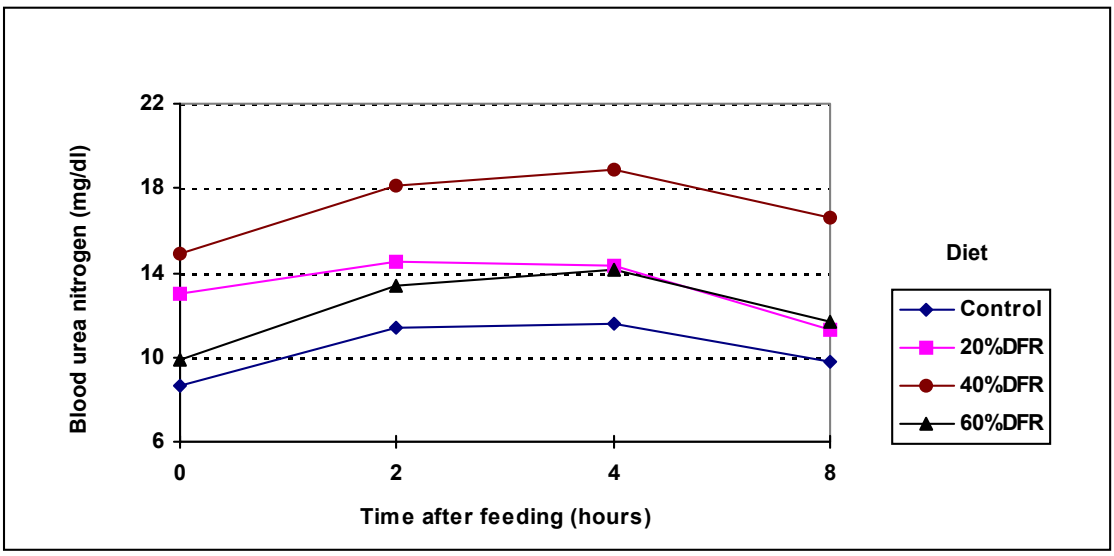

Figure 4.3 Blood urea nitrogen at different times after feeding.

4.3.4 Blood calcium

The level of blood calcium in the 4 groups were not significantly different (P>0.05) at any time period after feeding (See Appendix 2.1). The cattle fed on 40% DFR had the lowest blood calcium concentration through 8 hours post-feeding (See figure 4.4) but there was no significant difference between treatments (P>0.05). Hence, blood calcium was not affected by treatments. The level of calcium was normal and averaged 10.03±0.48 across treatments.

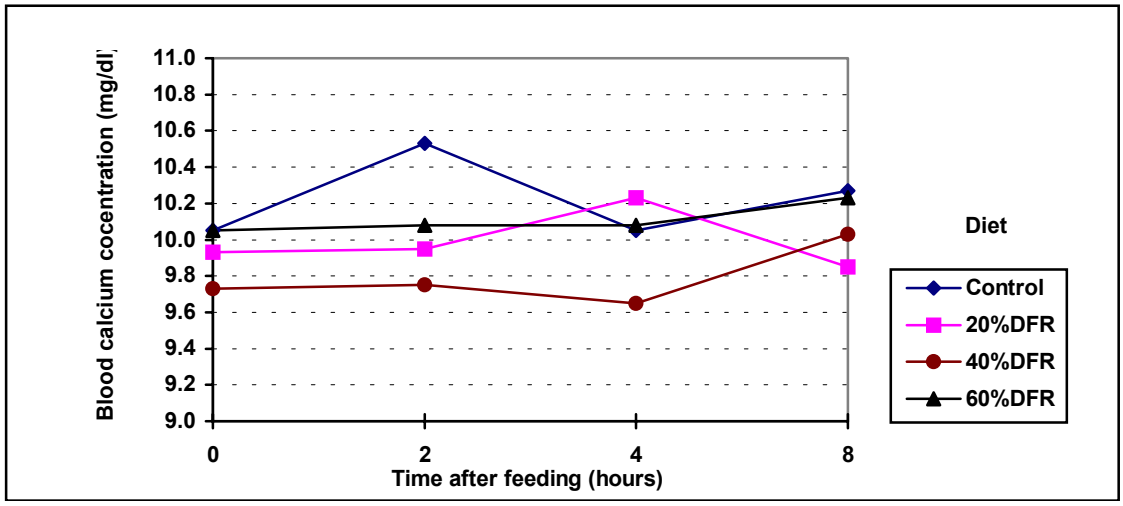

Figure 4.4 Blood calcium concentration at different times after feeding.

4.3.5 Blood phosphorus

Blood phosphorus were not significantly (P>0.05) different across treatments during the first 6 hours after feeding (See Appendix 2.1). At 8 hours post-feeding the levels were significantly different (P<0.05) across treatments means. Cattle fed on 40% DFR has the highest average levels of blood phosphorus throughout the 8 hours collecting period (See Figure 4.5). Blood phosphorus was considerably constant throughout the time periods and gave normal values. Blood calcium to phosphorus ratio was 1.31:1.

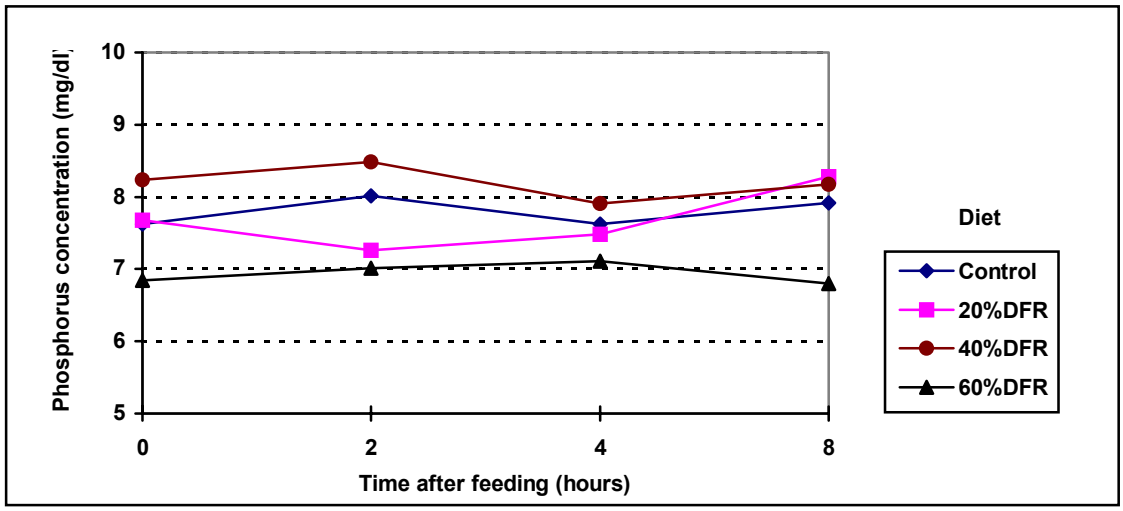

Figure 4.5 Blood phosphorus concentration at different times after feeding.

4.4 Discussion

Although the result measured both at 2 and 4 hours after feeding showed that the ruminal pH is affected by treatments ($P<0.05$) the pH levels are still close to the range which is optimal either for fibre (6.0-6.8) or starch (5.5-6.0) digestion (MASON, 1997). For all treatments the pH decreased at 2 hours post feeding and reached the lowest level at 8 hours for the control and 20% DFR inclusion diets and at 4 hours post feeding for the cattle fed on 40 and 60% DFR inclusion level. This changing profiles are inline with results by ARONEN and VANHATALO (1992), who report that the pH value of the rumen fluid of the cattle fed on different concentrate supplemented to grass silage were found to be lowest at 4 and 6 hours post feeding. In an experiment in sheep, however, the lowest pH level was found at 1.5-2.5 hours post feeding (SINCLAIR *et al.*, 1993, SINCLAIR *et al.*, 1995). The various pH values are suggested to be caused by differences in the secretion of saliva, which arise from the differences in the time required for the chewing of the feed and for rumination (ØRSKOV and RILE, 1990). The optimum pH again achieved at 8 hours post feeding imply, that the variation in nutrient composition among the diets due to the difference in DFR inclusion levels did not cause any adverse effects on rumen fermentation. The rumen parameters, especially the rumen pH range in the optimum level either for cellulose or amylose digestion throughout 8 hours post feeding.

The rumen ammonia concentrations were clearly affected by the treatments ($P<0.05$) both at 2 and 4 hours post feeding. The tendencies of the ruminal ammonia concentrations go along with

the amount of urea in the feed especially the ammonia concentration at 4 hours post feeding where the ruminal ammonia increase with increasing amount of urea in the diets. These results are similar with the previous results reported by Kang-Meznarich and Broderick (1981). The rate of increase in the ruminal ammonia concentration, normally have a very close relationship to the level of ruminal degradability of crude protein of the diets (PRESTON *et al.*, 1961). The rate of ammonia concentration, moreover, depends on the solubility of the nitrogen sources. It has long been recognised that urea has very high solubility in the rumen because the rumen fluid has a high urease activity (PEARSON and SMITH, 1943). Diets containing higher urea content, therefore, tend to have a higher ammonia concentration, especially at short time post feeding.

It is clearly indicated that the concentration of ruminal ammonia is within the normal pattern when compared with previous reports by other researchers who found that ammonia concentration reach the peak within 1 hour (MOORE and KING, 1958; NIKOLIC *et al.,* 1980) to three hours (BLACKBURN, 1965) post feeding, depending on the solubility of the nitrogen sources contained in the diet. The peak concentration of ruminal ammonia in this experiment was found within 2 hours post feeding for all diets. The concentration of ruminal ammonia through 8 hours post feeding ranged from 1.89 mg/100ml at 8 hours for the cattle offered the control diet to 26.65 mg/100 ml at 2 hours post feeding for the cattle offered 40% DFR inclusion level in the diets. These concentration levels, however, are still in the optimal range for microbial protein synthesis reported from previous experiments. It might be concluded that the use of DFR in cattle feed with 20 to 60% inclusion levels did not cause any depressive effects on rumen fermentation in terms of ruminal ammonia concentration.

The concentration of blood urea nitrogen (BUN) post feeding follow the same tendency as the concentration of the ruminal ammonia nitrogen, though the increase in BUN compared with the increase in ruminal ammonia nitrogen, is delayed with maximum 4 hours post feeding. Similarly the ruminal ammonia decreased more rapidly than the BUN. This results are inline with observation by RODRIGUEZ *et al.* (1997).

The profiles of the BUN concentration shows that inclusion of DFR up to 40% in the diets provided the highest BUN concentration at any time period post feeding. The cattle fed on diets containing higher or lower DFR inclusion level (60% and 20% DFR, respectively) have lower BUN at any time period post feeding. It is known that the concentration of BUN will vary not only dependent on ruminal ammonia nitrogen but also on the degradability of protein in the diets

(NOCEK and POLAN, 1984; ROPSTAD *et al.,* 1989). A diurnal fluctuation of the BUN, in relation to the change of the ruminal ammonia (GUSTAFSSON and PALMQUIST, 1993) and time of feeding (RODRIGUEZ *et al.,* 1997) will always occur. The present results might imply that the 40% DFR inclusion level diet contains crude protein with higher degradability than the other diets. The BUN measured from the cattle offered the 40% DFR diet, therefore, was higher than that of in others.

The result from the study on the effect of use of the DFR in cattle feed on blood calcium and blood phosphorus concentration show that the treatment didn't have any influences on blood calcium and phosphorus concentrations at any time period throughout 8 hours post feeding, except for the concentration of the blood phosphorus at 8 hours post feeding that it was significantly different ($P<0.05$) across treatments. The average plasma calcium and phosphorus concentration ratio (Ca: P) of the animal offered diet containing different DFR inclusion levels ranged from 1.31, 1.30, 1.19 and 1.45:1 respectively. This is the optimum ratio for the concentration of blood calcium and blood phosphorus in the ruminants. It is known that the theoretical ratio of the compound calcium and phosphorus within the animal was 1.67:1 (TERNOUTH, 1997) but the most acceptable ratio was 2:1 (CORBRIDGE, 1995). In the diets (Table 4.1), the content of total mineral or crude ash as well as calcium and phosphorus content are obviously different among the diets. The total ash content ranged from 35.22, 172.95, 305.43 and 437.12 g/kg respectively, whereas the content of calcium and phosphorus ranged from 3.77, 5.71, 9.27 and 11.88 and 3.05, 4.23 3.67 and 2.76 g/kg respectively.

The concentration of blood calcium and blood phosphorus, especially short time post feeding, did not show any different between the treatments. This might be due to the fact that the mineral contained in the DFR, especially the silica (SiO_2) which account for 89.2 to 92.8% of total diatomite (EAGLE-PICHER, 1988) have no interaction with other mineral during the metabolism neither in the digestive tract nor at the circulation system level, because the chemical properties of the diatomite is completely inert, non resorbable, non swelling and non toxic (SCHULENBERG and RABELING, 1996). An impact of the higher mineral content in diet on blood calcium and blood phosphorus content within short time, therefore, can not be observed.

The difference in blood phosphorus concentration at 8 hours post feeding might not only be ground on the fact that the content of phosphorus in the diet is different but it might also be ground on the moderately high level of DFR in the 60% DFR inclusion diet. The higher DFR

containing diets may have lower density than those contained with higher DFR inclusion levels. This is due to the diatomite has small particle size, bulky, very light and porosity (EAGLE-PICHER, 1988). In the ruminants, it is well documented that the rate of phosphorus secretion from the salivary gland was affected by the physical characteristic of diets (SCOTT and BUCHAN, 1985). The diatomite, although its chemical effect is inert shows some physiological activity when it is used in the diet (SCHULENBERG and RABELING, 1996). The small particle size may cause a higher out flow rate in the GI tract of the cattle. This may cause in lower secretion of phosphorus, especially, long time post feeding. The blood phosphorus content at 8 hours post feeding of the animals fed on a diet containing higher DFR inclusion levels (60% DFR), therefore, is lower ($P<0.05$) than that of others.

4.5 Conclusion

Inclusion of the DFR in cattle diets up to 60% level did not cause any adverse effects on fermentation or blood parameters. High mineral content in the DFR did not show interference effects with the rumen fermentation and metabolism of calcium and phosphorus in the blood circulation. This might be ground on the fact that the chemical properties of the diatomite is completely inert, non resorbable, non swelling and non toxic. These current results indicate that DFR can be used in the cattle diets at the optimum level without any adverse effects in rumen fermentation or blood parameter levels.

5. EXPERIMENT 2. EFFECT OF RESTRICTED FEEDING OF A BASAL DIET CONTAINING DIFFERENT LEVELS OF DIATOMITE FILTER AID RESIDUE ON FEEDLOT PERFORMANCE OF MALE CROSSBRED HOLSTEIN X THAI-INDIGENOUS CATTLE

5.1 Objectives

The objective of this study was to determine the effect of restricted feeding of diets containing different levels of diatomite filter aid residue on feedlot performance of male crossbred Holstein x Thai-indigenous cattle.

5.2 Materials and methods

5.2.1 Animals

Sixteen male Holstein x Thai-indigenous crossbred cattle averaging 191.20±16.68 kg initial body weight and 1.11±0.26 years initial age were used. The animals were born between November, 1994 and September, 1995 at the Rajamangala Institute of Technology, Nakhonsithammarat Campus, Thungsong District, Nakhonsithammarat Province, Thailand. All animals were vaccinated against Foot and Mouth Disease (FMD) and Haemorrhagic Septicaemia and were given anthelminthics and a Vitamin A, D_3 and E injection before the experiment.

5.2.2 Housing

The animals were housed in individual stalls of 1.4 m length, 1.4 m width and 1.2 m height. The stalls had separate feed troughs for both concentrate and roughage and automatic drinking water cups.

5.2.3 Diets

Four Isonitrogenous diets were formulated according to NRC (1980) recommendations to contain 148 g CP/kg DM (Table 5.1). DFR was included by 0, 20, 40 and 60% as in the previous experiment. All diets contained the same amount of soybean and peanut meal as protein sources. Other ingredients such as vitamin and minerals premix (RAMICAL®), normal salt, molasses,

urea, broken rice and palm kernel meal were used and were mixed with the same method as in the previous experiment. Feed was prepared every second day throughout the 84 days experimental period. Feed samples were collected every week.

5.2.4 Experimental procedure

5.2.4.1 Experimental design

The cattle were randomly allocated to the diets in a completely randomised design (CRD) (STEEL and TORRIE, 1981). There were four animals per treatment.

5.2.4.2 Feeding and management

The animals were fed with roughage and concentrate at 3% of body weight (BW) on dry matter basis. The concentrate to roughage ratio in the diet offered was maintained at 60: 40 respectively. The amount of feed offered was changed every second week based on the change in body weight of the animals. Feeds were offered to the animals twice a day at 07.30 and 16.30 hours. Light was kept on during the night. Refusals of both roughage and concentrate were weighed daily before new feed was offered. Net feed intake was calculated on a daily basis.

The animals were weighed three times at an interval of 12 hours before the experiment. The average body weight was used as the initial live body weight of each animal. The animals were weighed every two weeks at 07.00 hours. The final and initial weights were used to determine body weight gain.

5.2.5 Chemical analysis

The proximate analysis was carried out and calcium, phosphorus, sodium and potassium analyzed as described by NAUMANN and BASSLER (1976). All sample were analysed in triplicates. Gross energy measurement and Hohenheim gas test as described by MENKE *et al.* (1979) were also undertaken.

Table 5.1 Feed ingredients (g/kg DM) and chemical composition of the experimental rations containing either 0, 20, 40 or 60% diatomite filter aid residue (DFR).

	Diet			
	0% DFR	20% DFR	40% DFR	60% DFR
Ingredient				
Diatomite filter aid residue	0.00	200.00	400.00	600.00
Broken rice	600.00	0.00	0.00	0.00
Palmkernel meal	243.40	647.30	441.90	236.40
Urea[1]	6.60	2.70	8.10	13.60
Soybean meal	30.00	30.00	30.00	30.00
Peanut meal	80.00	80.00	80.00	80.00
Molasses	20.00	20.00	20.00	20.00
Vitamin-premix [2]	15.00	15.00	15.00	15.00
Normal salt	5.00	5.00	5.00	5.00
Total	1000.00	1000.00	1000.00	1000.00
Crude protein (calculated)	148.00	148.00	148.00	148.00
Chemical analysis				
Dry matter[3]	874.78	902.51	847.26	818.40
Organic matter	950.59	840.81	723.86	615.98
Crude protein	147.05	146.67	145.78	143.36
Crude fibre	70.09	180.30	153.92	107.39
Ether extract	19.76	43.66	37.17	31.63
Crude ash	49.41	159.19	276.14	384.02
Nitrogen free extract	713.70	470.19	387.00	333.60
Calcium	4.11	8.14	11.41	12.99
Phosphorus	3.14	4.71	4.25	3.30
Sodium	2.01	3.16	5.25	4.98
Potassium	5.15	4.98	2.28	1.03
Organic matter digestibility[4]	706.87	499.12	445.17	439.27
Gas production (ml/200mg DM)	61.67	37.50	30.58	29.14
Gross energy (MJ/kg)	18.14	17.72	16.03	13.86
Metabolisable energy (MJ/kg)[5]	10.87	7.04	5.84	5.51

DFR: Diatomite filter aid residue.
[1] Contained 46% Nitrogen.
[2] Contained Vitamin A, 1.250.000 iu; Vitamin D_3, 250.000 iu; Vitamin E, 1.000 iu; Zinc, 5.200 mg; Iron, 2.250 mg; Manganese, 2.500 mg; Copper, 1.000 mg; Cobalt, 60 mg; Iodine, 80 mg; Selenium, 25 mg and Sodium, 20 mg.
[3] Expressed as g/kg at air dry basis.
[4,5] Calculated according to Close and Menke (1986) on page 134.

5.2.6 Statistical analysis

The collected data were analysed using the general linear model procedure (SAS, 1988). Duncan's new multiple range test (DMRT) was used to compare differences between treatment means.

5.3 Results

5.3.1 Intake

Average DM intakes of concentrate, roughage and total feed are shown in Table 5.2. For concentrate, total dry matter intake, average dry matter intake/day and average dry matter intake as percentage of body weight were not significantly different across treatments ($P>0.05$), whereas average dry matter intake as percent of the metabolic body weight ($BW^{0.75}$) was significantly different across treatments ($P<0.01$). When concentrate intake was expressed as percentage of body weight, the intake reached the maximum level of feed offered in every treatment, which was calculated at 1.80 per cent of body weight, except for the cattle fed the diet with 60% DFR where the intake level was lower than 1.80 per cent.

The roughage intake, when expressed as total dry matter and average dry matter intake/day was significantly different ($P<0.05$), whereas it was highly significant different ($P<0.01$), when it was expressed as percentage of body weight and as percentage of metabolic body weight ($BW^{0.75}$). The cattle fed on 0% DFR had the highest ($P<0.05$) total dry matter intake and average dry matter intake/day whereas the cattle fed on 60% DFR had the highest ($P<0.01$) dry mater intake when expressed on percentage of body weight and percentage of metabolic body weight ($BW^{0.75}$).

Total feed intake expressed as total DM and average DM/day was not significantly different ($P> 0.05$), whereas total feed intake expressed as percentage of body weight and of metabolic body weight ($BW^{0.75}$) were highly significantly different ($P<0.01$). Inclusion of higher levels of DFR in feed, therefore, increase total feed intake expressed as percentage of body weight and of metabolic body weight ($BW^{0.75}$) whereas the concentrate to roughage ratio decreased.

Table 5.2 Average intake of concentrate, roughage and total feed of male Holstein x Thai-indigenous crossbred cattle offered rations containing either 0, 20, 40 or 60% diatomite filter aid residue (DFR).

Parameter	Diet				
	0% DFR	20% DFR	40% DFR	60% DFR	SEM
Number of animals	4	4	4	4	
Feeding Period (days)	84	84	84	84	
Concentrate intake (kg)					
Total dry matter intake	350.71	329.40	338.34	275.22	30.48
Dry matter intake/day	4.18	3.92	4.03	3.28	0.36
Dry matter intake as %BW/day	1.87	1.85	1.86	1.79	0.03
Dry matter intake g/$BW^{0.75}$/day	72.10^{A}	70.39^{AB}	71.02^{AB}	65.45^{B}	1.93
Roughage intake (kg)					
Total dry matter intake	202.79^{a}	124.99^{b}	163.67^{ab}	184.64^{ab}	20.47
Dry matter intake/day	2.42^{a}	1.50^{b}	1.95^{ab}	2.20^{ab}	0.24
Dry matter intake as %BW/day	1.08^{A}	0.71^{C}	0.88^{B}	1.20^{A}	0.05
Dry matter intake g/$BW^{0.75}$/day	41.64^{A}	26.82^{C}	33.84^{B}	43.92^{A}	2.18
Total feed intake (kg)					
Total dry matter intake	553.49	454.18	502.01	459.86	50.35
Dry matter intake/day	6.59	5.41	5.96	5.47	0.60
Dry matter intake as %BW/day	2.95^{A}	2.56^{B}	2.74^{B}	2.98^{A}	0.07
Dry matter intake g/$BW^{0.75}$/day	113.75^{A}	97.22^{B}	104.87^{AB}	109.36^{AB}	3.93
Concentrate to roughage dry matter intake ratio	1.74^{C}	2.67^{A}	2.13^{B}	1.49^{C}	0.11

Feed was limited to 3 per cent of animal live body weight over the feeding period.
$^{a, b, c, A, B, C}$ Means in the same row not having at least a common superscript differ significantly: $^{a, b, c}$ $P<0.05$, $^{A, B, C}$ $P<0.01$; DFR= Diatomite filter aid residue, SEM= Standard error of mean, BW= Live body weight in kg.

Organic matter, crude protein and metabolisable energy intakes are shown in Table 5.3. The organic matter and metabolisable energy intakes of concentrate across treatments were highly significantly different ($P<0.01$). The values decreased proportionally with increasing DFR in the diet, whereas the crude protein intake was not significantly different ($P>0.05$) across treatments. On the other hand, organic matter, crude protein and metabolisable energy intakes of roughage were significantly different ($P<0.05$).

Table 5.3 Profiles of average organic matter (g DM/day), protein (g DM/day) and energy (MJ ME/day) intakes of male Holstein x Thai-indigenous crossbred cattle fed rations containing either 0, 20, 40 or 60% diatomite filter aid residue (DFR).

Parameter	Diet				
	0% DFR	20% DFR	40% DFR	60% DFR	SEM
Number of animals	4	4	4	4	
Feeding period (days)	84	84	84	84	
Organic matter intake (g/day)					
Concentrate	3961.68^{A}	3262.33^{AB}	2910.18^{B}	2048.38^{C}	277.04
Roughage	2131.36^{a}	1313.69^{b}	1720.21^{ab}	1940.66^{ab}	215.17
Total	6093.04^{a}	4576.02^{ab}	4630.39^{ab}	3989.05^{b}	482.99
Crude protein intake (g/day)					
Concentrate	614.15	575.28	587.26	469.83	52.86
Roughage	89.56^{a}	55.20^{b}	72.29^{ab}	81.55^{ab}	9.04
Total	703.72	630.49	659.55	551.38	61.73
Metabolisable energy intake (MJ/day)					
Concentrate	45.38^{A}	27.60^{B}	23.31^{BC}	18.04^{C}	2.55
Roughage	15.38^{a}	9.47^{b}	12.41^{ab}	14.00^{ab}	1.55
Total	60.76^{A}	37.08^{B}	35.73^{B}	32.05^{B}	3.99
Crude protein/energy intake ratio					
(g CP/kg: MJ ME/kg)	11.58	17.01	18.57	17.21	

Feed was limited to 3 per cent of the animal's live body weight (DM) over the feeding period; Organic matter, crude protein and metabolisable energy intake were calculated from the organic matter, crude protein and metabolisable energy content of the diets as shown in Table 5.1.
$^{a, b, c, A, B, C}$ Means in the same row not having at least a common superscript differ significantly: $^{a, b, c}$ $P<0.05$, $^{A, B, C}$ $P<0.01$; DFR= Diatomite filter aid residue, SEM= Standard error of mean, BW= Live body weight in kg.

Crude ash and minerals intakes are shown in Table 5.4. Total minerals, calcium, phosphorus, sodium and potassium intakes obtained from concentrate were highly significantly different ($P<0.01$) across treatments and the intakes from roughage significantly different ($P<0.05$) across treatments. The intake ratio of calcium to phosphorus was higher by higher level of DFR inclusion.

Table 5.4 Profiles of average mineral intake (g DM/day) of male Holstein x Thai-indigenous crossbred cattle fed rations containing either 0, 20, 40 or 60% diatomite filter aid residue (DFR).

Parameter	Diet				
	0% DFR	20% DFR	40% DFR	60% DFR	SEM
Number of animals	4	4	4	4	
Feeding Period (days)	84	84	84	84	
Crude ash intake (g/day)					
Concentrate	206.20^{C}	624.30^{B}	1112.10^{A}	1258.10^{A}	96.96
Roughage	284.87^{a}	175.58^{b}	229.92^{ab}	259.38^{ab}	28.77
Total	491.10^{B}	799.90^{B}	1342.02^{A}	1517.50^{A}	125.73
Calcium intake (g/day)					
Concentrate	17.11^{C}	31.76^{B}	45.92^{A}	42.59^{AB}	3.80
Roughage	7.24^{a}	4.46^{b}	5.84^{ab}	6.59^{ab}	0.72
Total	24.36^{C}	36.22^{BC}	51.76^{A}	49.19^{AB}	4.51
Phosphorus intake (g/day)					
Concentrate	12.94^{B}	18.43^{A}	19.33^{A}	10.81^{B}	1.54
Roughage	1.93^{a}	1.19^{b}	1.56^{ab}	1.76^{ab}	0.19
Total	14.87^{bc}	19.62^{ab}	20.89^{a}	12.57^{c}	1.73
Sodium intake (g/day)					
Concentrate	8.35^{C}	12.55^{BC}	20.94^{A}	16.38^{AB}	1.63
Roughage	0.96^{a}	0.59^{b}	0.78^{ab}	0.88^{ab}	0.10
Total	9.31^{C}	13.14^{BC}	21.72^{A}	17.26^{AB}	1.73
Potassium intake (g/day)					
Concentrate	21.71^{A}	19.61^{A}	9.26^{B}	3.27^{C}	1.19
Roughage	52.14^{a}	32.14^{b}	42.09^{ab}	47.48^{ab}	5.26
Total	73.85^{a}	51.75^{b}	51.35^{b}	50.75^{b}	6.24
Total calcium : phosphorus intake ratio	1.63^{D}	1.84^{C}	2.47^{B}	3.91^{A}	0.007

Feed was limited to 3 per cent of the animal's live body weight (DM) over the feeding period; Nutrient intake was calculated from nutrient content values in Table 5.1.

$^{a, b, c, A, B, C}$ Means in the same row not having at least a common superscript differ significantly: $^{a, b, c}$ $P<0.05$, $^{A, B, C}$ $P<0.01$; DFR= Diatomite filter aid residue, SEM= Standard error of mean.

Table 5.5 Average bodyweight gain and feed efficiency of male Holstein x Thai-indigenous crossbred cattle fed rations containing either 0, 20, 40 or 60% diatomite filter aid residue (DFR).

Parameter	Diet				
	0% DFR	20% DFR	40% DFR	60% DFR	SEM
Number of animals	4	4	4	4	
Feeding period (days)	84	84	84	84	
Weight change (kg)					
Initial weight	200.80	190.68	201.82	171.55	16.68
Final weight	266.25	251.25	242.50	210.75	25.84
Weight gain	65.45	60.58	40.67	39.20	10.64
Average daily gain	0.78	0.72	0.48	0.47	0.13
Feed conversion ratio					
Concentrate	5.75	5.76	9.73	7.62	1.21
Roughage	3.32^{ab}	2.22^{b}	4.55^{a}	5.11^{a}	0.63
Total feed	9.06^{ab}	7.97^{b}	14.28^{a}	12.73^{ab}	1.82

Feed was limited to 3 per cent of the animal's live body weight (DM) over the feeding period.
[a, b, c,] Means in the same row not having at least a common superscript differ significantly at P< 0.05, DFR= Diatomite filter aid residue, SEM= Standard error of mean.

5.3.2 Weight gain and feed conversion ratio

Data on weight gain and feed conversion ratio is shown in Table 5.5. The initial weight, final weight, weight gain and average daily gain of the animals were not significantly different (P> 0.05) across treatments. Feed conversion ratio for roughage and total feed was significantly different (P<0.05), whereas it was not significantly different (P>0.05) for concentrate.

5.4 Discussion

The dry matter intake of the concentrate was 1.87, 1.85, 1.86 and 1.79% BW, respectively and there are no significant difference ($P>0.05$) across treatments. This indicates that various level of DFR in the diet did not influence the palatability of the rations and there is no obstruction for DFR to be used as a component in the diet for cattle. The roughage intake, on the other hand, was significant different ($P<0.05$) across treatments, when it was expressed as average dry matter intake/day and was highly significant different ($P<0.01$) across treatments, when it was expressed as %BW and $BW^{0.75}$. The trend was that roughage intake increased with increasing DFR inclusion level. For the cattle fed on 60% DFR inclusion level all offered roughage was consumed. This might mean that the amount of energy contained in the concentrate was not enough both for maintenance and for body weight gain for the cattle when the amount offered was limited at about 1.8% BW. The cattle therefore search to cover the deficient portion of their required energy from the roughage in order to meet their requirement. The roughage intake of the cattle fed on the diets containing higher DFR inclusion level, therefore was higher than that of the cattle fed on diets containing lower DFR inclusion level. This shows the influence of energy content within the diet on the voluntary feed intake and that the roughage intake of the ruminants was partly regulated by energy content of the diet (FORBES, 1986; NRC, 1987).

When the metabolisable energy content in the experimental diet (Table 5.1) is considered, it is found that the diets containing a higher DFR inclusion level contained a lower amount of metabolisable energy than the diets containing a lower DFR inclusion level. Therefore, when the concentrate was limited at 1.80% BW, the energy content derived from the concentrate is insufficient. The cattle which was offered diets containing higher DFR inclusion levels, therefore consumed more roughage than the cattle fed on diets containing lower DFR inclusion levels.

Whereas the amount of concentrate is similar for all treatments, the total feed intake when it was expressed as %BW and as $\%BW^{0.75}$ were highly significant different across treatments ($P<0.01$). This phenomena is based on the fact that the cattle fed on the diets containing higher DFR inclusion levels have a higher roughage intake. The total feed intake expressed as %BW and as $\%BW^{0.75}$, therefore was highly significantly different ($P<0.01$).

The intake of the organic matter, crude protein and metabolisable energy (Table 5.3), total ash as well as some minerals (Table 5.4) varied according to concentrate and roughage intake. Because the organic matter content of the diets containing higher DFR inclusion levels was lower than of the diets containing lower DFR inclusion levels, the organic matter intake of the cattle fed on those diets was lower and had a similar tendency as the organic matter content of the concentrate.

The body weight gain and average daily gain (ADG) (Table 5.5) of the cattle were not significant different across treatment ($P>0.05$), whereas the feed conversion ratio was significant different across treatments ($P<0.05$). The cattle fed on diets containing lower DFR inclusion levels tended to have a better growth performance than the cattle fed on diets containing higher DFR inclusion levels. This evidence might relate to the difference on nutrient content among the diets, especially the amount of metabolisable energy supplied to the cattle in each group. In Table 5.2, it can be seen that the control diet contains more metabolisable energy than the diets of the other treatment groups. It is found that limitation of the metabolisable energy intake might impair overall feedlot performance of the cattle (SAINZ *et al.*, 1995). Metabolisable energy is one of the most important components used as criteria for the determination of the body weight gain of the growing and finishing cattle. The cattle with higher weight gain will be offered the diets containing higher metabolisable energy content and vice versa (NRC, 1976). The cattle fed on the control diet which contained a higher metabolisable energy content, therefore, had more weight gain than the cattle in the other groups.

5.5 Conclusion

The different DFR contents of the diets didn't show any obstruction on feed intake. Due to the fact that the rations were limited and the diet was lower in metabolisable energy content, the cattle fed on diets containing higher DFR inclusion level, had a higher roughage intake than the cattle fed on diets containing lower DFR inclusion level. The cattle fed on diets containing a lower DFR inclusion level tended to have better growth performance and feed efficiency than the cattle fed on diets containing higher DFR inclusion level.

6. EXPERIMENT 3. EFFECT OF *AD LIBITUM* FEEDING OF A BASAL DIET CONTAINING DIFFERENT LEVELS OF DIATOMITE FILTER AID RESIDUE IN FEED ON FEEDLOT PERFORMANCE OF MALE CROSSBRED HOLSTEIN-THAI-INDIGENOUS CATTLE

6.1 Objectives

The objective of this study was to determine the effect of *ad libitum* feeding of diets containing different levels of diatomite filter aid residue on feedlot performance of male crossbred Holstein x Thai-indigenous cattle.

6.2 Materials and methods

6.2.1 Animals

Sixteen male Holstein x Thai-indigenous crossbred cattle averaging 194.81±21.06 kg initial body weight and 1.06±0.13 years initial age were used. The animal were born between February, 1995 and January, 1996 at the Co-operative of Dairy Production, Phatew District, Chumporn, 86160, Thailand. All animals were vaccinated against Foot and Mouth disease and Haemorrhagic Septicaemia and were given anthelminthics and a Vitamin A, D_3 and E injection before the experiment.

6.2.2 Housing

The animals were housed in individual stalls similar to the stalls used in experiment 2.

6.2.3 Diets

Four isonitrogenous diets were formulated according to NRC (1980) recommendations to contain 148 g CP/kg DM. Diatomite filter aid residue (DFR) was included at four levels varying from 0-60 per cent. Soybean meal was used as protein source. Premix (RAMICAL®) and normal salt were used as sources of vitamins and minerals. Molasses was used as a feed appetiser. Urea was used to adjust the level of crude protein in the rations. Palm kernel meal was

used for the substitution of diatomite filter aid residue in the feed. For the control diet, broken rice was used as an energy source instead of DFR. Urea was ground and mixed with part of the palmkernel meal, soybean meal, RAMICAL® as well as normal salt. This was to prevent urea from aggregating and also to facilitate the dispersion of vitamins and minerals within the diet. The diatomite filter aid residue was dried in a hot air oven at 105°C for 24 hours before being used in the rations. Palmkernel meal was ground with a hammer mill using a sieve with a diameter of 4 millimetres. All of the feed ingredients were mixed together using a horizontal feed mixture bin. Molasses was added during mixing. Feed was prepared every second day throughout the experiment. The diets used in this experiment is shown in Table 6.1.

6.2.4 Experimental procedure

6.2.4.1 Experimental design

The cattle were randomly allocated to the diets in a completely randomised design (CRD) (STEEL and TORRIE, 1981). There were four animals per treatment.

6.2.4.2 Feeding and management

The animals were fed both concentrate and roughage *ad libitum*. Feeds were offered to the animals twice a day at 07.30 and 16.30 hours. Light was kept on during the night. Refusals of both roughage and concentrate were weighed daily before new feed was offered. The animals were weighed three times at an interval of 24 hours before the experiment. The average was used as the initial live body weight of each animal. The animals were weighed every two weeks at 07.00 hours. The final and initial weights were used to determine body weight changes. Net feed intake was calculated on a daily basis.

6.2.5 Feed analysis

The proximate analysis was carried out and calcium, phosphorus, sodium and potassium analysed as described by NAUMANN and BASSLER (1976). All sample were analysed in triplicates. Gross energy measurement and Hohenheim gas test as described by MENKE *et al.* (1979) were also undertaken.

Table 6.1 Feed ingredients (g/kg DM) and chemical composition of the experimental rations containing either 0, 20, 40 or 60% diatomite filter aid residue (DFR).

	Diet			
	0% DFR	20% DFR	40% DFR	60% DFR
Ingredient				
Diatomite filter aid residue	0.00	200.00	400.00	600.00
Broken rice	600.00	0.00	0.00	0
Palmkernel meal	333.30	750.80	542.60	326.30
Urea[1]	16.70	15.00	20.00	23.70
Soybean meal	20.00	4.20	7.40	20.00
Molasses	20.00	20.00	20.00	20.00
Vitamin-premix[2]	5.00	5.00	5.00	5.00
Normal salt	5.00	5.00	5.00	5.00
Total	1000.00	1000.00	1000.00	1000.00
Crude protein (calculated)	148.00	148.00	148.00	148.00
Chemical analysis				
Dry matter[3]	879.03	896.88	852.04	801.35
Organic matter	964.78	827.05	694.57	562.88
Crude protein	147.86	136.05	134.73	125.22
Crude fibre	92.12	219.23	162.13	122.06
Ether extract	22.74	49.07	40.23	34.44
Crude ash	35.22	172.95	305.43	437.12
Nitrogen free extract	702.06	422.70	357.48	281.16
Calcium	3.77	5.71	9.27	11.88
Phosphorus	3.05	4.23	3.67	2.76
Sodium	1.71	3.45	4.44	3.91
Potassium	4.43	2.87	0.67	0.43
Organic matter digestibility[4]	677.27	485.67	489.07	376.83
Gas production (ml/200mg DM)	58.44	35.94	35.36	21.82
Gross energy (MJ/kg)	18.45	17.05	14.63	12.65
Metabolisable energy (MJ/kg)[5]	10.38	6.78	6.57	4.31

DFR: Diatomite filter aid residue.
[1] Contained 46% Nitrogen.
[2] Contained Vitamin A, 1.250.000 iu; Vitamin D_3, 250.000 iu; Vitamin E, 1.000 iu; Zinc, 5.200 mg; Iron, 2.250 mg; Manganese, 2.500 mg; Copper, 1.000 mg; Cobalt, 60 mg; Iodine, 80 mg; Selenium, 25 mg and Sodium, 20 mg.
[3] Expressed as g/kg at air dry basis.
[4,5] Calculated according to Close and Menke (1986) on page 134.

6.2.6 Statistical analysis

The collected data were analysed using the general linear model procedure (SAS, 1988). Analysis of variance (ANOVA) was undertaken. Duncan's new multiple range test was used to compare differences between treatment means.

6.3 Results

6.3.1 Intake

Average dry matter intakes of concentrate, roughage and the total feed are shown in Table 6.2. Total dry matter intake, average dry matter intake/day and average dry matter intake as percentage of body weight of concentrate were significantly different ($P<0.05$) across treatments, whereas average dry matter intake as percent metabolic body weight ($BW^{0.75}$) was highly significantly different ($P<0.01$) across treatments. The cattle fed on 60% DFR had highest concentrate dry matter intake. Average dry matter intake of concentrate when expressed either as total dry matter intake, average dry matter intake/day, average dry matter intake as percentage body weight or of metabolic body weight ($BW^{0.75}$) increased proportionately with increasing amount of DFR in the diet.

Average dry matter intake of roughage was not significantly different ($P>0.05$) between treatment means either when expressed as total dry matter intake, average dry matter intake/day, average dry matter intake as %BW or average dry matter intake on metabolic body weight ($BW^{0.75}$). The cattle fed on 40% DFR had the lowest ($P>0.05$) average dry matter intake of roughage, whereas the cattle from the group fed on 60% DFR had the highest.

Average intake of total feed when expressed as total dry matter intake and as average dry matter intake/day were significantly different ($P<0.05$) across treatment means, whereas it was highly significantly different ($P<0.01$) when expressed as average dry matter intake as percent of body weight (%BW) and of metabolic body weight ($BW^{0.75}$). The cattle fed on 60% DFR had the highest average dry matter intake either when expressed as total dry matter intake, average dry matter intake/day, average dry matter intake as %BW or average dry matter intake on percentage of metabolic body weight ($\%BW^{0.75}$).

Table 6.2 Average intake of concentrate, roughage and total feed of male Holstein x Thai-indigenous crossbred cattle fed rations containing either 0, 20, 40 or 60% diatomite filter aid residue (DFR).

Parameter	Diet				
	0% DFR	20% DFR	40% DFR	60% DFR	SEM
Number of animals	4	4	4	4	
Feeding period (days)	50	50	50	50	
Average concentrate intake (kg)					
Total dry matter intake	178.09^{c}	205.43^{bc}	271.16^{ab}	291.34^{a}	24.66
Dry matter intake/day	3.56^{c}	4.11^{bc}	5.42^{ab}	5.82^{a}	0.49
Dry matter intake as %BW/day	1.75^{c}	2.07^{bc}	2.75^{ab}	2.82^{a}	0.23
Dry matter intake g/$BW^{0.75}$/day	66.21^{B}	77.76^{B}	103.22^{A}	107.18^{A}	8.07
Average roughage intake (kg)					
Total dry matter intake	47.71	51.93	33.79	56.87	15.16
Dry matter intake/day	0.96	1.04	0.68	1.14	0.30
Dry matter intake as %BW/day	0.46	0.45	0.33	0.50	0.10
Dry matter intake g/$BW^{0.75}$/day	17.42	17.19	12.24	19.23	3.97
Average total feed intake (kg)					
Total dry matter intake	225.90^{b}	257.36^{ab}	304.96^{ab}	348.21^{a}	30.08
Dry matter intake/day	4.52^{b}	5.15^{ab}	6.10^{ab}	6.97^{a}	0.60
Dry matter intake as %BW/day	2.22^{C}	2.51^{BC}	3.08^{AB}	3.32^{A}	0.19
Dry matter intake g/$BW^{0.75}$/day	83.63^{C}	94.95^{BC}	115.47^{AB}	126.42^{A}	7.12
Concentrate to roughage dry matter intake ratio	4.05	5.74	8.48	6.63	1.41

Animals were fed *ad libitum* (10-20% over requirement) throughout the feeding period.
$^{a, b, c, A, B, C}$ Means in the same row not having at least a common superscript differ significantly : $^{a, b, c}$ $P<0.05$, $^{A, B, C}$ $P<0.01$; DFR= Diatomite filter aid residue, SEM= Standard error of mean, BW= Live body weight in kg.

The concentrate to roughage ratio of intaked feed ranged between 4.05 for cattle group fed on 0% DFR and 8.48 for cattle group fed on 40% DFR.

Organic matter, crude protein and metabolisable energy intakes are shown in Table 6.3. Organic matter, crude protein, and metabolisable energy intakes either for concentrate, roughage or total

Table 6.3 Average intake of organic matter (g DM/day), crude protein (g DM/day) and energy (MJ ME/day) of male Holstein x Thai-indigenous crossbred cattle fed rations containing either 0, 20, 40 or 60% diatomite filter aid residue (DFR).

Parameter	Diet				
	0% DFR	20% DFR	40% DFR	60% DFR	SEM
Number of animals	4	4	4	4	
Feeding period (days)	50	50	50	50	
Organic matter intake (g/day)					
Concentrate	3438.41	3398.30	3767.04	3279.94	366.29
Roughage	843.46	918.09	597.43	1005.43	268.28
Total	4281.86	4316.38	4364.47	4285.37	476.05
Crude protein intake (g/day)					
Concentrate	527.09	558.78	730.52	729.53	66.05
Roughage	35.40	38.53	25.07	42.20	11.25
Total	562.50	597.32	755.60	771.72	68.08
Metabolisable energy intake (MJ/day)					
Concentrate	36.99^{a}	27.86^{ab}	35.63^{ab}	25.06^{b}	3.33
Roughage	6.07	6.61	4.30	7.24	1.93
Total	43.07	34.47	39.93	32.30	4.07
Crude protein/energy intake ratio					
(g CP/kg:MJ ME/kg)	13.08^{C}	17.46^{B}	18.94^{B}	24.03^{A}	0.76

Animals were fed *ad libitum* (10-20% over requirement) over the feeding period; Organic matter, crude protein and metabolisable energy intake were calculated from the organic matter, crude protein and metabolisable energy content of the diets as shown in Table 6.1.
$^{a, b, c, A, B, C}$ Means in the same row not having at least a common superscript differ significantly : $^{a, b, c}$ $P<0.05$, $^{A, B, C}$ $P<0.01$; DFR= Diatomite filter aid residue, SEM= Standard error of mean, BW= Live body weight in kg.

feed were not significantly different ($P>0.05$) across treatments except metabolisable energy intake of concentrate. The cattle fed on the control diet had the highest ($P<0.05$) metabolisable energy intake, whereas those fed on 60% DFR had the lowest metabolisable energy intake.

The ratio between crude protein and metabolisable energy intake for each group increased proportionately with increasing DFR in the feed and ranged from 13.08 for the control group to 24.03 for the cattle fed on 60% DFR group.

Crude ash and mineral intakes are shown in Table 6.4 Total minerals, calcium, sodium and potassium intakes obtained from concentrate were highly significantly different ($P<0.01$) and significantly different ($P<0.05$) across treatments for phosphorus. Mineral intakes from roughage were not significantly different ($P>0.05$) across treatment except for crude ash ($P<0.05$). The ratio between calcium and phosphorus widened with higher inclusion level of DFR in the diet.

6.3.2 Weight change and feed conversion ratio

Data on weight change and feed conversion ratio is shown in Table 6.5. The initial weight, final weight, weight gain and average daily gain of the animals were not significantly different ($P> 0.05$) across treatments. Feed conversion ratio for concentrate and total feed was significantly different ($P<0.05$), whereas it was not significantly different ($P>0.05$) for roughage.

Table 6.4 Profiles of average ash and minerals intake (g DM/day) of male Holstein x Thai-indigenous crossbred cattle fed rations containing either 0, 20, 40 or 60% diatomite filter aid residue (DFR).

Parameter	Diet				
	0% DFR	20% DFR	40% DFR	60% DFR	SEM
Number of animals	4	4	4	4	
Feeding period (days)	50	50	50	50	
Crude ash intake (g/day)					
Concentrate	125.50^{D}	710.40^{C}	1656.30^{B}	2546.90^{A}	154.01
Roughage	108.50^{b}	335.00^{ab}	299.50^{ab}	663.30^{a}	127.01
Total	233.90^{D}	1045.40^{C}	1955.80^{B}	3210.20^{A}	204.25
Calcium intake (g/day)					
Concentrate	13.54^{C}	23.42^{C}	50.44^{B}	69.34^{A}	4.42
Roughage	2.86	3.11	2.03	3.41	0.91
Total	16.40^{C}	26.53^{C}	52.47^{B}	72.75^{A}	4.57
Phosphorus intake (g/day)					
Concentrate	10.69^{b}	17.26^{a}	20.06^{a}	15.73^{ab}	1.67
Roughage	0.76	0.83	0.54	0.90	0.24
Total	11.45^{b}	18.09^{a}	20.61^{a}	16.64^{ab}	1.70
Sodium intake (g/day)					
Concentrate	6.06^{C}	13.97^{B}	23.86^{A}	22.72^{A}	1.76
Roughage	0.38	0.41	0.27	0.45	0.12
Total	6.44^{C}	14.38^{B}	24.13^{A}	23.18^{A}	1.77
Potassium intake (g/day)					
Concentrate	15.68^{A}	11.91^{B}	3.79^{C}	2.33^{C}	1.19
Roughage	20.61	22.43	14.60	24.57	6.55
Total	36.29	34.34	18.39	26.90	6.78
Total calcium: phosphorus intake ratio	1.42^{C}	1.46^{C}	2.54^{B}	4.36^{A}	0.03

Animals were fed *ad libitum* (10-20% over requirement) throughout the feeding period. Nutrient intake was calculated from nutrient content values in Table 6.1.

$^{a, b, c, A, B, C}$ Means in the same row not having at least a common superscript differ significantly: $^{a, b, c}$ $P<0.05$, $^{A, B, C}$ $P<0.01$; DFR= Diatomite filter aid residue, SEM= Standard error of mean.

Table 6.5 Average body weight gain and feed efficiency of male Holstein x Thai-indigenous crossbred cattle fed rations containing either 0, 20, 40 or 60% diatomite filter aid residue (DFR).

Parameter	Diet				
	0% DFR	20% DFR	40% DFR	60% DFR	SEM
Number of animals	4	4	4	4	
Feeding period (days)	50	50	50	50	
Weight change (kg)					
Initial weight	190.75	195.25	191.25	202.00	21.06
Final weight	229.67	222.25	224.25	225.83	23.21
Weight gain	38.92	27.00	33.00	23.83	5.93
Average daily gain	0.78	0.54	0.66	0.48	0.12
Feed conversion ratio					
Concentrate	5.34^{b}	8.82^{ab}	8.36^{ab}	13.27^{a}	1.80
Roughage	1.53	1.82	1.07	2.43	0.50
Total feed	6.87^{b}	10.64^{ab}	9.42^{b}	15.70^{a}	1.94

Animals were fed *ad libitum* (10-20% over requirement) over the feeding period.
$^{a, b, c, A, B, C}$ Means in the same row not having at least a common superscript differ significantly: $^{a, b, c}$ $P<0.05$, $^{A, B, C}$ $P<0.01$; DFR= Diatomite filter aid residue, SEM= Standard error of mean.

6.4 Discussion

When the animals were fed *ad libitum*, the concentrate intake (Table 6.2) increased with increasing DFR in the diets. This result might relate to the content of energy contained in the diets. It is generally accepted that both digestible energy and crude protein content of a diet control the voluntary feed intake (FORBES, 1986; NRC, 1987). This mean that the feed intake is directed towards a constant intake of digestible energy and protein. The results of this study shows that the dry matter intake of concentrate increased with decreasing metabolisable energy content of the diet. This result have the same tendency as a previous report by DONEFER *et al.* (1963) who concluded that dry matter intake falls as the concentration of digestible energy increases.

Although the calculated protein content among the diets was the same (148 g/kg DM) the analytical value of crude protein content decreased with increasing DFR inclusion level (Table 6.2). Even though it is well documented that lower protein content of a feed depresses voluntary intake (ELLIOTT and TOPPS, 1963), the feed intake in this experiment increased with decreasing crude protein content of the diets. This might imply that the influence of the energy content on the voluntary feed intake was greater than the influence of crude protein content. Cattle group fed on the diets having a lower metabolisable energy content, therefore, had a higher dry matter intake.

Although the concentrate dry matter intake (Table 6.2) when expressed as average dry matter intake/day or as %BW and as $BW^{0.75}$ were significantly different ($P<0.05$) and highly significant different ($P<0.01$) across treatments, respectively, the total organic matter and total crude protein intake as well as total metabolisable energy intake (Table 6.3) were not significantly different ($P>0.05$) across treatments. This evidence is in line with previous studies where it was found that the voluntary feed intake of an animal was partly controlled by energy content of the diet (FORBES, 1986; NRC, 1987). The result also correspond with the results found in the previous experiment (experiment 2).

There are no significant difference ($P>0.05$) across treatment on roughage DM intake, although the cattle fed on the diet containing 60% DFR tend to have a higher roughage intake than the other groups. The total feed intake, however, was significant different across treatments ($P <0.05$) when it was expressed as average dry matter intake/day and as %BW and was highly significant ($P<0.01$) when it was expressed as $BW^{0.75}$. The influence of the roughage on the feed intake may be concealed by the influence of the concentrate intake. Therefore a significance across treatments of the total feed intake can be found similar to the result of the influence of the concentrate on dry matter intake.

The intake of the organic matter, crude protein, metabolisable energy (Table 6.3), total ash as well as some other minerals (Table 6.4) varied according to concentrate and roughage intake (Table 6.2). Although both crude protein and organic matter content of the diets containing higher DFR inclusion levels was lower than that of the diets containing lower DFR inclusion levels, the organic matter and crude protein intake among the cattle groups were not significant different ($P>0.05$). This is due to the fact that the animals fed on diets containing lower organic matter and crude protein content have a higher total feed intake than the cattle fed on diets containing lower DFR inclusion levels. The organic matter and crude protein intake among the groups of cattle, therefore, were not significant different ($P>0.05$).

The metabolisable energy intake from concentrate was significantly different across treatments ($P<0.01$). The diets containing higher DFR inclusion levels contained lower amounts of metabolisable energy, therefore, although feed intake of the animals of these groups was higher ($P<0.05$) than the control group, the metabolisable energy content obtained from the diets was lower ($P<0.05$). This shows that the feed intake was controlled by the metabolisable energy content of the diets. The animals will try to consume feed enough to meet their energy requirement (FORBES, 1986; NRC, 1987).

The ratio of crude protein and energy intake was highly significant ($P<0.01$) different across treatments. The ratio increases with increasing DFR inclusion levels. The increase in the ratio between crude protein and energy is due to the fact that both crude protein and energy content in the diets decreased with increasing DFR inclusion levels. However, the rate of decrease of metabolisable energy was greater than the crude protein decrease. The ratio of crude protein/energy intake, therefore increase with increasing DFR inclusion level.

The weight gain of the cattle of the different groups was not significant different ($P>0.05$) across treatments. The 14.80% crude protein content of the experimental diets is enough to provide a daily gain of the cattle of about 0.70 kg/day when the other nutrients also meet the requirements for this production (NRC, 1976). The average dairy gain of the cattle from each group, however, varied from 0.78, 0.54, 0.66 and 0.48 Kg/day, respectively. It is clearly indicated that growth performance of the cattle varied according to the concentration of the metabolisable energy intake. PAPSTEIN *et al.* (1991) performed feeding trails on growing Holstein Friesian bulls at low feeding levels and found that the growth and retention performance of the cattle were considerably influenced by the energy concentration of the ration and by daily energy intake.

Feed conversion ratio of the both concentrate and roughage as well as the total feed tend to increase with increasing the DFR inclusion levels. It is known that higher feed intake together with low weigh gain causes higher feed conversion ratio. The lower metabolisable energy content may be the most important factor influencing feed efficiency of the cattle in this experiment. Whereas the metabolisable energy content in the diet range from 10.38 to 4.31 MJ/kg of feed, respectively, the optimum amount of metabolisable energy required for growing beef cattle is 12 MJ/kg (MEISSNER *et al.,* 1995). The most limiting factor for the use of the DFR in cattle feed, therefore is it's energy content.

6.5 Conclusion

With *ad libitum* feeding, the animals fed on diets at any DFR inclusion level have a total dry matter intake higher than that of the cattle fed on the control diet. The cattle fed on a diet containing 40 and 60% DFR inclusion levels, moreover, have a total feed intake of more than 3% of their live body weight, the maximum level of feed offered in the previous experiments. Although the total feed intake was greater, the metabolisable energy intake of the animals fed on diets containing DFR tend to be lower than that of the animals fed on the control diet. This cause lower average daily gain and feed efficiency of the cattle fed on diets containing DFR at any inclusion level. Lowering the metabolisable energy content of the diets containing DFR at any inclusion level is the most disadvantageous feature on the use of DFR in cattle feed.

7. EXPERIMENT 4. RUMINAL DRY MATTER DEGRADABILITY OF FEEDS CONTAINING DIFFERENT LEVELS OF DIATOMITE FILTER AID RESIDUE

7.1 Objectives

The objective of this study was to evaluate the effect of different levels of diatomite filter aid residue in feed on ruminal dry matter degradability.

7.2 Materials and methods

7.2.1 Animals

Four rumen fistulated heifers were used. The animals were housed in conventional stanchions. Feed troughs and automatic drinking water cups were located at the front-end of each stanchion. The pens had concrete floors and were roofed. The animals were fed with commercial concentrate and rice straw throughout the experimental period.

7.2.2 Diets

Four isonitrogenous diets were formulated according to NRC (1980) recommendations to contain 148 g CP/kg DM. Diatomite filter aid residue (DFR) was included at four levels: 0, 30, 40 and 50 per cent. The diatomite filter aid residue was dried in a hot air oven at 105°C for 24 hours before being used in the rations. The proportions of the different ingredients and chemical composition of the experimental rations used is shown in Table 7.1.

7.2.3 Nylon bag technique equipment

The nylon bag technique used was modified from the method described by ØRSKOV and McDONALD (1979). 112 nylon bags of 7.5 cm width and 15 cm length were used. The bags have 28μ in pore size. All the bags were numbered at the upper-end and were soaked in distilled water overnight and then washed under running tap water. The bags were dried at 65°C in hot air oven for 48 hours and later cooled in desiccators and weighed.

Table 7.1 Feed ingredients (g/kg DM) and chemical composition of experimental rations containing either 0, 30, 40 or 50% diatomite filter aid residue (DFR) used for rumen degradability study.

	Diet			
	0% DFR	30% DFR	40% DFR	50% DFR
Ingredient				
Diatomite filter aid residue	0.00	300.00	400.00	500.00
Broken rice	300.00	0.00	0.00	0.00
Palmkernel meal	629.30	625.80	523.20	420.40
Urea[1]	10.70	14.20	16.90	19.60
Soybean meal	30.00	30.00	30.00	30.00
Molasses	20.00	20.00	20.00	20.00
Vitamin-premix [2]	5.00	5.00	5.00	5.00
Normal salt	5.00	5.00	5.00	5.00
Total	1000.00	1000.00	1000.00	1000.00
Crude protein (calculated)	148.00	148.00	148.00	148.00
Chemical analysis				
Dry matter[3]	884.73	866.55	852.41	842.75
Organic matter	953.77	758.38	707.36	627.12
Crude protein	156.86	167.09	161.24	151.37
Crude fibre	120.39	151.84	140.73	110.20
Ether extract	48.17	46.35	41.38	36.76
Crude ash	46.23	241.63	292.64	372.88
Nitrogen free extract	628.36	393.10	364.01	328.80
Calcium	3.61	7.83	8.88	10.87
Phosphorus	4.55	4.24	3.91	3.28
Sodium	1.89	4.38	4.47	4.10
Potassium	6.46	1.36	0.86	0.54
Organic matter digestibility[4]	657.06	495.80	497.52	444.24
Gas production (ml/200mg DM)	56.04	36.42	36.27	29.74
Gross energy (MJ/kg)	19.07	15.70	15.08	13.90
Metabolisable energy (MJ/kg)[5]	10.06	6.82	6.74	5.64

DFR: Diatomite filter aid residue.
[1] Contained 46% Nitrogen.
[2] Contained Vitamin A, 1.250.000 iu; Vitamin D_3, 250.000 iu; Vitamin E, 1.000 iu; Zinc, 5.200 mg; Iron, 2.250 mg; Manganese, 2.500 mg; Copper, 1.000 mg; Cobalt, 60 mg; Iodine, 80 mg; Selenium, 25 mg and Sodium, 20 mg.
[3] Expressed as g/kg at air dry basis.
[4,5] Calculated according to Close and Menke (1986) on page 134.

Feed samples were grinded through a 2 mm screen. About 5 g of the samples were weighed for the dry matter determination. 3 g of samples were weighed and transferred to the bags after they were taken from the desiccators and weighed. The bags were tied and attached to 28 about 50 cm long nylon cords using rubber bands which are resistant to the rumen micro-organism. To each cord was tied 4 bags with 4 different feed samples containing 4 different levels of DFR inclusion. The part of the cord with the samples was tied into a loop to assure that all samples have the same position in the rumen.

The bags containing the feed samples were placed deep into the rumen through the rumen fistula. The upper ends of the nylon cords were tied to the covers of the rumen fistulae. The bags were incubated for 2, 4, 8, 12, 24 and 48 hours. The sequential withdrawal method was used to withdraw the incubated samples from the rumen.

The bags which were attached to the nylon cords were immediately placed in a bucket of cold water to prevent further fermentation and to wash off feed particle adhering to the out side of the bags. Each bag was washed under running tab water while they were rubbed gently between thumb and fingers until the water was clear. The zero hour samples were soaked and washed together with the incubated samples. The washed bags were dried at 65°C for 48 hours, then allowed to cool down in the desiccators and weighed with the dried feed residue.

7.2.4 Experimental design

A Randomised Completed Block Design (RCBD) (STEEL and TORRIE, 1981) was assigned for this experiment. The treatments consist of 4 feed formulas containing four different levels of DFR inclusion (Table 7.1).

7.2.5 Proximate analysis

The proximate analysis was carried out and calcium, phosphorus, sodium and potassium analysed as described by NAUMANN and BASSLER (1976). All samples were analysed in triplicates. Gross energy measurement and Hohenheim gas test as described by MENKE *et al.* (1979) were also undertaken.

7.2.6 Statistical analysis

The data calculated from new way excel computer programs (CHEN,1997) were analysed using the general linear model procedure (SAS, 1988). Analysis of variance was undertaken. Duncan's new multiple range test was used to compare differences between treatment means.

7.3 Results

7.3.1 Degradation constant for dry matter

The pattern of dry matter degradation of the diets containing different levels of DFR inclusion is shown in Figure 7.1, while the degradation constant for dry matter of the diets containing different levels of DFR inclusion is shown in Table 7.2.

Dry matter degradation of the soluble fraction, the 'a' constant, was significantly different ($P < 0.05$) across treatments. The control diet has a lower dry matter degradation of soluble fraction ($P < 0.05$) than the other groups.

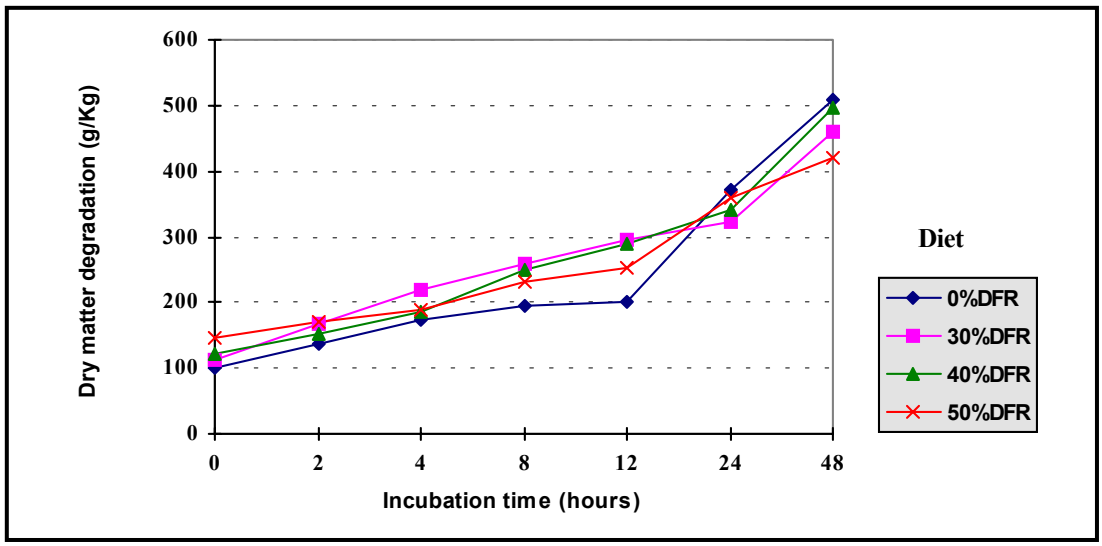

Figure 7.1 The pattern of dry matter degradation of diets containing different levels of diatomite filter aid residue incubated in nylon bags in the rumen of cows at different time periods.

Table 7.2 Least square means of rumen degradation (g DM/kg) of experimental feed containing either 0, 30, 40 or 50% diatomite filter aid residue (DFR).

Parameter	Diet				
	0%DFR	30%DFR	40%DFR	50%DFR	SEM
a	113.00^{b}	172.75^{a}	138.25^{b}	139.50^{b}	0.92
b	842.00^{a}	504.50^{b}	576.25^{b}	363.00^{b}	6.63
P (a+b)	955.00^{a}	677.25^{b}	714.50^{b}	502.50^{b}	6.88
c	0.0135^{b}	0.0248^{b}	0.0273^{ab}	0.0405^{a}	0.0039
ed1	451.50^{a}	401.25^{ab}	421.75^{a}	367.00^{b}	1.43
ed2	291.50	305.25	307.75	289.00	0.80
ed3	234.25^{b}	266.75^{a}	260.50^{a}	251.50^{ab}	0.69

Degradation constants derived from the Ørskov and McDonald (1979) equation $P= a + b (1-e^{-ct})$ where P is degradability at time 't', 'a', the rapidly soluble fraction, 'b', the potentially degradable fraction, 'c', the degradation rate of the 'b' fraction. Effective degradation in the rumen at 0.02, 0.05 and 0.08 fraction/h passage rate is represented by ed1, ed2 and ed3 respectively and is calculated by using the Excel Application Programs for processing feed degradability data written by Chen (1997).

$^{a, b, c,}$ Means in the same row not having at least one common superscript differ significantly ($P <0.05$), SEM = Standard error of mean.

The potential degradable fractions, 'b' values, of the diets containing different levels of DFR inclusion were significantly different ($P<0.05$) across treatments. The control diet has the highest ($P<0.05$) potential degradable fraction, while the diet with the maximum level of the DFR inclusion (50% DFR) has the lowest. The potential degradable fraction of the feed decreased when DFR increased.

The potential degradability ('a'+'b') of the diets containing different levels of DFR inclusion was significantly different ($P<0.05$) across treatments. The control diet has the highest ($P<0.05$) potential degradability. The potential degradability decreased when the DFR levels were increased. The trend of the potential degradability value of the diets was similar to the value of potential degradable fraction or the 'b' value.

Rate of degradation of the potential degradable fraction, the 'c' values, was significantly different ($P<0.05$) across treatments. The diet containing 50% DFR inclusion level had the highest rate of degradation of the potential degradable or the 'b' fraction, whereas the control

diet had the lowest. The rate of degradation of the potential degradable fraction increased proportionately with increasing DFR in the diets.

The effective degradation in the rumen at 0.02 fraction/hour passage rate (ed1) was significantly different ($P<0.05$) across treatments. The control diet had the highest ($P<0.05$) effective degradation in the rumen at 0.02 fraction/hour passage rate, whereas the diet with 50% DFR inclusion level had the lowest degradation. The effective degradation in the rumen at 0.02 fraction/hour passage rate decreased with increasing DFR inclusion level in the diet, while a significant different was not found for the effective degradation in the rumen at 0.05 fraction/hour passage rate (ed2). The diet with 40% DFR inclusion level had the highest effective degradation in the rumen at 0.05 fraction/hour passage rate but it was not significantly different across treatment means, whereas the effective degradation in the rumen at 0.08 fraction/hour passage rate (ed3) was significantly different ($P<0.05$) across treatments. The diet with 30% DFR inclusion level has highest ($P<0.05$) effective degradation in the rumen at 0.08 fraction/hour passage rate (ed3), whereas the control diet had the lowest.

7.4 Discussion

The nylon bag technique is a simple and low cost method where a rather large number of samples can be analysed in a short time (ØRSKOV *et al.,* 1980). Therefore it has been extensively used in the last decade as an indicator for estimation of the degradability of particular feed ingredients and diets. The dry matter degradability of such a substance varies dependent on various factors in the ruminal environment. These include the level of crude protein content of the feed (VOIGT and PIATKOWSKI, 1987), the grain content in the diet, the pore size of the bag (WEAKLEY *et al.,* 1983), the concentration of rumen ammonia (MEHREZ *et al.,* 1977; ERDMAN *et al.,* 1986; ODLE and SCHAEFER, 1987), particle size of the tested feed and type of feed sources (CERNEAU and MICHALET-DOREAU, 1991).

There is a discrepancy on the effect of ruminal ammonia nitrogen concentration on dry matter degradability determined by the nylon bag technique between different authors. Whereas MEHREZ *et al.* (1977) pointed out, that the minimal ammonia concentration for maximal rate of fermentation of the barley starch in the nylon bag was estimated at 23.50 mg/100 ml, ERDMAN *et al.* (1986) argued from his experiment that the concentration of rumen ammonia required for maximum degradation are not constant, but rather a function of the fermentability of

the diet with the equation: minimum ammonia concentration (mg/100ml) = 0.452 fermentability%-15.71. This function accounts for 50% of the variation in the minimum ammonia requirements. GRUMMER *et al.*(1984) found that concentration of ammonia nitrogen from 4.8 to 17.3 mg/100 ml did not effect the rates of dry matter and nitrogen disappearance from the polyester bags with soybean protein supplemented with 10.2 or 50.1% soluble nitrogen.

The results on dry matter degradability in the rumen of the diets from the present experiment indicates that the water soluble fraction (by other authors called the rapidly soluble fraction), the ´a` fraction, from the control diet are lower than the others. This might corresponds with the difference in chemical composition of the ingredients contained in the diet. It was concluded by ODLE and SCHAEFER (1987) that chemical or structural characteristics of feed ingredients influences the optimum level of ammonia concentration which determine the rate of dry matter degradation within the rumen. The not water soluble but potential rumen degradation fraction i.e. the ´b` fraction and the total potential ruminal degradation fraction i.e. the ´a+b` fraction of the control diet, however, was higher than other diets. This might be due to the fact that organic matter content in the control diet was higher than in the other diets. The ´b` and ´a+b` fraction, therefore were higher. It is generally accepted that diets with higher organic matter content provide higher potential degradability within the rumen. The effective degradability (ed) of the control diet at low passage rate (0.02 fraction/hour passage: ed1), was higher than the effective degradability of diets containing differing DFR inclusion levels, whereas at higher passage rate the higher DFR inclusion level tend to have a higher effective degradability than the control diet. The diet containing higher DFR content, therefore have higher effective degradability at higher passage rate.

7.5 Conclusion

With increasing DFR in the diets the rapidly soluble fraction (a) tended to increase. However, the potential degradation declined. Similarly the total potentially degradable fraction (a + b) decreased with increased levels of DFR in the diets. The rate of degradation of the ‘b’ fraction increase with increase in DFR level. As a result the effective degradation at higher flow rates were higher with increased levels of DFR in the diets. This is due to the higher ‘a’ fraction and higher ‘c’ fraction.

8. EXPERIMENT 5. ECONOMIC ASSESSMENT OF THE USE OF DIATOMITE FILTER AID RESIDUE IN FEED OF MALE CROSSBRED HOLSTEIN-THAI-INDIGENOUS CATTLE UNDER FEEDLOT CONDITIONS

8.1 Objectives

The objective of the study was to determine the effect of feeding diets containing different levels of diatomite filter aid residue on feedlot performance and economic return of male crossbred Holstein x Thai-indigenous cattle.

8.2 Materials and methods

8.2.1 Animals

Sixteen male Holstein x Thai-indigenous crossbred cattle averaging 230.48±23.45 kg initial body weight and 1.21±0.14 years initial age were used. All animals were vaccinated against Foot and Mouth disease and Haemorrhagic Septicaemia and were given anthelminthics and a Vitamin A, D_3 and E injection, before the experiment.

8.2.2 Housing

The animals were housed in individual stalls similar to the stalls used in the experiment 2.

8.2.3 Feeds

Four isonitrogenous diets were formulated according to NRC (1980) recommendations to contain 148 g CP/kg DM. Diatomite filter aid residue (DFR) was included at four levels varying from 0, 30, 40 and 50 per cent. The diatomite filter aid residue was dried in a hot air oven at 105° C for 24 hours before being used in the rations. The feed ingredients and chemical composition of the experimental rations used is shown in Table 8.1.

Table 8.1 Feed ingredients (g/kg DM) and chemical composition of experimental rations containing either 0, 30, 40 or 50% diatomite filter aid residue (DFR).

	Diet			
	0% DFR	30% DFR	40% DFR	50% DFR
Ingredient				
Diatomite filter aid residue	0.00	300.00	400.00	500.00
Broken rice	300.00	0.00	0.00	0.00
Palm kernel meal	629.30	625.80	523.20	420.40
Urea[1]	10.70	14.20	16.90	19.60
Soybean meal	30.00	30.00	30.00	30.00
Molasses	20.00	20.00	20.00	20.00
Vitamin-premix [2]	5.00	5.00	5.00	5.00
Normal salt	5.00	5.00	5.00	5.00
Total	1000.00	1000.00	1000.00	1000.00
Crude Protein (calculated)	148.00	148.00	148.00	148.00
Chemical analysis				
Dry matter[3]	884.73	866.55	852.41	842.75
Organic matter	953.77	758.38	707.36	627.12
Crude protein	156.86	167.09	161.24	151.37
Crude fibre	120.39	151.84	140.73	110.20
Ether extract	48.17	46.35	41.38	36.76
Crude ash	46.23	241.63	292.64	372.88
Nitrogen free extract	628.36	393.10	364.01	328.80
Calcium	3.61	7.83	8.88	10.87
Phosphorus	4.55	4.24	3.91	3.28
Sodium	1.89	4.38	4.47	4.10
Potassium	6.46	1.36	0.86	0.54
Organic matter digestibility[4]	657.06	495.80	497.52	444.24
Gas production (ml/200mg DM)	56.04	36.42	36.27	29.74
Gross energy(MJ/kg)	19.07	15.70	15.08	13.90
Metabolisable energy(MJ/kg)[5]	10.06	6.82	6.74	5.64

DFR: Diatomite filter aid residue.
[1] Contained 46% Nitrogen.
[2] Contained Vitamin A, 1.250.000 iu; Vitamin D_3, 250.000 iu; Vitamin E, 1.000 iu; Zinc, 5.200 mg; Iron, 2.250 mg; Manganese, 2.500 mg; Copper, 1.000 mg; Cobalt, 60 mg; Iodine, 80 mg; Selenium, 25 mg and Sodium, 20 mg.
[3] Expressed as g/kg at air dry basis.
[4,5] Calculated according to Close and Menke (1986) on page 134.

8.2.4 Experimental design

The cattle were allocated randomly to their diets in a completely randomised design (CRD) (STEEL and TORRIE, 1981). There were four animals per treatment.

8.2.5 Feeding and management

The animals were fed both concentrate and roughage *ad libitum*. Feeds were offered to the animals twice a day at 07.30 and 16.30 hours. Light was kept on during the night. Refusals of both roughage and concentrate were weighed daily before new feed was offered. The animals were weighed three times at an interval of 24 hours before the experiment. The average body weight was used as the initial live body weight. Thereafter, the animals were weighed every two weeks at 07.00 hours. The final and initial weights were used to determine body weight changes. Net feed intake was calculated on a daily basis.

8.2.6 Proximate analysis

The proximate analysis was carried out and calcium, phosphorus, sodium and potassium analysed as described by NAUMANN and BASSLER (1976). All sample were analysed in triplicates. Gross energy measurement and Hohenheim gas test as described by MENKE *et al.* (1979) were also undertaken.

8.2.7 Statistical analysis

The collected data were analysed for analysis of variance (ANOVA) using a model specific for a CRD. A general linear model procedure (SAS, 1988) was used for the analysis. Duncan's new multiple range test was used to compare differences between treatment means.

8.3 Results

8.3.1 Intake

Average dry matter intakes of concentrate, roughage and the total feed are shown in Table 8.2. Total dry matter intake and average dry matter intake/day of concentrate were significantly

different ($P<0.05$) across treatment means, whereas average dry matter intake as percentage of body weight (%BW) and of metabolic body weight ($BW^{0.75}$) were highly significantly different ($P<0.01$) across treatments. The cattle fed on 60% DFR had the highest total dry matter intake, average dry matter intake/day, dry matter intake as percentage of body weight (%BW) and of metabolic body weight ($BW^{0.75}$). Dry matter intake of concentrate increased proportionately with increasing amount of DFR in the diet.

Total dry matter intake, average dry matter intake and average dry matter intake as percentage of body weight of roughage were significantly different ($P<0.05$) across treatment means, whereas dry matter intake expressed on metabolic body weight ($BW^{0.75}$) was highly significantly different ($P<0.01$) across treatments. The cattle fed on the control diet had the highest roughage dry matter intake. The intake decreased with increasing DFR in the feed and the cattle fed on 60% DFR had the lowest dry matter intake.

Total dry matter intake and average dry matter intake of total feed were significantly different ($P<0.05$) across treatments, whereas dry matter intake as percentage of body weight (%BW) and dry matter intake as metabolic body weight ($BW^{0.75}$) were highly significantly different ($P<0.01$) across treatments. Cattle fed on 60% DFR inclusion level in the diets had the highest intake. Dry matter intake of total feed increased proportionately with increasing DFR in the diet.

The concentrate to roughage dry matter intake ratio increased with increasing DFR in the diets and ranged from 5.81 for control diet to 18.50 for the cattle group fed on the diet containing 60% DFR.

Total organic matter, crude protein and metabolisable energy intakes of concentrate, roughage and total feed are shown in Table 8.3. The CP intake from concentrate, roughage and total feed were significantly different across treatments ($P<0.05$). Also when the intake of concentrate was expressed as percentage of body weight (%BW) and of metabolic body weight ($BW^{0.75}$), there were highly significant ($P<0.01$) treatment differences. With increasing DFR content in the diet, the concentrate dry matter intake increased ($P<0.05$), whereas the roughage intake decreased ($P<0.05$), but not proportionately. Total feed intake increased ($P<0.05$) with increasing DFR in the diet.

Table 8.2 Average intake of concentrate, roughage and total feed of male Holstein x Thai-indigenous crossbred cattle fed rations containing either 0, 30, 40 or 50% diatomite filter aid residue (DFR).

Parameter	Diet				
	0% DFR	30% DFR	40% DFR	50% DFR	SEM
Number of animal	4	4	4	4	
Feeding period (days)	84	84	84	84	
Concentrate feed intake (kg)					
Total dry matter	408.76^{c}	509.45^{bc}	626.31^{ab}	700.46^{a}	53.93
Dry matter/day	4.87^{c}	6.06^{bc}	7.46^{ab}	8.34^{a}	0.64
Dry matter as %BW/day	1.71^{C}	2.44^{B}	2.85^{AB}	3.21^{A}	0.20
Dry matter g/$BW^{0.75}$/day	70.08^{C}	96.63^{B}	114.33^{AB}	128.49^{A}	7.46
Roughage feed intake (kg)					
Total dry matter	72.86^{a}	46.26^{b}	49.42^{b}	37.65^{b}	6.48
Dry matter/day	0.87^{a}	0.55^{b}	0.59^{b}	0.45^{b}	0.08
Dry matter as %BW/day	0.30^{a}	0.22^{b}	0.23^{b}	0.18^{b}	0.02
Dry matter g/$BW^{0.75}$/day	12.38^{A}	8.77^{B}	9.11^{B}	7.10^{B}	0.86
Total feed intake(kg)					
Total dry matter	481.62^{b}	555.72^{ab}	675.72^{a}	738.11^{a}	56.82
Dry matter/day	5.73^{b}	6.62^{ab}	8.04^{a}	8.79^{a}	0.68
Dry matter as %BW/day	2.01^{C}	2.67^{B}	3.08^{AB}	3.39^{A}	0.20
Dry matter g/$BW^{0.75}$/day	82.47^{C}	105.40^{BC}	123.44^{AB}	135.59^{A}	7.61
Concentrate and roughage dry matter intake ratio	5.81^{C}	11.92^{B}	12.82^{B}	18.50^{A}	1.26

Animals were fed *ad libitum* (10-20% over requirement) over the feeding period.

$^{a, b, c, A, B, C}$ Means in the same row not having at least a common superscript differ significantly: $^{a, b, c}$ $P<0.05$, $^{A, B, C}$ $P<0.01$; DFR= Diatomite filter aid residue, SEM = Standard error of mean, BW= Live body weight in kg.

Organic matter intake of concentrate and total feed were not significantly different ($P>0.05$) across treatments, whereas for roughage, it was significantly different ($P<0.05$) across treatments. The cattle fed on 40% DFR had the highest organic matter intake either from concentrate or total feed, whereas the cattle fed on the control diet (0% DFR) had the highest organic matter intake of roughage.

Crude protein intake of concentrate, roughage and total feed were significantly different ($P < 0.05$) across treatments. The cattle fed on 60% DFR had the highest crude protein intake either for concentrate or total feed, whereas the cattle fed on control diet (0% DFR) had the highest crude protein intake of roughage. Crude protein intake of concentrate and total feed increased with increasing DFR in diets.

Metabolisable energy intake of either concentrate or total feed were not significantly different ($P > 0.05$) across treatments, whereas it was significantly different ($P < 0.05$) across treatments for roughage. The cattle fed on the control diet (0% DFR) had the highest metabolisable energy intake of total feed.

Crude protein to metabolisable energy intake ratio ranged from 14.60 to 25.61 (g CP/kg: MJ ME/kg).

Crude ash and minerals intakes are shown in Table 8.4. Crude ash, calcium, sodium, and potassium intakes obtained either from concentrate or total feed in each treatment were highly significantly different ($P < 0.01$) across treatments. It was not significantly different ($P > 0.05$) for phosphorus, whereas all measured minerals obtained from roughage were significantly different ($P < 0.05$), except for sodium that was highly significant ($P < 0.01$) across treatments. The total eaten ratio between calcium and phosphorus ranged from 0.89 in the control diet to 3.31 in the 60% DFR diet and it increased with increasing DFR in diet.

8.3.2 Weight gain and feed conversion ratio

Data on weight gain and feed conversion ratio are shown in Table 8.5. The initial weight, final weight, weight gain and average daily gain of the animals were not significantly different ($P > 0.05$) across treatments. Feed conversion ratio for concentrate and total feed was significantly different ($P < 0.01$), whereas for roughage, it was not significantly different ($P > 0.05$).The cattle fed on control diet had the lowest feed conversion ratio for concentrate and total feed, whereas the cattle fed on 60% DFR diet had the lowest feed conversion ratio for roughage. Feed conversion ratio increased proportionately with increasing DFR in the diets.

Table 8.3 Profiles of average organic matter (g DM/day), protein (g DM/day) and energy intake (MJ ME/day) of male Holstein x Thai-indigenous crossbred cattle fed rations containing 0, 30, 40 and 50% diatomite filter aid residue (DFR).

Parameter	**Diet**				
	0% DFR	30% DFR	40% DFR	50% DFR	SEM
Number of animals	4	4	4	4	
Feeding period (days)	84	84	84	84	
Organic matter intake (g/day)					
Concentrate	4641.39	4599.64	5274.40	5229.24	432.83
Roughage	766.75^{a}	486.86^{b}	520.06^{b}	396.22^{b}	68.16
Total	5408.14	5086.50	5794.46	5625.46	464.16
Crude protein intake (g/day)					
Concentrate	763.50^{b}	1013.45^{ab}	1201.91^{a}	1262.49^{a}	99.96
Roughage	32.18^{a}	20.43^{b}	21.82^{b}	16.63^{b}	2.86
Total	795.68^{b}	1033.90^{ab}	1223.73^{a}	1279.12^{a}	101.17
Metabolisable energy intake (MJ/day)					
Concentrate	48.95	41.36	50.25	47.03	3.98
Roughage	5.52^{a}	3.51^{b}	3.75^{b}	2.86^{b}	0.49
Total	54.48	44.87	54.00	49.89	4.19
Crude protein/energy intake ratio					
(g CP/kg:MJ ME/kg)	14.60^{D}	25.61^{A}	22.67^{C}	23.06^{B}	0.13

Animals were fed *ad libitum* (10-20% over requirement) over the feeding period. Organic matter, crude protein and metabolisable energy intake were calculated from the organic matter, crude protein and metabolisable energy content of diets as shown in Table 8.1.

$^{a, b, c, A, B, C}$ Means in the same row not having at least a common superscript differ significantly: $^{a, b, c}$ $P<0.05$, $^{A, B, C}$ $P<0.01$; DFR= Diatomite filter aid residue, SEM= Standard error of mean, BW= Live body weight in kg.

8.3.3 Feed cost value and economic return

Data on feed cost and economic return are shown in Table 8.6. Total feed consumed increased proportionately with increasing level of DFR in the diets. The cattle fed on 30% DFR diet had the lowest cost for total feed consumption. When cost of feed was the only cost considered, the economic return was highest for 40% DFR inclusion followed by 30% DFR, 50% DFR and lowest for the control diet.

Table 8.4 Profiles of average mineral intake (g/day) of male Holstein x Thai-indigenous crossbred cattle fed rations containing 0, 30, 40 and 50% diatomite filter aid residue (DFR).

Parameter	Diet				
	0% DFR	30% DFR	40% DFR	50% DFR	SEM
Number of animals	4	4	4	4	
Feeding period (days)	84	84	84	84	
Crude ash intake (g/day)					
Concentrate	224.80^{D}	1465.30^{C}	2181.60^{B}	3109.50^{A}	216.56
Roughage	102.35^{a}	64.99^{b}	69.42^{b}	52.89^{b}	9.10
Total	327.20^{D}	1530.30^{C}	2251.12^{B}	3162.40^{A}	220.21
Calcium intake (g/day)					
Concentrate	17.52^{C}	47.31^{B}	66.36^{B}	90.89^{A}	6.43
Roughage	2.60^{a}	1.65^{b}	1.76^{b}	1.35^{b}	0.23
Total	20.12^{C}	48.96^{B}	68.12^{B}	92.24^{A}	6.52
Phosphorus intake (g/day)					
Concentrate	21.89	25.47	29.07	27.51	2.31
Roughage	0.69^{a}	0.44^{b}	0.46^{b}	0.35^{b}	0.06
Total	22.59	25.91	29.54	27.87	2.33
Sodium intake (g/day)					
Concentrate	9.25^{B}	26.68^{A}	33.55^{A}	34.18^{A}	2.67
Roughage	0.35^{A}	0.22^{B}	0.24^{B}	0.18^{B}	0.03
Total	9.59^{B}	26.92^{A}	33.79^{A}	34.36^{A}	2.69
Potassium intake (g/day)					
Concentrate	31.63^{A}	8.49^{B}	6.71^{BC}	4.17^{C}	0.88
Roughage	18.73^{a}	11.89^{b}	12.70^{b}	9.68^{b}	1.66
Total	50.36^{A}	20.38^{B}	19.41^{BC}	13.85^{C}	1.94
Total calcium : phosphorus intake ratio	0.89^{D}	1.89^{C}	2.30^{B}	3.31^{A}	0.006

Animals were fed *ad libitum* (10-20% over requirement) over the feeding period. Nutrient intake was calculated from nutrient content values in Table 8.1.

$^{a, b, c, A, B, C}$ Means in the same row not having at least a common superscript differ significantly : $^{a, b, c}$ $P<0.05$, $^{A, B, C}$ $P<0.01$; DFR= Diatomite filter aid residue, SEM= Standard error of mean.

Table 8.5 Average bodyweight gain and feed efficiency of male Holstein x Thai-indigenous crossbred cattle fed rations containing either 0, 30, 40 or 50% diatomite filter aid residue (DFR).

Parameter	Diet				
	0% DFR	30% DFR	40% DFR	50% DFR	SEM
Number of animals	4	4	4	4	
Feeding period (days)	84	84	84	84	
Weight gain (kg)					
Initial weight	250.00	217.08	224.67	230.17	23.45
Final weight	345.25	302.00	324.75	313.50	25.24
Weight gain	95.25	84.92	100.08	83.33	8.62
Average daily gain	1.13	1.01	1.19	0.99	0.10
Feed conversion ratio					
Concentrate	4.31^{C}	6.16^{B}	6.28^{B}	8.59^{A}	0.53
Roughage	0.77	0.58	0.49	0.48	0.10
Total feed	5.08^{B}	6.74^{B}	6.77^{B}	9.07^{A}	0.61

Animals were fed *ad libitum* (10-20% over requirement) throughout the feeding period.

$^{A, B, C}$ Means in the same row not having at least a common superscript differ significantly ($P < 0.01$).

Table 8.6 Mean gross returns of male Holstein x Thai-indigenous crossbred cattle fed rations containing either 0, 30, 40 or 50% diatomite filter aid residue (DFR).

Parameter	Diet			
	0% DFR	30% DFR	40% DFR	50% DFR
Number of animals	4	4	4	4
Feeding period (days)	84	84	84	84
Initial mean weight	250.00	217.08	224.67	230.17
Initial value[1] (B)	6250.00	5427.00	5616.75	5754.25
Final live weight	345.25	302.00	324.75	313.50
Final value[1] (B)	8631.25	7550.00	8118.75	7837.50
Change in value[2]	2381.25	2123.00	2502.00	2083.25
Total feed consumed (kg)				
Concentrate	408.76	509.45	626.31	700.46
Roughage	72.86	46.26	49.42	37.65
Total	481.62	555.72	675.72	738.11
Feed cost /kg (B)				
Concentrate	4.17	2.45	2.29	2.14
Roughage	1.25	1.25	1.25	1.25
Total value of feed consumed (B)				
Concentrate	1906.20	1443.30	1683.80	1813.90
Roughage	98.88[a]	62.78[b]	67.07[b]	51.10[b]
Total	2005.08	1506.08	1750.87	1865.00
Economic return[3] (B)	376.17	616.92	751.13	218.25

DFR= Diatomite filter aid residue.
[1] Assumed 25 Baht /Kg of live weight.
[2] Final value - initial value.
[3] Change in value - value of feed consumed.
B (Baht) Thai monetary unit (15.20 Baht/1 DM at the time of the experiment: December 1996-March 1997).

8.4 Discussion

Based on the fact that the nutritional content of the experimental diets (Table 8.1) indicates the magnitude of the nutrients that the animal from each group will be supplied, the animal fed on diets containing higher DFR inclusion level tend to confront with consuming the diets containing lower energy content. Because the content of the metabolisable energy in the diets decrease with increasing the DFR inclusion level. The metabolisable energy in the diet ranged from 10.06, 6.82, 6.74 and 5.64 MJ/kg, respectively. The diets containing higher DFR inclusion level, therefore, contain lower the metabolisable energy. The content of crude protein of the diets ranged from 156.86, 167.09, 161.24 and 151.37 g/kg, respectively. The level of crude protein content were higher than the recommendation by the NRC (1976) which is recommended at 148 g/kg for the cattle gained 700 g/day. The phenomena on decreasing of the metabolisable energy content according to increasing of DFR inclusion within the diets was similar to the other experiments. It can be supposed that the metabolisable energy content of the DFR was lower than the mixtures of other components of the diets. When higher levels of the DFR were included, the metabolisable energy content of diets, therefore, tend to decrease.

The alternative energy source used in this experiment was palm kernel meal (Table 8.1), the local, low-cost feed ingredient, generally used as energy source in cattle feed. The metabolisable energy value of palm kernel mean was about 12.18 (AHMAD, 1988a) to 12.53 (AHMAD, 1988b) MJ/kg. The chemical content, the in vitro digestibility value (IVDMD) and the ruminal degradability of this feedstuff had been well elucidated by MARCHIEW (1993). The dry matter, crude protein, NDF, ADF, ash, ether extracted, IVDMD and the ruminal degradability value at 72 hours of the palm kernel meal from the Thai oil palm industry and garden Co. Ltd., the same source of feedstuff used in this experiment, were 90.75, 13.70, 72.85, 46.90, 3.90, 12.70, 63.97 and 64.33% respectively. Palm kernel meal and the DFR are the major components of the total mixed rations which are account for 62.93, 92.58, 92.32 and 92.40%, respectively (Table 8.1).

When data on feed intake (Table 8.2) was considered, It was found that the average dry matter intake/day of the concentrate, roughage and total feed intake were significantly different across treatments ($P<0.05$). The tendency of the intake of the concentrate was similar to the result in the experiment 3. This mean that the cattle fed on diets containing higher DFR inclusion levels consumed more feed ($P<0.05$) than those fed on diets containing lower DFR inclusion levels. However, the average dry matter intake of the roughage decrease ($P<0.05$) with increasing DFR

inclusion levels. The tendency on feed intake of the total feed was similar to the concentrate. This mean that the total feed intake increase with increasing DFR inclusion levels. The most important factors influencing on feed intake might be the energy content of the diets. Because it was only one parameter that varied tremendously between treatments and it was known that energy play an essential role on the regulation of feed intake (CONRAD *et al.,* 1964; DINIUS and BAUMGARDT, 1970; GROVUM, 1984, FORBES, 1986; NRC, 1987; FORBES, 1993).

When the metabolisable energy (ME) content of the diets (Table 8.1) was considered, it was found that the ME decrease with increasing DFR inclusion levels. Concerning with the understanding on the control of feed intake in the ruminants, it is generally accepted that the intake was controlled to match energy requirements when physical constraints were not important (CONRAD *et al.,* 1964; FORBES, 1993). According to the analytical value of the ME of the diets in this experiment (Table 8.1), it is clearly indicated that the diets containing higher DFR inclusion levels contained lower ME content. To meet the ME requirement, the cattle fed on diet containing higher DFR inclusion levels, therefore have to consume more feed than the cattle fed on diets containing lower DFR inclusion levels. The cattle fed on diet containing higher DFR inclusion levels, therefore, have higher average dry matter intake ($P<0.05$), dry matter intake as %BW ($P<0.05$) and dry matter intake as $BW^{0.75}$ ($P<0.01$) than those fed on diets containing lower DFR inclusion levels.

The control of feed intake, however, may not be controlled only by the content of energy in the diets , the physiologically control may also play a role in the regulation of feed intake (FORBES, 1993). It had been reported that the diatomite grade suitable for industrial feed manufacturing has some physiological activities when it was included in the diets (SCHULENBERG and RABELING, 1996). The most prominent capacity of the diatomite (diamol) used in animal feed manufacture was to improve flowability of feedstuffs rich in fat and/or water. The diatomite, moreover, can be use in animal feed as the carrier of some chemical such as organic acid, cholinechloride, antioxidants and amino acids (SCHULENBERG and RABELING, 1996). This event might ground on the fact that the diatomite is bulky, light and have a small particle size (EAGLE-PICHER, 1988). In the gastro-intestinal tract of the ruminants, the particle size of the feed influences out flow rate of the ingested feed. It was found that diets containing smaller particle size of feeding ingredient will leave the fore stomachs faster than the larger one and leads to increase intake (BALCH and CAMPLING, 1962). On this ground, the cattle fed on diets containing larger proportion of DFR, therefore, have higher passage rate and higher dry

matter intake (P<0.05) than those fed on diets containing lower DFR inclusion levels. The regulation of feed intake in the cattle when they were fed with diets containing different levels of DFR, therefore, might be considered both on physiological and chemical controls in terms of the metabolic mechanism control (FORBES, 1993).

The tendency of the average dry matter intake of the roughage (Table 8.2) was decreased with increasing DFR inclusion levels and was significantly different (P<0.05) across treatments. The cattle fed on the control diet had higher (P<0.05) dry matter intake of roughage than that of others. The characteristic of the intake of roughage was a good exhibition on rumen fill mechanism regulation. Because of the cattle fed on diets containing higher DFR inclusion levels consumed more concentrate (P<0.05) than those fed on diets containing lower DFR inclusion levels. The remained space in the reticulorumen for the roughage, therefore, was smaller. The cattle, therefore, consumed less (P<0.05) roughage. It had been reported that the intake of roughage by the ruminants is through to be controlled largely by distension or the volume of contents in the reticulorumen (BAILE and FORBES, 1974; GROVUM, 1984).

The tendency of the total feed intake (Table 8.2) was similar to the concentrate for every measurement criteria. This might mean that the factors affecting the regulation of concentrate intake dominate over the factors affecting the regulation of the roughage intake. It can be concluded from this result that the lowering in ME content due to higher DFR inclusion levels of the diets play a crucial role on regulation of intake of both concentrate and roughage.

The amount of intake of the organic matter, crude protein and metabolisable energy (Table 8.3), total ash as well as some other minerals (Table 8.4) varied according to the amount of both concentrate and roughage intake (Table 8.2). The total intaked calcium to phosphorus ratio (Table 8.4) varied from 0.9:1 to 3.3:1 and was highly significant different (P<0.01) across treatments.

Although the organic matter content among the treatments of the concentrate (Table 8.1) was considerably different in which the diets containing higher DFR inclusion levels tend to have lower organic matter content, the organic matter intake of the concentrate, however, was not significantly different (P>0.05) across treatment. The organic matter intake of the roughage, on the other hand, was significantly different (P<0.05) across treatments. The amount of organic matter intake from the roughage tend to decrease (P<0.05) with increasing DFR inclusion levels

in the diets. The explanation on this phenomena was similar to the explanation on roughage intake which was described previously in this chapter. The occurrence of the distension limiting the roughage intake of the cattle fed on diets containing higher DFR inclusion levels causes the cattle consuming less ($P<0.05$) organic matter from roughage than the control diet. The cattle fed on the control diet, therefore, consumed more ($P<0.05$) organic matter from roughage than that of others.

The crude protein intake (Table 8.3) from both concentrate and roughage as well as total feed were significantly different ($P<0.05$) across treatments. The tendency of crude protein intake was similar to dry matter intake of the diets. The cattle fed on the control diet tend to have lower ($P<0.05$) total crude protein intake than that of others.

Although the metabolisable energy content among the diets (Table 8.1) varied from 10.06, 6.82, 6.74 and 5.64 MJ/kg, respectively and the dry matter intake of the concentrate (Table 8.2) was significant different ($P<0.05$) across treatments, the total ME intake (Table 8.3) between the treatments, however, was not significant different ($P>0.05$) across treatments. This was ground on the fact that the intake was controlled to match energy requirements when physical constraints were not important (Forbes, 1993). To meet the energy requirement, the animal fed on diets containing lower ME content, therefore, have to consume more ($P<0.05$) diet than those fed on diet containing higher ME content (Table 8.2). Although the ME intake of the roughage (Table 8.3) was significant different ($P<0.05$) across treatments, the total ME intake, however, were not significant different ($P>0.05$) across treatments. This might mean that the animal from each group had adjusted their ME intake both from concentrate and roughage. Lowering in ME intake from the roughage of the animal fed on diet containing higher DFR inclusion level had been compensated by the ME derived from the concentrate, the total ME intake among the cattle group therefore, were not significant different ($P>0.05$) across treatments. The ratio between crude protein and ME (Table 8.3) tend to increase with increasing DFR inclusion level.

The body weight gain of the cattle (Table 8.5) fed on diets containing different DFR inclusion levels were not significantly different ($P>0.05$) across treatments. The average dairy gain (ADG) ranged from 1.13, 1.01, 1.19 and 0.99 kg/day, respectively. Although the average daily gain was not significant different ($P>0.05$) across treatments, feed efficiency in terms of feed conversion ratio (FCR) of both the concentrate and total feed were highly significant different ($P<0.01$) across treatments. The FCR ratio of the concentrate increase with increasing DFR inclusion

levels the similar tendency was also found in the FCR of the total feed. Both the ADG and FCR obtained from this experiment indicated the positive tendency of the use of the DFR in feedlot enterprise. Because growth performance of the male crossbred Holstein-Thai-indigenous cattle is better than the expected weight gain recommended by the NRC (1976) at about 700 g/day. The maximum growth rate found in this experiment was 1.19 g/day for the cattle fed on diet containing 40% DFR inclusion level.

When the mean gross returns (table 8.6) was considered, it is found that average economic return on the use of DFR in cattle feed at different inclusion levels range from 376.17, 616.92, 751.13 and 218.25 Baht/head, respectively. The cattle fed on diet containing 40% DFR inclusion has the highest economic return. From the economic return point of view, it can be concluded that the use of DFR in cattle feed on fattening the male crossbred Holstein-Thai-indigenous cattle have positive tendency. The use of DFR at 40% inclusion level together with palmkernel meal, the local and low cost feedstuff give the maximise mean gross return.

8.5 Conclusion

Inclusion of the DFR in cattle feed caused the diet lowering in the metabolisable energy content. The lowering in the ME content caused the animal to have higher concentrate intake whereas the roughage was suppressed. The ME obtained from the total feed intaked, however, was not significantly different ($P>0.05$). The intake of crude protein tend to increase with increasing DFR inclusion levels. The average daily gain of the cattle offered diets with different in DFR inclusion levels was similar whereas the feed conversion ratio increase with increasing DFR inclusion levels. The cattle fed on diet containing 40% DFR inclusion levels gave the best economic return. The suggestion level of the use of DFR in cattle feed found in this experiment, therefore, was at 40% DFR inclusion level.

The chemical and physical properties of DFR are of particular interest as a feed resource. Although the crude protein content of the DFR is low at 5.1% of DM but the content of readily available carbohydrate, in terms of nitrogen free extract (NFE) is high with a proportion of 46.20 per cent (FEED ANALYSIS DIVISION, 1991). The high mineral content in the DFR can present problems to its utilisation. However, incorporation of DFR in the diet in this study did not have an adverse effects on the animals. In fact, DFR inclusion led to increased dietary intakes. Incorporation of DFR at 50% led to depressed economic performance. This could be

attributed to imbalance crude protein and energy supply which might lead to uncoupled fermentation and inefficient diet utilisation at tissue level (PERRY, 1980). At higher levels of DFR inclusion level therefore, incorporation of readily fermentable energy sources might be necessary. Under the conditions in the study, the optimum level of DFR inclusion was found to be 40%.

9. GENERAL DISCUSSION AND RECOMMENDATIONS FOR FUTURE WORK

9.1 General discussion

Considering its nutritional profile, the industrial waste product diatomite filter aid residue (DFR) shows a positive potential for its use as an energy source in animal feed since it contains about 42.69% nitrogen free extract (FEED ANALYSIS DIVISION, 1991). When a mixture of DFR and ground growers swine feed were fed to Wistar Albino rats, it was found that the rats fed on diets containing higher proportions of DFR had lower weight gain as the animals fed on mixed diets added less DFR. This might be related to the protein and energy content in the mixed rations, since the diets containing higher DFR inclusion level had a lower content of both crude protein and energy. The use of DFR in the feed of Wistar Albino rats at inclusion levels of 75% or less, however, did not cause any adverse effects to the animals. According to the positive tendency found in this experiment, the next step of the evaluation process were, therefore, undertaken.

The evaluation of the use of the DFR in cattle feed began with an experiment on the use of different levels of DFR as a component of cattle feed on rumen fermentation and blood parameters. The DFR didn't show any negative effects at any inclusion level in the experiment. Inclusion of up to 60% of DFR in cattle diets did not cause any adverse effects neither on rumen fermentation nor on blood parameters. Although a significant difference ($P<0.05$) across treatments were found both in the rumen and in blood parameters, the value of those parameters were still within the optimum range for normal rumen function. The urea in the ration rather than the diatomite may influence the BUN and the ruminal ammonia because the diatomite is completely inert, non resorbable, non swelling and non toxic (SCHULENBERG and RABELING, 1996). Most of the curves of the rumen and blood parameters of the cattle fed on diets containing different DFR inclusion levels showed similar patterns as the curves from cattle fed on control diet. The small particle size of diatomite (EAGLE-PICHER, 1988) may influence the rate of passage in the GI tract. The cattle fed on the diets containing higher DFR inclusion levels, therefore, have a higher passage rate. This physiological function may affect the secretion of phosphorus from the saliva. A significant difference ($P<0.05$) across treatment on blood phosphorus, therefore, were found at 8 hours post feeding.

An evaluation of the effect of restricted feeding of a basal diet constituting different levels of diatomite filter aid residue on feedlot performance of male crossbred Holstein x Thai-indigenous cattle was subsequently undertaken. When the diet was limited, all the feed offered was consumed, though the cattle fed on diets containing higher DFR inclusion levels consumed more ($P<0.05$) roughage than those fed on diets containing lower DFR inclusion levels. This intake characteristic indicates the energy content of the DFR where the moderately low metabolisable energy content of the diets cause this feed intake behaviour of the cattle. The content of NFE which were analysed to account for 42.69% in newly produced DFR (DM) may not have this value at the time of feeding. It has been previously reported that both the moisture and the residual sugar contained in the wet DFR decreased within the storage period (AJINOMOTO, personal communication). This change might be due to the fact that the micro-organism utilise the residual sugar content in the DFR. To avoid the change of the chemical constituents of the DFR, an appropriate drying method may be needed.

The impact of restricted feeding of a basal diet constituting different levels of diatomite filter aid residue on feedlot performance of male crossbred Holstein x Thai-indigenous cattle (Table 5.5) was that the cattle fed on diets containing higher DFR inclusion levels tend to have lower growth performance and feed efficiency than the cattle fed on diets containing lower DFR inclusion levels.

An evaluation of the effect of *ad libitum* feeding of a basal diet constituting different levels of diatomite filter aid residue in feed on feedlot performance of male crossbred Holstein-Thai-indigenous cattle was later conducted. According to the profile of the intake of both concentrate and roughage (Table 6.2), it is indicated that when the diets were not limited, the cattle fed on diets containing higher DFR inclusion levels consumed more concentrate and total feed ($P <0.05$) than the cattle fed on diets containing lower DFR inclusion levels. These intake characteristics may confirm the previous expectation that the DFR has a lower energy content than the other ingredients mixed into the diets. The diets containing higher DFR inclusion levels, therefore, contain a lower ME content (Table 6.1).

Due to the ad libitum feeding system of both concentrate and roughage, a difference on growth performance was not found in this experiment. However a difference on feed efficiency of the concentrate and the total feed was found in which the cattle fed on diets containing higher DFR inclusion levels had lower ($P<0.05$) feed efficiency than the cattle fed on diets containing lower

DFR inclusion levels. Both the growth performance and the feed utilisation efficiency were used to plan the subsequent experiment to evaluate the economic assessment.

The results from the last two experiments which were performed simultaneously and which used the same feed formula, elucidated both the degradability characteristics of the diets used in the experiment (Experiment4) and gave an economic assessment on the use of the DFR in feedlot enterprise (Experiment5). The result of the experiment with the nylon bag technique indicates that the diets containing higher DFR inclusion levels tend to have a more rapidly soluble fraction, however, the potential degradation as well as the potentially degradable fraction declined. The inorganic matter plays a crucial role on the declining of the potentially degradable fraction (a+b fraction). The particle size of the diatomite may influence the flow rate of the diets. Therefore, the effective degradation at higher flow rates were higher with increased DFR contents in diets.

The result on the economic assessment indicates that 40% inclusion level is the optimal level for inclusion of DFR in the diets for pen-fattening of male crossbred Holstein-Thai-indigenous cattle under similar conditions as in this study.

9.2 Recommendations for future work

Although the results generated from this research work in which some physiological functions and an economic evaluation is considered show a positive tendency for the use of DFR as an alternative feed resource in the diet for cattle, further research work needs to be carried out to:

1. Characterise the influence of the use of DFR in cattle feed on carcass traits and qualities.

2. Assess the chemical composition of meat and some essential visceral organs of the cattle fed on diet containing DFR compared to those fed on standard diets.

3. Evaluate the differences on the economic return of the use of DFR in cattle feed as compared to other animals feed.

4. Determine a suitable method to be used to assure minimum changes in physical and chemical properties of the DFR after it is produced, deposited and included in the cattle feed.

10. CONCLUSION

The properties of the diatomite filter aid residue (DFR) that meet our interest when we look at it as a possible alternative feed source rather than disposing it into water or in the fields which may cause environmental problems are 1.) The content of the nitrogen free extract (NFE) which accounts for 46.69% DM. This NFE is the easily fermentable carbohydrate that may be a good source of energy. 2.) The molasses-like flavour that may function as an appetiser of the mixed diets. 3.) Since it is chemically inert to other components or minerals in the diet it may not interfere on the utility of the other feed ingredients. The metabolisable energy from the analysis of both the DFR and the diet containing the DFR at different levels, however, depicted that the DFR have a moderately to low metabolisable energy content compared to the expected content. The most important reason for this lower ME content is that the fermentation of the DFR go on after it is produced and collected from the factory. To prevent the declination on the chemical composition and the physical properties of the DFR, an appropriate preservation method such as drying must be applied.

The effect of inclusion of DFR in feed for the rats found in the preliminary study indicate that the rats fed on diets containing higher DFR inclusion levels have a lower body weight gain. The use of the DFR in feed up to 75% level or less, however, did not cause a decrease in the body weight of the animals. The measured increase in the body weight of the rats is reverse proportional with the inclusion levels of the DFR in the diets. The most disadvantageous property of the DFR when it was used as a component of the animal feed is the ME content. However, with an appropriate preservation method, the decline in the ME might be less.

The influence of the DFR in diet on rumen fermentation and blood parameters show that the diet containing DFR up to 60% level did not cause any adverse effects neither on rumen fermentation nor on blood parameters. The high mineral content of the DFR did not interfere with normal rumen fermentation and metabolism of the calcium and phosphorus in the blood circulation. This might ground on the chemical properties of the diatomite: It is completely inert, non resorbable, non swelling and non toxic.

The result from the feeding trial with the restricted feeding shows that the diet containing different DFR inclusion levels didn't show any obstruction on feed intake. Due to that the diets containing higher DFR inclusion levels have a lower ME content and the concentrate offered to

the animals was limited, the cattle fed on the higher DFR inclusion diets have a higher roughage intake than the cattle fed on diets containing lower DFR inclusion levels. The cattle fed on diets containing a lower DFR inclusion levels tended to have a better growth performance and feed efficiency than those fed on diets containing higher DFR inclusion levels.

With *ad libitum* feeding, the animals fed on diets at any DFR inclusion levels have total dry matter intake higher than the cattle fed on the control diet. The cattle fed on diets containing 40 and 60% DFR inclusion levels, moreover, have a total feed intake of more than 3% of live body weigh. Although the total feed intake was greater, the metabolisable energy intake of the animals fed on diets containing any of the DFR inclusion levels was lower than for the animals fed on the control diet. This is the reason for the lower average daily gain and feed efficiency of the cattle fed on diets containing DFR at all inclusion levels. The lowering in the metabolisable energy content of the rations containing DFR in any inclusion level is the most disadvantageous property for the use of DFR in cattle feed.

The result from the degradability study depicted that the rapidly soluble fraction or the 'a' fraction of the diet tended to increase with increasing DFR in the diets. However, the potential degradation fraction or the 'b' fraction declined. Similarly the total potentially degradable fraction (a+b) decreased with increasing levels of DFR in the diets. The rate of degradation of the ‘b’ fraction which is presented as the 'c' parameter of the model increased with increasing amounts of DFR in the diet. The effective degradation at higher flow rates were higher with increased levels of DFR in the diets. This is due to the higher ‘a’ and ‘c’ fractions.

Up to 50% inclusion of DFR in the diets for pen-fattening Holstein x Thai-indigenous crossbred male cattle did not lead to any depression in performance. The diet with 40% DFR inclusion lead to the highest economic return. Therefore DFR should find ready application in fattening rations for cattle in terms of reduction in feed costs and as one way of disposal.

11. SUMMARY

The evaluation of the industrial waste product diatomite filter aid residue (DFR) as an alternative feed source for the cattle was carried out in a series of a preliminary study and five experiments.

Preliminary study: The effect of DFR in feed on growth performance of Wistar Albino rats was evaluated. It was found that the rats fed on diets containing higher DFR inclusion levels have a lower body weight gain. The use of the DFR in feed up to 75% level or less, however, did not cause a decrease in the body weight of the animals.

Experiment 1: The effect of the DFR on rumen fermentation and blood parameters was evaluated by 4-rumen fistulated heifer offered diets containing 4 different DFR inclusion levels. Both the ruminal pH and the rumen ammonia concentrations as well as blood urea nitrogen were affected by treatments ($P<0.05$). Blood calcium and blood phosphorus at short time post feeding was not affected by the treatments ($P>0.05$). The concentration of blood phosphorus at 8 hours post feeding, however, was significant different ($P<0.05$). The use of the DFR up to 60% level in the cattle diet did not cause any adverse effects neither on rumen fermentation nor on blood parameters.

Experiment 2: The effect of restricted feeding of diets containing different levels of diatomite filter aid residue on feedlot performance of cattle was evaluated. The dry mater intakes of concentrate when measured as total dry matter intake, average dry matter intake/day and average dry matter intake as percentage of body weight were not significantly different across treatments ($P>0.05$), whereas average dry matter intake as per cent of the metabolic body weight ($BW^{0.75}$) was significantly different across treatments ($P<0.01$). The final weight, weight gain and average daily gain of the animals were not significantly different ($P>0.05$) across treatments. Feed conversion ratio for roughage and total feed was significantly different ($P<0.05$), whereas it was not significantly different ($P>0.05$) for concentrate. The result indicates that the diet containing different DFR inclusion levels didn't show any negative effect on feed intake and weight gain.

Experiment 3: The effect of *ad libitum* feeding of diets containing different levels of diatomite filter aid residue on feedlot performance of male crossbred Holstein x Thai-indigenous cattle was evaluated. Total dry matter intake, average dry matter intake/day and average dry matter intake as percentage of body weight of concentrate were significantly different ($P<0.05$) across

treatments, whereas average dry matter intake as per cent metabolic body weight ($BW^{0.75}$) was highly significantly different ($P<0.01$) across treatments. The cattle fed on 60% DFR had the highest concentrate dry matter intake. Weight gain and average daily gain of the animals were not significantly different ($P>0.05$) across treatments. Feed conversion ratio for concentrate and total feed was significantly different ($P<0.05$). The cattle fed on 60% DFR had the highest feed conversion ratio ($P<0.05$).

Experiment 4: The effect of different levels of diatomite filter aid residue in feed on ruminal dry matter degradability was evaluated. The potential degradability ('a'+'b') of the diets containing different levels of DFR inclusion was significantly different ($P<0.05$) across treatments. The control diet has the highest ($P<0.05$) potential degradability. The potential degradability decreased when the DFR levels were increased.

Experiment 5: The effect of feeding diets containing different levels of diatomite filter aid residue on feedlot performance and economic return of male crossbred Holstein x Thai-indigenous cattle was evaluated. The cattle fed on 60% DFR had the highest total dry matter intake ($P<0.05$), average dry matter intake/day ($P<0.05$), dry matter intake as percentage of body weight ($P<0.01$) and of metabolic body weight ($P<0.01$). Weight gain and average daily gain of the animals were not significantly different ($P>0.05$) across treatments whereas the feed conversion ratio for concentrate and total feed was significantly different ($P<0.01$). Feed conversion ratio increased proportionately with increasing DFR in the diets. The economic return was highest for 40% DFR inclusion followed by 30% DFR, 50% DFR and lowest for the control diet. Under the conditions in the study, the optimum level of DFR inclusion was found to be 40%. This experiment shows that DFR can be mixed into fattening rations of cattle to reduce feed cost.

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13. APPENDIX

Appendix 2.1 Mean levels of pH and ammonia nitrogen (mg/dl) in the rumen fluid, urea nitrogen (mg/dl), calcium (mg/dl) and phosphorus concentration (mg/dl) in blood plasma at 0, 2, 4, and 8 hours post-feeding in cattle offered feed containing either 0, 20, 40 or 60% diatomite filter aid residue (DFR).

Parameter	Diet					
Time	0% DFR	20% DFR	40% DFR	60% DFR	Average	SEM
pH						
0 hr	7.04	6.99	7.23	7.24	7.12^{A}	0.01
2 hr	7.03^{a}	6.75^{b}	7.06^{a}	6.94^{ab}	6.95^{B}	0.07
4 hr	7.04^{a}	6.77^{b}	6.89^{ab}	6.89^{ab}	6.90^{B}	0.06
8 hr	6.97	6.86	7.02	6.90	6.94^{B}	0.05
Average	7.02^{AB}	6.84^{B}	7.05^{A}	6.99^{AB}	6.98	0.11
Ammonia nitrogen						
0 hr	1.93^{c}	6.03^{ab}	7.74^{a}	3.45^{bc}	4.78^{BC}	1.10
2 hr	13.71^{b}	16.33^{b}	26.95^{a}	22.99^{ab}	20.24^{A}	3.08
4 hr	4.82^{c}	5.12^{bc}	9.51^{ab}	12.25^{a}	7.50^{B}	1.02
8 hr	1.89	4.24	4.19	2.00	3.08^{C}	0.05
Average	5.59^{B}	7.37^{AB}	12.27^{A}	10.03^{AB}	8.76	3.71
Blood urea nitrogen						
0 hr	8.63^{c}	13.05^{ab}	14.90^{a}	9.88^{bc}	11.61^{C}	1.17
2 hr	11.35^{b}	14.55^{ab}	18.15^{a}	13.38^{b}	14.36^{A}	1.15
4 hr	11.58^{b}	14.33^{b}	18.83^{a}	14.13^{b}	14.71^{A}	1.27
8 hr	9.75^{b}	11.30^{b}	16.58^{a}	11.65^{b}	12.32^{B}	1.32
Average	10.33	13.31	17.11	12.26	13.25	4.53
Blood calcium						
0 hr	10.05	9.93	9.73	10.05	9.94	0.13
2 hr	10.53	9.95	9.75	10.08	10.08	0.29
4 hr	10.05	10.23	9.65	10.08	10.00	0.14
8 hr	10.27	9.85	10.03	10.23	10.09	0.23
Average	10.22	9.99	9.79	10.11	10.03	0.48
Blood phosphorus						
0 hr	7.62	7.68	8.24	6.84	7.59	0.43
2 hr	8.01	7.26	8.48	7.01	7.70	0.56
4 hr	7.62	7.48	7.91	7.11	7.53	0.63
8 hr	7.92^{ab}	8.28^{a}	8.17^{a}	6.80^{b}	7.80	0.33
Average	7.79	7.68	8.20	6.95	7.65	0.82

$^{a, b, c, A, B, C}$ Means in the same row not having at least a common superscript differ significantly: $^{a, b, c}$ $P<0.05$, $^{A, B, C}$ $P<0.01$; DFR= Diatomite filter aid residue, SEM= Standard error of mean.

Appendix 4.1 The Hohenheim gas test

The Hohenheim gas test (MENKE *et al.*, 1979) was undertaken to evaluate the organic matter digestibility (OMD) and the metabolisable energy (ME) content of the samples. The organic matter digestibility and metabolisable energy content were estimated using the results of the proximate analysis and of the Hohenheim gas test using the equations proposed by Close and Menke (1986) as following:

1. OM degradability (%)= 14.88+0.889*%GP+0.045*%CP+0.065*%crude ash

and

2. Metabolisable energy (ME)=1.06+0.157GP+0.0084*%CP+0.022*%EE-0.0081*crude ash

Where:
OM degradability (%)=Percentage digestibility of organic matter (%OMD)
GP= Gas production of each 200 mg sample during 24 hours (ml)
CP= Crude protein content of the sample (N x 6.25)
EE= Ether extract or crude fat content of the sample.

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ACKNOWLEDGEMENTS

I wish to express my deepest thank to my supervisor, Prof. Dr. Udo ter Meulen who initially accepted me as a Ph D candidate, and who has over the past five years, given me all the encouragement and the conditions necessary to carry out this work. Similarly, I am thankful to Prof. Dr. Therdchai Vearasilp, the co-supervisor from Thailand and Prof. Dr. Hans-J. Langholz in the Institute of Animal Breeding and Genetics.

Deep appreciation is also expressed to Mrs. G. ter Meulen for the correction of this work. I would also like to thank Mr. Klaus Grow for conducting the laboratory analysis.

I would like to thank all the staff members of the Rajamangala Institute of Technology (RIT) Nakhonsithammarat Campus, particularly to Mr. Jim Nooyimsia, the director of the RIT Nakhonsithammarat Campus for all the support given and allowing me access to all facilities during the 18 months of the research work who to my deepest sadness passed away last year. I am also thankful my students for their support.

My grateful thanks also to all my colleagues at the Institute of Animal Physiology and Animal Nutrition, particularly Sebastian Chakeredza, Aporn Songsang, Dahrul Syah, John David Kabasa and Donna Gultom for their help and encouragement.

I am entirely grateful to the German Academic Exchange Service (DAAD) who financially supported my studies during my whole stay in Germany and the Rajamangala Institute of Technology (RIT) who sponsored my research work in Thailand. I am also thankful to the Ajinomoto, Thailand Co. Ltd. who sponsored the diatomite filter aid residue (DFR) for this work.

Last but not least, I owe my warmest thanks to my dear wife Poonsub Insung, my parents and my sisters and brothers whose encouragement and patience helped me to attain this goal.