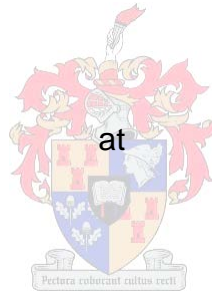# THE EFFECT OF FINE PARTICLE REMOVAL ON THE ESTIMATION OF PROTEIN DEGRADABILITY PARAMETERS IN DAIRY CATTLE

by

Magdel Nel

Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Agriculture (Animal Science)

at

Stellenbosch University

Department of Animal Sciences

Faculty of AgriScience

Supervisor: Prof CW Cruywagen

Date: March 2012

DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: March 2012

ABSTRACT

Title: The effect of fine particle removal on the estimation of protein
             degradability parameters in dairy cattle

Name: Magdel Nel

Supervisor: Prof. C.W. Cruywagen

Institution: Department of Animal Sciences, Stellenbosch University

Degree: MScAgric

Grinding of feedstuffs prior to *in sacco* incubation results in fine particles that could escape from dacron bags without being degraded. The objective of this study was to determine the effect of the removal of these fine particles on protein degradation parameters. The feedstuffs that were used were protein feedstuffs that are commonly used in dairy cattle diets in South Africa. Feedstuffs were soybean oilcake, sunflower oilcake, maize gluten 20, maize gluten 60 and fish meal. Treatments were; 1. grinding through a 2 mm screen with no subsequent sieving, 2. grinding through a 2 mm screen followed by sieving through a 106 μm mesh, and 3. grinding through a 2 mm screen followed by sieving through a 150 μm mesh. In the first trial, the material that was lost through sieving and the chemical composition of the different treatments were determined. Protein solubility was also determined. Between 20 and 60% of the material dry matter was lost with the sieving. The chemical composition for the soybean and sunflower oilcake and maize gluten 60 was similar between the three different treatments (or fractions). The CP content of fish meal and maize gluten 20 differed somewhat between the treatments. The 106 μm mesh seemed to be most suitable for fish meal, but a suitable mesh size could not be found for maize gluten 20. In the second trial, the degradability parameters were determined according to the *in sacco* degradation procedure. Three lactating Holstein cows that were fitted with rumen cannulae were used. The cows received a commercial lactation diet and oat hay that

was supplied *ad libitum*. Samples of all the protein sources were placed in dacron bags and incubated in the rumen. The following removal times were used: 0, 2, 4, 8, 12, 24 or 48 hours. Dry matter and CP disappearances were determined, and the values were used to estimate DM and CP degradability parameters using a non-linear model. Effective CP degradability was also determined. The a-values were affected most of all. On average, the a-values were 39.4 and 40.3% higher for the un-sieved treatments than for the sieved treatments, for DM and CP, respectively. The effective CP degradability was also, on average, 43% higher for the un-sieved treatments. Grinding without the subsequent sieving of samples appears to result in an overestimation of DM and CP degradation in the rumen. It is therefore recommended that after grinding, feedstuffs should be sieved through at least a 106 µm mesh in preparation for *in sacco* incubations.

UITTREKSEL

Titel:	Die invloed van die verwydering van fynmateriaal op die beraming van proteïen-degradeerbaarheidsparameters.
Naam:	Magdel Nel
Studieleier:	Prof. C.W. Cruywagen
Instansie:	Departement Veekundige Wetenskappe, Universiteit van Stellenbosch
Graad:	MScAgric

Die maal van grondstowwe ter voorbereiding van *in sacco* inkubasies lei tot 'n potensiële verlies van klein partikels uit die dakronsakkies sonder dat hulle gedegradeer word. Die doel van hierdie studie was om die invloed van die verwydering van fynmateriaal op proteïen-degradeerbaarheidsparameters te bepaal. Die grondstowwe wat in hierdie studie gebruik is, is proteïengrondstowwe wat algemeen in melkbeesdiëte in Suid-Afrika gebruik word. Die grondstowwe was soja-oliekoek, sonneblom-oliekoek, mielie gluten 20, mielie gluten 60 en vismeel. Behandelings was; 1. maal deur 'n 2 mm sif sonder verdere sifting, 2. maal deur 'n 2 mm sif gevolg deur sifting deur 'n 106 µm sif en 3. maal deur 'n 2 mm sif gevolg deur sifting deur 'n 150 µm sif. In die eerste proef is die hoeveelheid materiaal wat verlore gaan as gevolg van sifting van die grondstowwe bepaal en die chemiese samestelling van die verskillende behandelings. Proteïenoplosbaarheid is ook bepaal. Tussen 20 en 60% van die materiaal het verlore gegaan as gevolg van sifting. Die chemiese samestelling van die soja- en sonneblom oliekoek, asook dié van die mielie gluten 60 was soortgelyk vir al drie die behandelings. Die ruproteïeninhoud (RP) van die vismeel en mielie gluten 20 het verskil tussen die drie behandelings. Dit wil voorkom asof die 106 µm sif die mees geskikte is vir vismeel, maar 'n geskikte sif kon nie vir mielie gluten 20 gevind word nie. In die tweede proef is die degradeerbaarheidsparameters bepaal met behulp van die *in sacco*-metode. Drie lakterende Holsteinkoeie met rumen kannulas is gebruik. Die koeie het 'n kommersiële melkbeesdieët ontvang en hawerhooi *ad libitum*. Die monsters is in dakronsakkies in die rumen geïnkubeer. Die sakkies is na die volgende inkubasietye verwyder: 0, 2, 4, 8, 12, 24 of 48 uur. Die DM- en RP-verdwyning is bereken en die waardes is gebruik om die

DM- en RP- degradeerbaarheidsparameters te bereken met behulp van 'n nie-lineêre model. Effektiewe RP- degradeerbaarheid is ook bereken. Die waardes wat die meeste beïnvloed is, is die a-waardes. Die a-waardes was gemiddeld 39.4 en 40.3% hoër vir die ongesifte behandelings as vir die gesifte behandelings, vir DM en RP, onderskeidelik. Die effektiewe RP-degradeerbaarheid was ook gemiddeld 43% hoër vir die ongesifte behandelings. Dit wil voorkom asof DM- en RP-degradeerbaarheid oorskat word wanneer voermonsters slegs gemaal word. Dit word aanbeveel dat grondstowwe ten minste deur 'n 106 µm sif gesif word ter voorbereiding vir *in sacco*-studies.

ACKNOWLEDGEMENTS

On the completion of this work, I would like to thank my Heavenly Father for His grace and for giving me the courage and perseverance to complete this thesis.

I would also like to express my sincerest appreciation and gratitude to the following people, without whom this work would have been impossible:

- Funding: NRF (Grant-holders' bursary) and Stellenbosch University (Postgraduate merit bursary)
- Prof. Cruywagen, my supervisor, for his guidance and support during my studies
- Ms. B. Ellis and the technical staff of the Department of Animal Sciences, Stellenbosch University, for their assistance during this work
- Mr. W. Van Kerwel and the technical staff of the Welgevallen Experimental Farm, Stellenbosch University, for the use of their facilities and their assistance during this work
- Nutrolab (University of Pretoria), for determining protein solubility
- Dr. Paul Weimer, for his assistance with determining protein solubility
- My parents, for their support, encouragement and prayers
- My friends and family, for their support.

LIST OF ABBREVIATIONS

a	Rapidly soluble fraction
AA	Amino acids
b	Fraction potentially degraded over time
BS	Bone-and-scales fraction
c	Rate of degradation
CF	Crude fibre
CO_2	Carbon dioxide
CP	Crude protein
D_{eff}	Effective degradability
DM	Dry matter
EE	Ether extract
k	Passage rate
MCP	Microbial crude protein
N	Nitrogen
NH_3	Ammonia
NPN	Non-protein nitrogen
OM	Organic matter
Y	Degradation at specific time point
RDP	Rumen degradable protein
SE	Standard error
SU	Stellenbosch University
UDP	Ruminally undegradable protein
UP	University of Pretoria
VFA	Volatile fatty acids

TABLE OF CONTENTS

List of Figures

List of Tables

CHAPTER 1

General Introduction

Dairy cattle are used exclusively for milk production, and milk production is mainly influenced by nutrition. Nutrients that are ingested are subjected to digestion before they can be utilised by the cow. Protein is digested in the rumen as well as in the rest of the digestive tract. Protein digestion occurring in the rumen is referred to as degradation (Erasmus et al., 1988). Various factors affect degradation and one of these is particle size (Satter, 1986). Degradation parameters are determined to enable nutritionists to formulate well-balanced diets, to optimise production and minimise nutrient losses.

The global demand for food is increasing drastically, and as the world population is still growing, it will continue to increase for at least another 40 years (Godfray et al., 2010). In the past, the problem of producing more food was addressed by using more land for agriculture. However, with numerous challenges facing producers because of the limited amount of arable land, they will have to increase production on the same amount of land or even less (Godfray et al., 2010). Thus, production will need to be intensified even more, including animal production.

Dairy cattle produce large amounts of milk, which serves as a protein source for humans. In South Africa, the number of dairy cattle farmers has decreased dramatically over the last few years. In 1997, there were 7077 producers in comparison to the 2686 producers in January 2011 (Lactodata, 2011). Farmers are consequently compelled to increase production; concurrently profitability also needs to increase.

Nutrition and milk yield per cow are the main factors determining the profitability of a dairy farm. Feeding costs are responsible for the largest component production costs, and this could be as high as 70% of the total farm costs (Ho et al., 2005). This highlights the importance of farming with animals that have the genetic potential to

produce large amounts of milk. These animals require a balanced diet to achieve their production potential (Clark & Davis, 1980).

A balanced diet is required, with the two most important nutrients for milk production being protein (or N) and energy (Clark & Davis, 1980). Proteins are the most expensive ingredients in animal diets (Hristov & Jouany, 2005).

Dietary protein or crude protein (CP) can be divided into rumen degradable protein (RDP) and ruminally undegradable protein (UDP). The RDP fraction is degraded in the rumen by micro-organisms, while UDP passes intact to the small intestine, where it becomes available for digestion and absorption (NRC, 2001; Bach et al., 2005). The genetic potential of the high-producing dairy cow will only be met if the diet supplies sufficient RDP for the micro-organisms in the rumen, as well as sufficient UDP that will pass to the lower digestive tract to supply additional amino acids (Erasmus et al., 1988). Because the two fractions, RDP and UDP, are affected by the extent of degradation occurring in the rumen, it is important to quantify the amount of rumen degraded protein (Ørskov & McDonald, 1979; Stern et al., 1994; NRC, 2001).

Protein degradation can be estimated *in vivo* or *in vitro* (Van der Walt & Meyer, 1988). *In vivo* measurements are done in animals and may include an estimation of AA absorption, but this requires animals that are surgically prepared with cannulae in both the rumen and the duodenum. These experiments are very expensive (Erasmus et al., 1990). For RDP estimations, *in vivo* trials usually include *in situ* procedures, also referred to as the *in sacco* method. *In vitro* measurements are made in the laboratory, and the rumen environment is merely imitated, and therefore this method might be less accurate (Van der Walt & Meyer, 1988). The *in sacco* method (or dacron bag technique) provides a combination of *in vitro* and *in vivo* estimations of protein degradation. It is the most popular technique and the best technique available to estimate protein degradation on a regular basis (Ørskov & McDonald, 1979).

The model that is used most frequently to describe *in sacco* ruminal degradation involves dividing feed crude protein into three fractions, A, B and C. Fraction A represents the amount of the total CP, consisting of non-protein nitrogen (NPN) and true protein, which quickly escapes from the *in sacco* bag because of its high solubility or fine particle size. It is assumed that NPN is rapidly degraded. Fraction C's crude protein is completely undegradable and remains in the bag at the defined end point of degradation. Fraction B represents the rest of the CP and includes the proteins that are potentially degradable (NRC, 2001).

For the *in sacco* estimation of protein degradation, samples are incubated in the rumen in dacron bags. The designated average pore size of dacron bags is 53 μm. However, microscopic image analyses done at Stellenbosch University's laboratory showed that the pore sizes range from 31 to 99 μm and that the average pore size is actually 63 μm (Cruywagen, 2007; unpublished data). Thus, any particle that is smaller than 100 μm can potentially be washed out at 0 hours. This can lead to an overestimation of fraction A, since fraction A mainly represents the soluble protein fraction (NRC, 2001).

Throughout the study, no results could be found for the effect of fine particle removal on protein degradation parameters. Removing the fine particles could result in a more accurate estimation of protein degradation, especially for the A fraction. The objective of this study was to determine whether the standard *in sacco* method described by the NRC (2001) to estimate protein degradation overestimates protein degradation. Results would enable nutritionist to better formulate balanced diets to optimise production.

The literature review for this study is presented in Chapter 2, focusing on the factors that affect protein degradation. In Chapter 3, the effect of fine particle removal from various protein feedstuffs was investigated in terms of chemical composition. Protein solubility was also determined. Chapter 4 is devoted to an *in sacco* study of the different fractions of the different feedstuffs. Crude protein (CP) and dry matter (DM) disappearance were determined as well as the effective degradability of CP. A general conclusion is presented in Chapter 5.

1.1. References

Bach, A., Calsamiglia, S. & Stern, M.D., 2005. Nitrogen metabolism in the rumen. J. Dairy Sci. 88: E9-E21.

Clark, J.H. & Davis, C.L., 1980. Some aspects of feeding high producing dairy Cows[1]. J. Dairy Sci. 63(6): 873-885.

Erasmus, L.J., Prinsloo, J., Botha, P.M. & Meissner, H.H., 1990. Establishment of a ruminal protein degradation data base for dairy cattle using the *in situ* polyester bag technique. 2. Energy sources. S. Afr. J. Anim. Sci. 20: 124-129.

Erasmus, L.J., Prinsloo, J. & Meissner, H.H., 1988. The establishment of a protein degradability data base for dairy cattle using the nylon bag technique. 1. Protein sources. S. Afr. J. Anim. Sci. 18: 23-29.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M. & Toulmin, C., 2010. Food security: The challenge of feeding 9 billion people. Science. 327(5967): 812.

Ho, C., Nesseler, R., Doyle, P. & Malcolm, B., 2005. Future dairy farming systems in irrigation regions. Australian Farm Business Management Journal. 2(1): 59-68.

Hristov, A.N. & Jouany, J.P., 2005. Factors Affecting the Efficiency of Nitrogen Utilization in the Rumen. In: Nitrogen and Phosphorus Nutrition of Cattle: Reducing the Environmental Impact of Cattle Operations. Eds. Pfeffer, E. & Hristov, A.N. CABI Publishing, Oxfordshire, UK. pp. 117-166.

Lactodata. 2011. Lactodata Statistics: A Milk SA publication compiled by the Milk Producers' Organisation. http://www.dairyconnect.co.za/dairyMail/LACTO_DATA_MAY_11.pdf. Accessed October 19, 2011.

National Research Council (NRC), 2001. *Nutrient Requirements of Dairy Cattle*. 7th Rev. ed. National Academy Press. Washington, D. C.

Ørskov, E.R. & McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92(02): 499-503.

Satter, L.D., 1986. Protein supply from undegraded dietary protein. J. Dairy Sci. 69(10): 2734-2749.

Stern, M.D., Varga, G.A., Clark, J.H., Firkins, J.L., Huber, J.T. & Palmquist, D.L., 1994. Evaluation of chemical and physical properties of feeds that affect protein metabolism in the Rumen[1]. J. Dairy Sci. 77(9): 2762-2786.

Van der Walt, J.G. & Meyer, J.H.F., 1988. Protein digestion in ruminants. S. Afr. J. Anim. Sci. 18(1): 30-41.

CHAPTER 2

Literature review

2.1. Introduction

In comparison with the single stomach of monogastric animals, the stomach of ruminant animals consists of four compartments, which provide them with more space for digestion and also serve as a habitat for a large number of micro-organisms (Ensminger, 1993). Micro-organisms enable ruminants to use fibre-rich plant material and also NPN sources that other animals and humans cannot use directly. Therefore, ruminants play an important role in effectively using the worlds declining nutrient resources (Chalupa, 1977).

Protein digestion in the rumen via micro-organisms is referred to as degradation (Van der Merwe & Smith, 1991). Micro-organisms in the rumen ferment and digest the ingested feed in order to provide nutrients for the organisms to grow and proliferate (Hungate, 1966). The resultant microbial protein is one of the main sources of AA supply to the animal as it moves to the small intestine and becomes available for digestion and absorption. However, the micro-organisms have the ability to change the AA composition of the feed so that it differs from the ingested feed at the time that it reaches the small intestine (Van der Merwe & Smith, 1991).

Dairy cattle are used for intensive milk production, and to enable cows to produce optimally, highly specialised diets are required. Therefore, it is important to determine accurately the level of degradation that occurs in the rumen so that optimal amounts of nutrients can be included in the diet to supply the needs of the micro-organisms and therefore the animal itself (Van der Merwe & Smith, 1991).

Various factors determine degradation in the rumen; some are related to the diet and some are related to the animal itself (Scott et al., 1991; Tice et al., 1993). Feed factors include chemical structure, protein solubility, processing, etc., while animal

6

factors include rumen pH, feed intake, passage rate from the rumen, etc. The chemical and physical properties of feed determine the degradation thereof, but they also determine the types of micro-organisms that inhabit the rumen (Lykos & Varga, 1995). The activity of the micro-organisms and the approachability of the protein are also important for degradation, which are influenced by the characteristics of the protein and the characteristics of the feed particle in which the protein occurs (Satter, 1986).

Protein degradation could be determined using various methods. Each of these has its advantages and disadvantages. Nylon and dacron bags are the most suitable to do this determination. Even though it is an imperfect and empirical approach, it combines animal and microbial factors in a useful way to measure the protein degradation taking place in the rumen in a relatively fast and easy way (Ørskov et al., 1980; Erasmus et al., 1988).

2.2. Protein metabolism

2.2.1. Various forms of protein

Dietary protein in feedstuffs usually refers to crude protein (CP), which consists of true protein as well as non-protein nitrogen (NPN). Dietary protein can be divided into rumen degradable protein (RDP) and rumen undegradable protein (UDP; NRC, 2001; Bach et al., 2005). Rumen degradable protein (RDP) represents non-protein N (NPN) and true protein N. True protein can be degraded into peptides, amino acids (AA) and ammonia in the rumen. Non-protein nitrogen (NPN) consists of N, which occurs in DNA, RNA, ammonia, urea, AA and small peptides and is assumed to be completely degraded in the rumen. The N in ammonia, AA and peptides are used by the micro-organisms for the production of microbial crude protein (MCP) (Bach et al., 2005).

The protein value of feedstuffs is more precisely determined in terms of the amount of protein and individual AA that reach the small intestine, where it is absorbed (Miller, 1982). The protein and amino acids (AA) that reach the small intestine come

from the following sources: MCP that is synthesised in the rumen, undegradable dietary protein (UDP) and endogenous proteins (Stern et al., 1994; Stern et al., 2006). Microbial crude protein is the most important source of AA and supplies 50 to 80% of the total absorbable protein that reaches the small intestine. It is a high-quality protein that is highly digestible in the small intestine (Stern et al., 2006), although it is limited in terms of certain essential AA (Van der Merwe & Smith, 1991).

Although the contribution of UDP in the supply of AA is relatively small compared with that of MCP, it is still important, as the UDP should balance any deficiencies that may occur in terms of AA supplied by MCP and the requirements of the dairy cow (NRC, 2001). As production increases, the cow's requirements for UDP increases, and thus the degradability of protein gets more important (Erasmus et al., 1988; Stern et al., 1994; NRC, 2001). Degradation of protein in the rumen is one of the most important factors that affect the AA supply to the small intestine of the animal (Stern et al., 2006).

2.2.2. Protein degradation

The ruminant possesses two types of protein digestion processes, namely, active digestion or degradation via the micro-organisms in the rumen and enzymatic hydrolysis of the protein substances in the lower digestive track (Van der Merwe & Smith, 1991). It is important to distinguish between potential degradability and effective degradability. Potential degradability refers to the amount of protein that could be dissolved and degraded in the rumen if there were sufficient time. Effective degradability refers to the amount of protein that would actually be degraded, and is therefore dependent on the time that the protein is retained in the rumen (Ørskov et al., 1980).

Degradation in the rumen includes two steps. First, the protein chain is broken by hydrolysis of the peptide bonds (proteolysis), and peptides and amino acids are formed as products (Tamminga, 1979). The second step is the decarboxylation and/or deamination of the amino acids, and this results in volatile and branched chain fatty acids, as well as CO_2 and NH_3 (Van Straalen & Tamminga, 1990).
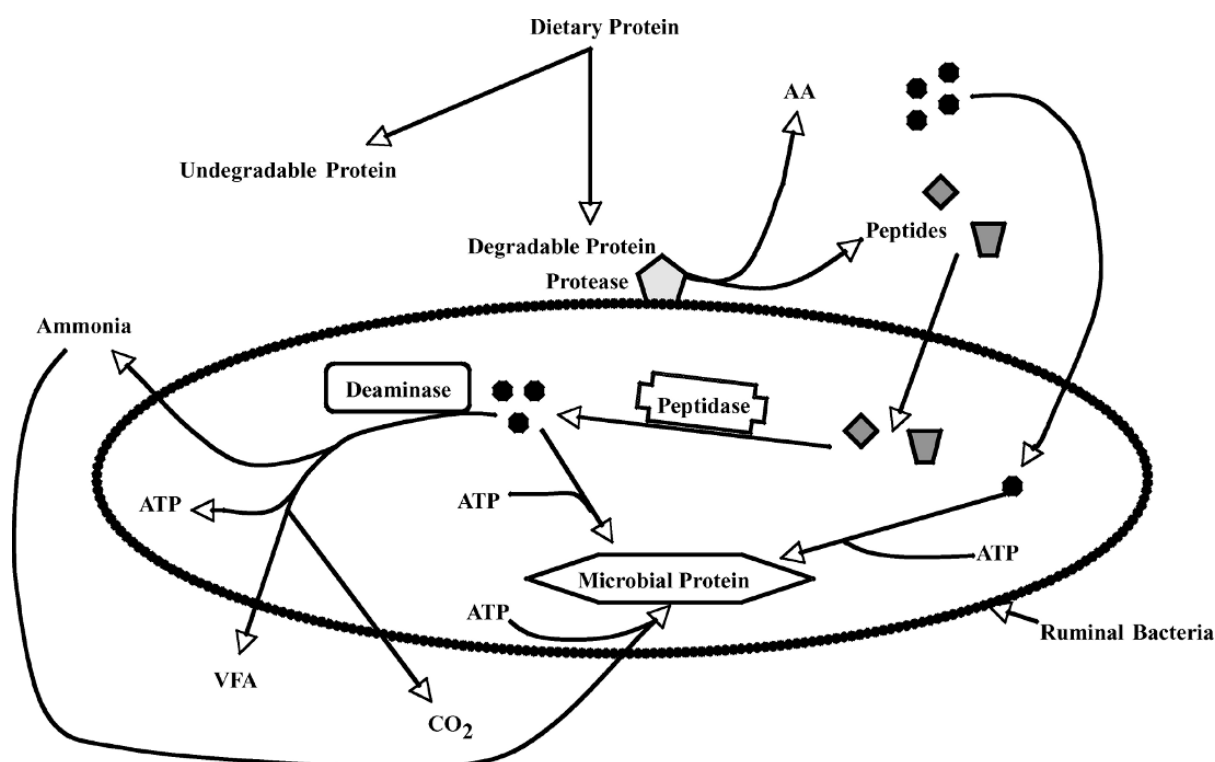
Figure 2.1 Protein degradation in the rumen (Bach et al., 2005).

Figure 2.1 gives a schematic representation of the degradation taking place in the rumen and shows what happens with the end products of degradation. The peptides and AA that are produced through extracellular proteolytic activity in the rumen are transported into the microbes (Bach et al., 2005). The peptides can then be degraded to form AA. These AA can then be used to synthesise MCP, or it can be degraded even further into volatile fatty acids (VFA), carbon dioxide (CO_2), ammonia (NH_3), methane (CH_4) and fermentation heat (Tamminga, 1979).

The fate of the AA and the peptides that are transported into the microbial cells depend on the availability of energy. When the diet provides enough energy, the AA are used for the synthesis of MCP. If, however, there is an energy shortage, the AA are deaminated and the carbon skeletons that are produced are used for the production of VFA, and the N is excreted (Bach et al., 2005).

When protein degradation exceeds the rate at which AA and ammonia are incorporated into microbial protein, the catabolism of peptides and AA will result in a

very high ammonia concentration in the rumen that exceeds the optimum levels of 17 to 25 mg/100 ml. This may cause an increase in the ruminal pH (Hibbit, 1988). Some of the AA and peptides may escape ruminal degradation and pass into the small intestine where they become available for absorption. However, excessive amounts of AA and ammonia cause a lot of dietary CP to be wasted and reduce the efficiency of usage of RDP for production (NRC, 2001). Another problem with excessive amounts of CP is that it lowers the performance of the animals, especially their reproductive performance (Schwab et al., 2005).

2.2.3. Nitrogen (N) requirements

The dairy cow's requirement for crude protein is the amount of protein that would maintain maximum milk production (Clark & Davis, 1980). The diet should supply enough N to meet the requirements of the micro-organisms, as well as those of the host animal itself. The total milk production of a cow, and also the protein content of the milk, are associated with the MCP yield (Nocek & Tamminga, 1991) and the flow of AA (Clark et al., 1992) to the small intestine. However, protein only becomes available to increase milk production once the need of the animal is met (Crish et al., 1986).

During early lactation, dairy cows are often in a negative energy balance as they are often unable to consume enough feed to meet their high nutrient requirements, even though they may be receiving a balanced diet (Clark & Davis, 1980; Ørskov et al., 1981). Some protein and energy can be drawn from storage sites in the body, but protein that is stored in the body has limited potential to supply the animal's needs. A shortage will cause a decrease in production, and in acute cases, it can result in a metabolic disorder such as ketosis (Clark & Davis, 1980).

MCP alone is not enough for the early lactating cow, and UDP of a high quality is needed to maintain optimal production (Clark et al., 1992). If peak milk production is low, it will result in a decrease in the milk produced during the entire lactation cycle (Clark & Davis, 1980). According to Broster (1975), each 1 kg decrease in peak milk production will lead to a 150 kg decrease in total milk production.

10

The ratio of RDP to UDP is very important. A diet that is low in protein or with an oversupply of UDP and too little RDP, will cause a deficiency in the N supply to the microbes and so the production of MCP will be decreased as well as the degradation of organic matter in the rumen, in particular the breakdown of cellulose-rich cell walls (Tamminga, 1979; McDonald et al., 2002). A shortage of nitrogen (N) can easily be overcome by supplying additional non-protein nitrogen (NPN). In such circumstances, the microbes must have enough energy available (Tamminga, 1979).

If N is oversupplied, it would lead to high levels of ammonia in the rumen. This will require additional energy for detoxification in the liver and so energy utilisation will be less efficient (Clark & Davis, 1980). Some excess ammonia is absorbed into the blood and transported to the liver, where it is converted into urea and excreted via the urine. It can also be recirculated back to the rumen via the saliva, and a small amount of the urea will also diffuse back into the rumen through the rumen wall (Hibbit, 1988).The advantages of urea recycling are that it can provide additional N when the N concentration in the rumen is low, and thus the urea would promote cellulose digestion. It can also increase the amount of AA that reaches the lower digestive tract (Van der Merwe & Smith, 1991).

Despite the higher UDP requirements of the dairy cow, diets that are supplemented with a source of undegradable protein have resulted in variable responses (Scott et al., 1991; Tice et al., 1993). These may be caused by insufficient RDP in the diet or insufficient fermentable carbohydrates, which cause the synthesis of MCP to be less effective, or they could be the result of an imbalance of AA caused by the AA profile of the UDP (Lykos & Varga, 1995).

2.3. Factors affecting protein degradability

The most important factors that affect the degradability of protein in the rumen are the type of protein, the interactions between the nutrients (mainly carbohydrates within the same feedstuff and within the rumen contents), and the predominant microbial population in the rumen. The predominant microbial population depends on

the type of diet, the passage rate of the feed ingested and the pH in the rumen (Bach et al., 2005).

2.3.1. Structure of the protein

The structure of the dietary protein is an important aspect of protein degradability, as it affects the accessibility of the protein to the microbial proteases and so determines the degradability of the protein. The bonds, both between and within protein chains (the tertiary and quaternary structures of the proteins), play an important role in determining the degradability of the protein. Some specific peptide bonds are more resistant to degradation than others (Bach et al., 2005).

The three-dimensional structure of the protein molecule will affect the entrance of proteolytic enzymes into the molecule (Satter, 1986). It is very hard for these enzymes to enter a protein with wide-ranging cross-linkages, for example the cross-linkages occurring in disulfide bonds, so these proteins offer a degree of resistance against degradation (Nungent & Mangan, 1978). Examples of such proteins are those occurring in hair and feathers.

Proteins that are soluble but also cyclic, for example ovalbumin, have no terminal AA or carboxyl groups, so the activity of the proteolytic enzymes is reduced (Mangan, 1972). Fibre content can also affect the degradability of protein, as the fibre in vegetable or plant proteins can form some sort of protection against bacterial degradation in the rumen (Ganev et al., 1979).

2.3.2. Solubility of protein

Solubility plays an important role in determining the degradability of the protein, as it determines the susceptibility of the protein to microbial proteases (Satter, 1986; Bach et al., 2005). Cereal grains and protein supplements have four different protein types, namely, albumins, globulins, prolamins and glutelins (Clark et al., 1987). The solubility of proteins is partly determined by the soluble albumins and globulins and

the amount of less soluble prolamins and glutelins (Tamminga, 1979). Albumins and globulins have a low molecular weight and are soluble in the rumen fluid, whereas prolamins and glutelins have a higher molecular weight and contain disulphide bonds, rendering them less soluble in rumen fluid. The prolamins and glutelins are therefore harder to access by the microbes and are more undegradable than the albumins and the globulins. The problem is that albumins and globulins have a much better AA composition and biological value than prolamins and glutelins, and it would be beneficial if they were not degraded so rapidly (Clark et al., 1987).

The solubility of feed protein is also affected by the treatment of feed during the manufacturing process (Tamminga, 1979). When proteins are treated with heat, it causes the proteins to denature. During denaturation, the structure of the protein is changed, and this exposes more of the hydrophobic amino acids, leading to a decrease in solubility (Russell & Hespell, 1981).

A lower N-solubility is associated with more AA available for absorption in the small intestine. However, one cannot only consider solubility; the structure of the protein is also important. Some soluble proteins contain disulfide bonds and are thus slowly degraded in the rumen (Bach et al., 2005). It is also possible for some proteins to be hydrolysed in the solid phase (Satter, 1986).

Solubility is not necessarily a good indication of degradation, as has been shown that some soluble proteins are degraded at a slower rate than some insoluble proteins (Mahadevan et al., 1980; Stern et al., 1994). This could be used as an indicator of degradation, but it cannot be used to compare a wide variety of feedstuffs differing in chemical and physical composition. Soluble proteins can be degraded rapidly or slowly and insoluble proteins are also degraded at various rates (Stern et al., 1994). For example, casein is readily degraded in the rumen but it is not readily soluble, whereas albumin is resistant to degradation, although it is readily soluble (McDonald et al., 2002). Combining solubility and the rate of degradation can be a better estimate of protein degradation (Stern & Satter, 1984).

2.3.3. Retention time and Intake

Protein degradation is inversely related to the rate of passage through the rumen (Ørskov & McDonald, 1979). Thus, the degree to which protein will be degraded in the rumen is greatly influenced by the time that the ingested feed is retained in the rumen (Satter, 1986). Retention time also affects the microbial growth occurring in the rumen (Isaacson et al., 1975; Russell et al., 1992).

Retention time varies greatly and differs from diet to diet but also between animals (Balch & Campling, 1965) and between species (Church, 1970; Tamminga, 1979). According to Hungate (1966), the retention time in cattle ranges from 1.3 to 3.7 days. The turnover rate of rumen fluid is usually much higher than the retention time, but most likely also influences the passage rate (Tamminga, 1979; Ørskov & McDonald, 1979).

Any factor that increases the rate of passage of digesta from the rumen would decrease the digestion taking place in the rumen, as it decreases the N and energy that are available to the micro-organisms (Erasmus et al., 1988). This could be advantageous for the digestion of nutrients such as protein and starch, as these could be more efficiently digested in the lower gut, but it has a negative effect on fibre digestion (McDonald et al., 2002).

The rate of passage of feed from the rumen is affected by both feed and animal factors. Feed factors can influence passage rate and is faster for smaller particles, particles with a higher density, hydrated particles and highly digested particles. The following animal factors would increase the passage rate: (i) in the last trimester of pregnancy, rumen fill is limited, and passage would increase and (ii) during lactation, intake increases and therefore passage rate also increases. Passage rate is also influenced by the environment. In a trial undertaken with sheep, it was found that colder environmental temperatures led to an increase in passage rate (Kennedy et al., 1976). Passage rate would decrease under the following circumstances: (i) fat animals have a lower intake and hence a lower passage rate and (ii) an increase in

environmental temperatures will decrease intake and also the passage rate (McDonald et al., 2002).

Roughage based diets are associated with a higher rate of passage of liquids through the rumen in comparison with concentrate diets. This is because roughage requires more rumination, so more saliva is added to the digesta. A diet with a high salt content will also increase the passage rate, as the salt would cause the water intake of the animal to increase (McDonald et al., 2002).

In Table 2.1 a comparison is given of the passage rates of two cows with different intakes and differences in the ratio of forage to concentrate in the diet. It is clear that the higher intake resulted in higher passage rates.

Table 2.1 Influence of intake on passage rate (NRC, 2001).

	Passage rates per hour	
	Cow A[1]	Cow B[1]
Wet forages	0.049	0.057
Dry forages	0.04	0.046
Concentrates	0.056	0.068

[1]With cow A: DMI = 18kg per day; Forage:Concentrate = 70:30 and cow B: DMI = 26kg per day; Forage:Concentrate = 40:60

Higher levels of feed intake lead to a decrease in the extent of protein degradation. This is probably due to a decrease in retention time that is associated with an increase in intake (Tamminga, 1979). As the passage rate is increased, the feed is exposed to the digestive enzymes for a shorter time, and this might decrease digestibility. Tamminga (1979) found that the percentage of dietary protein that was undegraded increased as intake increased. Cows that consumed 12.9 kg DM daily had 45% UDP while that of cows consuming 8.2 kg DM daily was 29%.

Increases in intake will either increase the amount of digesta retained in the rumen (rumen-fill) or increase the rate of passage, or both, but the animal has a limited intake (McDonald et al., 2002). In the high-producing dairy cow, intake is not only

15

influenced and regulated by the particle size of the forage but is also determined by other factors. Some of these factors include the type of forage and the fermentable carbohydrates in the diet as they affect the metabolism in the rumen (Tafaj et al., 2007).

Intake plays a role in the retention time of the feed, but it also plays a role in the degradation of protein, besides its influence on retention time. Intake also affects the pH in the rumen (Satter, 1986). Highly soluble proteins would be degraded independent of the feeding level, while more resistant proteins would be affected more by higher passage rates, which are associated with higher feeding levels (Miller, 1973). Another important factor that affects intake is the fibre content of the diet as well as the hydrolysis of the fibre (Allen, 2000).

Frequency of feeding also plays an important role in MCP synthesis. Feed intake is one of the most important factors affecting microbial protein yield (Sniffen & Robinson, 1987). In a trial with sheep, Al Attar et al. (1976) found that feeding at two-hour intervals resulted in greater MCP synthesis compared with feeding only once daily.

2.3.4. Rumen pH

Rumen pH influences the degree of protein degradation and, together with the type of diet, the pH will determine the microbial population of the rumen (Bach, 2005). The pH in the rumen usually ranges between 5.5 and 7, which is close to the optimum of between 6 and 7 for degradation (Blackburn & Hobson, 1960). It is influenced by both type of diet (Johnson & Sutton, 1968; Tremere et al., 1968) and frequency of feeding (Moir & Somers, 1957; Tremere et al., 1968; Kaufmann, 1976).

The rumen is buffered by bicarbonate, phosphate and proteins. During fermentation, acids are produced, and these acids can sometimes exceed the buffering capacity of the rumen. When the buffering capacity is exceeded, it will cause the pH in the rumen to decrease. If the pH drops too low for a sustained period, the rumen's functioning would be negatively affected, and this could cause a decrease in animal production (Russell; Hespell, 1981). A low pH will result in the inhibition of cellulolytic

16

micro-organisms, and hence, fibre digestibility will decrease (McDonald et al., 2002). According to Lewis and Emery (1962), deamination by the rumen bacteria becomes insignificant when the pH drops below 4.5.

A low pH is usually associated with a build-up of lactic acid in the rumen, causing an increase in the number of *Streptococcus bovis* and lactobacilli (Mackie et al., 1978), which also produce lactic acid. This increase in lactic acid production, together with a suppression of growth of the other micro-organisms in the rumen, plays an important role in the onset of rumen acidosis (Russell et al., 1979).

When the pH increases too much, it also has a negative effect on degradation. According to Chalmers (1969), when ruminal pH rose higher than 7.2, deamination ceased.

2.3.5. Feed processing

The form, as well as the amount of protein required by the animal and its microbes, may change dramatically when the feed is changed through processing. Processing is often used to increase the UDP fraction of the feed (Prigge et al., 1978). Processing methods that are used to decrease degradability are grinding, heat treatment or treatment with chemicals such as aldehydes, tannins and volatile fatty acids (Tamminga, 1979).

2.3.5.1. Heat processing

Sometimes feeds are subjected to heat during processing and/or storage. For example, by-product feeds that are dried before they are sold and ensiled feeds are sometimes exposed to high temperatures for long periods. Heat treatment results in the denaturation of the protein, and this changes the three-dimensional structure of the protein (Lykos & Varga, 1995). Processing methods used, such as pelleting, extrusion, steam rolling or flaking, can also expose the feed to sufficient heat to change the protein and the degradability thereof (Satter, 1986).

When a protein source is heat-treated, this leads to the Maillard reaction. This reaction takes place between the sugar aldehyde groups and the amino groups of the protein, and in the process an amino-sugar complex is produced. This complex is less degradable in the rumen as it is more resistant to enzymatic hydrolysis. The degree to which this reaction can be turned around would depend on the time and the temperature of the heat treatment (Stern et al., 1994).
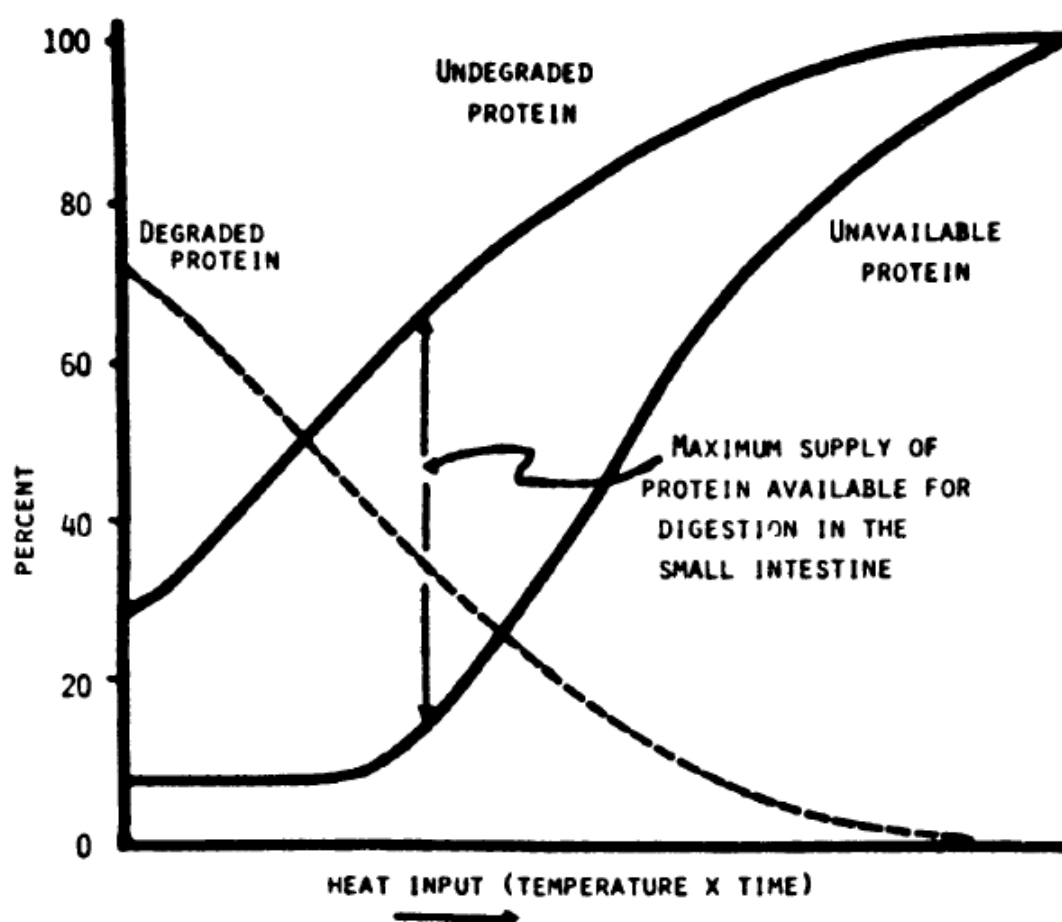
Figure 2.2 Influence of heat treatment on protein utilisation (Satter, 1986).

Figure 2.2 illustrates that, as the heat input increases, it causes the degradation of protein to decrease and so the amount of UDP is increased. At the same time, the amount of unavailable protein also increases. The optimum degradation would most likely be reached where there is a certain amount of heat damage to the protein so

that more protein can reach the small intestine in the form of UDP and become available to the animal for absorption (Satter, 1986).

The temperature and the duration of heat treatment are important as they will determine the amount of protein that would escape ruminal degradation (Stern et al., 1985). The efficiency of heat in protecting proteins from degradation in the rumen is influenced by a number of factors, such as the moisture content, the amount of soluble carbohydrates that are present, and the maximum temperature that is reached during processing (Satter, 1986).

Sources such as cottonseed, sunflower and soybean oilcake, with their high degradability, have a limited level of inclusion in dairy cattle diets, but these can be increased if the oilcakes are treated with heat (Cros et al., 1992). Treating these protein sources with heat usually causes a decrease in the amount of rapidly soluble N, the rate of degradation, and also the extent of CP degradation, but the amount of slowly degradable protein increases (Mosimanyana & Mowat, 1992).

When cottonseed oilcake (CSOC) was compared with soybean oilcake (SBOC), it was found that the CSOC can be processed at much higher temperatures than SBOC before heat damage occurs. Protein solubility decreases if heat processing is applied for long periods, especially for CSOC. It was found that the effective degradation of CSOC decreased from 73.7% to 45.5% when the oilcake was heat-treated, while that of SBOC decreased from 73.3% to 31.4% at an outflow rate of 8% per hour (Schroeder et al., 1995).

Stern et al. (1985) found that whole soybeans that were extruded at 149°C had lower protein degradation in the rumen and there was an increase in the flow of AA to the small intestine compared with soybeans extruded at 139°C or whole raw soybeans. Heating did not decrease digestion in the small intestine, probably due to it causing the trypsin inhibitor in the soybeans to become denatured (Stern & Hoover, 1979).

2.3.5.2. Chemical treatment

Chemical treatment is used to create a pH-dependent chemical modification so that the protein is not broken down at the pH in the reticulo-rumen, which is usually neutral, but which can be broken down at the lower pH occurring in the abomasum and in the proximal part of the duodenum (Tamminga, 1979). Some chemicals form linkages with amino and amide groups, and this may decrease their degradability in the rumen. These linkages are destructed when they are exposed to the acidious environment of the abomasum, and so the amount of UDP increases (Chalupa, 1975).

However, proteins should not be overprotected. This would cause the proteins to be undegradable in the rumen, but they would also be indigestible in the small intestine, excreted and thus wasted. Another problem with the overprotection of proteins is that, although this would cause the flow of dietary protein into the small intestine to increase, the flow of microbial protein may decrease if there are insufficient amounts of NH_3 for the micro-organisms. Less efficient micro-organisms would also cause a decrease in the degradation of other substrates such as the cellulose-rich cell walls of plants, and therefore, this would result in a general reduction of the digestibility of the feed (Tamminga, 1979).

Formaldehyde treatment

The most research has been done on the chemical treatment of feedstuffs with formaldehyde (Ferguson, 1975). Formaldehyde treatment is known to decrease the degradation of protein occurring in the rumen. It reacts with the terminal amino groups of protein and with the epsilon amino groups of lysine and forms methylene bridges or cross linkages. These bonds cause the protein to be more resistant to microbial degradation as they make the proteins less accessible to proteolysis (Barry, 1976).

In a trial done by Freer and Dove (1984) with cannulated sheep, it was found that formaldehyde treatment reduced the degradation from 80 to 15% for sunflower meal

20

and from 72 to 19% for canola meal when the fractional outflow from the rumen was 0.0416 h^{-1} (Freer & Dove, 1984).

A problem with the formaldehyde treatment of soybean meal is that it was shown that it decreased the availability of lysine and tyrosine (Erfle et al., 1986). In a study done by Weakley et al. (1983) on soybean meal, treatment with formaldehyde resulted in the decreased degradation of amino acids. This was also reported for canola and soybean meal (Rooke et al., 1983). Formaldehyde potentially also has human health hazards (Lundquist et al., 1986).

2.3.5.3. Diet composition

Any factor that has the ability to influence the microbial population of the rumen can potentially influence digestion in the rumen (Vanzant et al., 1998). The digestibility of a feed is not only affected by its own composition but also by the composition of the other feeds consumed with it (McDonald et al., 2002). Differences in the availability of nutrients for microbes in the rumen have an impact on their efficiency in producing MCP (Stern et al., 1994).

While the amounts of the different nutrients are important, the rate at which these nutrients become available in the rumen is also important. When protein degradation exceeds the rate of carbohydrate fermentation, excessive N will be produced and lost as ammonia. Likewise, if carbohydrate fermentation exceeds protein degradation, MCP synthesis will be negatively affected (Nocek & Russell, 1988).

Ensiled feeds, for example, usually contain high amounts of RDP and should be combined with feedstuffs that have low degradability, for optimal digestion (Erasmus et al., 1988). If the carbohydrate supply to the rumen varies constantly, it may cause a reduction in MCP synthesis, so the animal would require more UDP to meet the AA requirements. Feedstuffs that contain highly fermentable substances, such as the starch and sugars in barley, wheat, molasses, etc., should rather be fed frequently instead of just twice a day, as this would decrease the need for UDP (Erasmus et al., 1988).

21

Fat content also affects degradation in the rumen, mainly because of the influence it has on the microbial population in the rumen (Ørskov et al., 1978) and not because of the protective coating it forms on the surfaces of feeds. It especially affects fibre digestion (Brooks et al., 1954) and cellulose digestion (Kowalczyk et al., 1977).

According to Assoumani et al. (1992), starch influences the degradation of protein. When additional amylase was added to cereal grains, protein degradation increased from 6 to 20%. In many of the plant protein sources, the proteins are 'protected' by a fibre matrix. These proteins cannot be degraded unless the fibre matrix is degraded first, as it prevents the proteases from accessing the proteins. Thus, the degradation of protein requires several proteolytic as well as non-proteolytic enzymes in the rumen (Bach et al., 2005). A diet with high forage content is associated with increased cellulolytic activity in the rumen. This increase in cellulolytic activity would result in more protein being exposed to the micro-organisms for degradation in the rumen (Weakley et al., 1983).

2.3.5.4. Particle size

Micro-organisms in the rumen can only utilise feedstuffs once the polymers in the feedstuffs are degraded by extracellular enzymes. As the extracellular enzymes act on the surface of the feedstuffs, particle size is important for ruminal fermentation. Particle size in the rumen is determined by the treatment of the feed prior to ingestion (pelleting, grinding, chopping etc.), rumination and digestion (Russell & Hespell, 1981). Fermentation is enhanced by smaller particle sizes, as small particles have a greater surface to mass ratio (Hungate, 1966). Smaller particles also result in better exposure of the substrate to micro-organisms.

Large and irregular shaped feed particles with a low specific gravity, move to the top of the rumen and are maintained in the rumen. This ensures that large particles are subjected to mechanical and microbial breakdown before they leave the rumen through the reticulo-omasal orifice (Balch & Campling, 1965). The smaller and

22

denser particles are washed out of the rumen with the rumen liquor (McDonald et al., 2002).

Freer & Dove (1984) compared the degradation of different particle sizes of lupin seeds in cannulated sheep. Three particle sizes were used: those milled with a 0.8 mm screen (fine), those milled with a 4.0 mm screen (medium) and those milled three times without a screen (coarse). After two hours of incubation, 85%, 45% and 10% of the N had disappeared from the fine, medium and coarse samples, respectively. In a trial with finely ground corn (FGC), cracked corn (CC) and chick-cracked corn (CCC), smaller particle sizes, through grinding, caused a higher solubility and degradation rate of DM, CP and total non-structural carbohydrates (TNC) for FGC compare with CC and CCC (Lykos & Varga, 1995).

Although a decrease in particle size causes an increase in the degradability of CP and non-structural carbohydrates (Lykos & Varga, 1995), the reduction of particle sizes should be exercised with caution. The formation of acids in the rumen needs to be neutralised in order to maintain an optimum environment for the rumen microbes. This requires a minimum daily amount of chewing activity and saliva secretion, which will only be maintained if the diet contains enough structural fibre (Tafaj et al., 2007).

Another problem is clumping of the feed. If the particles are too small, it could cause clumping of the particles and so degradability would decrease. Figroid (1972) found that particles smaller than 0.6 mm resulted in clumping and hence lower degradability. Smaller particles can also pass unfermented from the rumen. If the passage rate exceeds the increase in fermentation, ruminal fermentation would be lowered (Waldo et al., 1972).

Particle size also affects DM intake. The degree to which intake is influenced by particle size is affected by the diet and its content. Factors that have an effect are the type of forage in the diet, the ratio of forage to concentrate, and the type of concentrate included (Tafaj et al., 2007).

23

2.3.6. Natural resistance to degradation

The simplest way to increase the UDP fraction in a diet is to use feedstuffs with a natural resistance to degradation in the rumen. Some protein sources in their natural state are more resistant to degradation than others (Weakley et al., 1983; Stern et al., 1994). This makes these proteins good sources of UDP. One of these sources is distiller's grain (DG). In a trial done by Weakley et al. (1983), DG was compared with soybean meal (SBM), and it was found that DG had a lower degradation than SBM. This could be due to the increased resistance of DG to micro-organisms (Weakley et al., 1983).

According to the National Research Council (NRC, 1985), 50% or more of the protein in corn gluten meal (gluten 60), and fish meal escapes microbial degradation in the rumen and passes to the SI. In contrast, the protein in soybean meal and corn gluten feed (gluten 20) is extensively degraded in the rumen (Firkins et al., 1984).

2.4. Protein sources for dairy cattle

The quality of the proteins provided to dairy cattle is very important, especially during early lactation (Ørskov et al., 1981). The protein sources that were selected for this study are common protein sources for dairy cattle diets in South Africa. They are soybean oilcake, sunflower oilcake, maize gluten 20, maize gluten 60, and fish meal.

2.4.1. Specific protein sources for this study

2.4.1.1. Oilcakes

With the removal of oil from the oilseeds, oilseed cakes or meals are produced as by-products. The oil is usually removed from the oilseeds with one of two processes. It is either removed with pressure that forces the oil out, or with an organic solvent, such as hexane, that dissolves the oil from the seed (McDonald et al., 2002). These

by-products have relatively high protein content (200 to 500 g/kg), so it is a good source of protein for farm animals (McDonald et al., 2002).

Soybean oilcake/meal

Soybeans contain 160 to 210 g/kg oil, and the oil is usually extracted with a solvent. The residual meal is one of the best sources of protein for animal feeding, as it contains more or less 45% to 50% protein. It is one of the most common protein feedstuffs that are used in the diets of dairy cattle. Although it provides a relatively high quality protein, it is also quite degradable in the rumen and therefore it is often treated before it is fed to cattle (Stern et al., 1994). Soybean oilcake usually contains all the essential amino acids, but the concentrations of cysteine and methionine are suboptimal (McDonald et al., 2002).

Sunflower oilcake/meal

Sunflower oil is removed from the seeds by hydraulic pressure or solvent extraction. Sunflower seeds have a thick coat or hull that has a high fibre content and low digestibility and this lowers its nutritional value. The hulls can be removed partly or completely by a process of decortication, so the nutritive value of the oilcake could be raised. The hulls are usually only partly removed, and it is best to grind these oilcakes finely to make them more palatable for the animals (McDonald et al., 2002).

Sunflower oil is prone to oxidation, and therefore, the meals have a short shelf life, as the oxidation causes rancidity that makes the meal unpalatable. It normally has a low lysine content, which is the first limiting amino acid, but it contains twice as much methionine as soybean protein. The maximal inclusion rates of sunflower oilcake for cattle are 200 kg/t (McDonald et al., 2002). It is highly degradable, and when it was compared with cottonseed oilcake, the degradability of sunflower was 86.2% (±2.1) compared to 54.5% (±1.9) for cottonseed oilcake (Erasmus et al., 1988).

2.4.1.2. Maize gluten

When maize is processed to produce starch, it results in by-products that can be used in animal feeds. One of these by-products is gluten. Cleaned maize is soaked in a diluted acid solution and then ground through a coarse screen. The maize germ floats on the surface and is removed for further processing. The de-germed maize is then ground with a fine screen and the bran is removed with the aid of wet screening. The remainder of the maize subsequently consist of starch and protein or gluten, and these form a suspension in the liquid. The starch is removed with centrifugation, resulting in gluten as a by-product (McDonald et al., 2002).

Maize gluten has a very high protein content, which can be as high as 700 g/kg DM (McDonald et al., 2002). The maize gluten used in this study was gluten 20 (or maize gluten feed), which has a CP content of more or less 20%, and gluten 60 (or maize gluten meal), which has a CP content of more or less 60%.

2.4.1.3. Fish meal

Fish meal is usually resistant to degradation in the rumen, but its degradability varies, as the processing of different fish meals varies (Stern et al., 1994). If the fish meal is treated with moderate heat, it will cause the formation of disulfide bridges and so the rate of ruminal proteolysis would decrease. Hence, fish meal can serve as a good source of UDP (Chen et al., 1987).

Fish meal is produced by cooking fish, and then pressure is used to remove most of the oil and water. This liquor is then concentrated and added back to the pressed mass, and all of it is dried. The protein quality of fish meal is largely influenced by the processing conditions, especially the degree and time of heating. Fish meal is a good source of all the essential amino acids, especially lysine, cysteine, methionine and tryptophan (McDonald et al., 2002).

26

2.5. The *in sacco* technique (Dacron bag or nylon bag technique)

It is important to measure protein degradation as it determines the protein that would be available to the host animal as well as the N supply to the micro-organisms in the rumen (Mehrez & Ørskov, 1977). Therefore, the N content of feed should be expressed in terms of degradability in the rumen (Freer & Dove, 1984).

Various methods or techniques are used to determine protein degradability. The ideal is *in vivo* measurements. With this method, the amount of protein that passes to the abomasum or duodenum can be measured. Thus, the animals need to be surgically prepared with cannulae. This technique is labour intensive, time consuming and subject to a significant amount of error (Stern & Satter, 1984; Erasmus et al., 1988), and therefore, it is not suitable to determine degradability routinely or on a large scale (Erasmus et al., 1988).

Protein degradation can also be determined *in vitro* in a laboratory. One such a method is the determination of protein solubility. Protein solubility is not equal to degradability, and therefore, it cannot be used to compare different feedstuffs with one another. It can however, be useful to compare different treatments of the same feedstuff (NRC, 2001). As *in vitro* methods can only imitate the rumen environment, they are subject to errors, and values obtained are very variable (Mohamed & Chaudhry, 2008). An alternative method is the *in sacco* technique.

The *in sacco* technique was first used in 1938 by Quin, Van Der Wath and Myburgh (Quin *et al.*, 1938; cited by Van Keuren & Heinemann, 1962; Mehrez & Ørskov, 1977; Weakley et al., 1983). The bags used were cylindrical and made of natural silk, and the animals for the experiment were cannulated sheep. Ever since 1938, this technique has been used by many workers (Mehrez & Ørskov, 1977). The test diet is contained in a bag that is incubated in the rumen for various periods, and then the disappearance of the feed from the bag is measured (Mehrez & Ørskov, 1977). This technique provides a relatively easy and fast way to measure the rate and extent of degradation taking place in the rumen, and it enables one to understand

27

better the fermentation taking place in the rumen (Ørskov et al., 1980; Stern et al., 1994).

The nylon bag technique is the best method available to determine protein degradability on a regular basis (Erasmus et al., 1988). Digestion can be studied in the rumen without the need to simulate the rumen environment (Vanzant et al., 1998). It is not a very expensive method, and rumen cannulation is also not very difficult. Once the animals are cannulated, they can be used in a number of trials (Ørskov et al., 1980).

The proportion of protein that will escape degradation can be determined by combining the rate of disappearance from the nylon bag with the fractional rate of out-flow (Ganev et al., 1979). One of the advantages of the *in sacco* technique over *in vitro* methods is that the *in sacco* technique involves digestion occurring in the rumen of a live animal (Stern et al., 1994).

Although the nylon bag technique is the best technique available for determining degradation on a regular basis, its use does have some causes for concern. In the first place, the samples are not subjected to chewing and rumination. Samples are placed in a bag that is directly placed into the rumen, so no mechanical breakdown can occur (Ørskov et al., 1980; Erasmus et al., 1988). Another limitation is that the feed will normally leave the rumen when broken down to a certain extent and size. However, the samples are trapped in a bag, and therefore, feed is unable to leave the rumen (Ørskov et al., 1980). This may lead to microbial contamination, which would cause considerable error, as CP degradation would be underestimated (Kennedy et al., 1984).

Lastly, this technique measures the breakdown of material to a size small enough to leave the bag, and this is not necessarily the same as degradation into simple chemical compounds (Ørskov et al., 1980; Erasmus et al., 1988). It measures the rate of disappearance from the bag and not actual degradation. Therefore, small particles could be washed out of the bag without necessarily being degraded (Erasmus et al., 1990). The rates at which the particles are degraded are also

unknown. If the particles that escape from the bags are degraded at the same rate as the particles that remain in the bags, N degradability could be overestimated (Michalet-Doreau & Cerneau, 1991).

2.6. References

Al Attar, A., Evans R.A., & Axford R.F., 1976. The effect of frequency of feeding and of the dietary energy source upon microbial protein synthesis in the rumen of sheep. Proc. Nutr. Soc. 35(2): 108A-109A.

Allen, M.S., 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83(7): 1598-1624.

Assoumani, M.B.F., Vedeau L.J. & Sniffen C.J., 1992. Refinement of an enzymatic method for estimating the theoretical degradability of proteins in feedstuffs for ruminants. Anim. Feed Sci. Technol. 39: 357-368.

Bach, A., Calsamiglia, S. & Stern, M.D., 2005. Nitrogen metabolism in the rumen. J. Dairy Sci. 88: E9-E21.

Balch, C.C. & Campling, R.C., 1965. Rate of Passage of Digesta through the Ruminant Digestive Tract. In: Physiology of Digestion in the Ruminant. Ed. Dougherty, R. W.Butterworth, Washington, DC. pp. 108-123.

Barry, T.N., 1976. The effectiveness of formaldehyde treatment in protecting dietary protein from rumen microbial degradation. Proc. Nutr. Soc. 35(02): 221-229.

Blackburn, T.H. & Hobson P.N., 1960. Proteolysis in the sheep rumen by whole and fractionated rumen contents. Microbiology. 22(1): 272.

Brooks, C.C., Garner, G.B., Gehrke, C.W., Muhrer, M.E. & Pfander, W.H., 1954. The effect of added fat on the digestion of cellulose and protein by ovine rumen microorganisms. J. Anim. Sci. 13(4): 758.

Broster, W.H., 1975. Principles and practice in level of feeding for the dairy cow. S. Afr. J. Anim. Sci. (5): 143-148.

Chalupa, W., 1977. Manipulating rumen fermentation. J. Anim. Sci. 45(3): 585.

Chalupa, W., 1975. Rumen bypass and protection of proteins and amino acids. J. Dairy Sci. 58(8): 1198-1218.

Chalmers, M.I., 1969. Nitrogen Nutrition for Lactation. In: Lactation. Ed. Falconer, I. R.Butterworth, London. pp. 379-391.

Chen, G., Sniffen, C.J., & Russell, J.B., 1987. Concentration and estimated flow of peptides from the rumen of dairy cattle: Effects of protein quantity, protein solubility, and feeding Frequency. J. Dairy Sci. 70(5): 983-992.

Church, D.C., 1970. Passage of Digesta through the Gastro-Intestinal Tract. In: Digestive Physiology and Nutrition of Ruminants, Ed. Church, D. C. Vol. I ed. Oregon State University Book Stores, Oregon. pp. 85-99.

Clark, J.H. & Davis, C.L., 1980. Some aspects of feeding high producing dairy Cows1. J. Dairy Sci. 63(6): 873-885.

Clark, J.H., Klusmeyer, T.H. & Cameron, M.R., 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy Cows1. J. Dairy Sci. 75(8): 2304-2323.

Clark, J.H., Murphy, M.R. & Crooker, B.A., 1987. Supplying the protein needs of dairy cattle from by-product Feeds1. J. Dairy Sci. 70(5): 1092-1109.

Crish, E.M., Wohlt, J.E. & Evans, J.L., 1986. Insoluble nitrogen for milk production in holstein cows via increases in voluntary intake and nitrogen Utilization1. J. Dairy Sci. 69(6): 1576-1586.

Cros, P., Moncoulon, C., Bayourthe, C. & Vernay, M., 1992. Effect of extrusion on ruminal and intestinal disappearance on amino acids in white lupin seed. Can. J. Anim. Sci. 72: 89-96.

Ensminger, M.E., 1993. Dairy cattle science. Third ed. Interstate Publishers. United States of America.

Erasmus, L.J., Prinsloo, J., Botha, P.M. & Meissner, H.H., 1990. Establishment of a ruminal protein degradation data base for dairy cattle using the *in situ* polyester bag technique. 2 energy sources. S. Afr. J. Anim. Sci. 20: 124-129.

Erasmus, L.J., Prinsloo, J. & Meissner, H.H., 1988. The establishment of a protein degradability data base for dairy cattle using the nylon bag technique. 1. protein sources. S. Afr. J. Anim. Sci. 18: 23-29.

Erfle, J.D., Sauer, F.D., Mahadevan, S. & Teather, R.M., 1986. Response of lactating dairy cows to formaldehyde-treated soybean meal when fed with control or urea-treated corn silage. Can. J. Anim. Sci. 66(1): 85-95.

Ferguson, K.A., 1975. The Protection of Dietary Proteins and Amino Acids Against Microbial Fermentation in the Rumen. In: Digestion and Metabolism in the Ruminant. Eds. McDonald, I. W., and Warner,A. C. I. The University of New England Publishing Unit, Armidale. pp. 448-464.

Figroid, W., Hale W.H. & Theurer B. 1972. An evaluation of the nylon bag technique for estimating rumen utilization of grains. J. Anim. Sci. 35(1): 113.

Firkins, J.L., Berger, L.L., Fahey, Jr G.C. & Merchen, N.R., 1984. Ruminal nitrogen degradability and escape of wet and dry distillers grains and wet and dry corn gluten feeds. J. Dairy Sci. 67(9): 1936-1944.

Freer, M. & Dove, H., 1984. Rumen degradation of protein in sunflower meal, rapeseed meal and lupin seed placed in nylon bags. Anim. Feed Sci. Technol. 11(2): 87-101.

Ganev, G., Ørskov, E.R. & Smart, R., 1979. The effect of roughage or concentrate feeding and rumen retention time on total degradation of protein in the rumen. J. Agric. Sci. 93: 651-656.

Hibbit, K.G., 1988. Effect of Protein on the Health of Dairy cows. In: Recent Developments in Ruminant Nutrition 2. Eds. Haresign, W., and Cole, D. J. A. First ed. Butterworths, England. pp. 184-195.

Hungate, R.E., 1966. *The rumen and its microbes*. Academic Press. New York and London.

Isaacson, H.R., Hinds, F.C., Bryant, M.P. & Owens, F.N., 1975. Efficiency of energy utilization by mixed rumen bacteria in continuous culture. J. Dairy Sci. 58(11): 1645-1659.

Johnson, V.W. & Sutton, J.D., 1968. The continuous recording of the pH in the bovine rumen. Br. J. Nutr. 22: 303-307.

Kaufmann, W., 1976. Influence of the composition of the ration and the feeding frequency on ph-regulation in the rumen and on feed in-take in ruminants. Livest. Prod. Sci. 3(2): 103-114.

Kennedy, P.M., Christopherson, R.J. & Milligan, L.P.,1976. The effect of cold exposure of sheep on digestion, rumen turnover time and efficiency of microbial synthesis. Br. J. Nutr. 36(2): 231-242.

Kennedy, P.M., Hazlewood, G.P. & Milligan, L.P., 1984. A comparison of methods for the estimation of the proportion of microbial nitrogen in duodenal digesta, and of correction for microbial contamination in nylon bags incubated in the rumen of sheep. Br. J. Nutr. 52(02): 403-417.

Kowalczyk, J., Ørskov, E.R., Robinson, J.J. & Stemend, C.S., 1977. Effect of fat supplementation on voluntary food intake and rumen metabolism in sheep. Br. J. Nutr. 37(2): 251-257.

Lewis, T.R. & Emery R.S., 1962. Intermediate products in the catabolism of amino acids by rumen Microorganisms1. J. Dairy Sci. 45(11): 1363-1368.

Lundquist, R.G., Otterby, D.E. & Linn, J.G. 1986. Influence of formaldehyde-treated soybean meal on milk production, 2. J. Dairy Sci. 69: 1337-1345.

Lykos, T. & Varga, G.A., 1995. Effects of processing method on degradation characteristics of protein and carbohydrate sources *in situ*. J. Dairy Sci. 78(8): 1789-1801.

Mackie, R.I., Gilchrist, F., Robberts, A.M., Hannah, P.E. & Schwartz, H.M., 1978. Microbiological and chemical changes in the rumen during the stepwise adaptation of sheep to high concentrate diets. J. Agric. Sci. 90(02): 241-254.

Mahadevan, S., Erfle, J.D. & Sauer, F.D., 1980. Degradation of soluble and insoluble proteins by bacteroides amylophilus protease and by rumen microorganisms. J. Anim. Sci. 50(4): 723.

Mangan, J.L., 1972. Quantitative studies on nitrogen metabolism in the bovine rumen: The rate of proteolysis of casein and ovalbumin and the release and the metabolism of free amino acids. Br. J. Nutr. 27: 261-283.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. & Morgan, C.A., 2002. Animal Nutrition. 6th ed. Pearson Education Limited. Harlow.

Mehrez, A.Z. & Ørskov, E.R., 1977. A study of artificial fibre bag technique for determining the digestibility of feeds in the rumen. The Journal of Agricultural Science. 88(03): 645-650.

Michalet-Doreau, B. & Cerneau, P., 1991. Influence of foodstuff particle size on *in situ* degradation of nitrogen in the rumen. Anim. Feed Sci. Technol. 35(1-2): 69-81.

Miller, E.L., 1982. Methods of Assessing Proteins for Ruminants, Including Laboratory Methods. In: *Protein Contribution of Feedstuffs for Ruminants*. Eds. Miller, E.L., Pike, I.H. & Van Es, A.J.H. 1st ed. Butterworth Scientific, England: 18-19.

Miller, E.L., 1973. Evaluation of foods as sources of nitrogen and amino acids. Proc. Nutr. Soc. 32(02): 79-84.

Mohamed, R. & Chaudhry, A.S., 2008. Methods to study degradation of ruminant feeds. Nutrition Research Reviews. 21(1): 68.

Moir, R.J. & Somers, M., 1957. Ruminal flora studies. VIII. the influence of rate and method of feeding a ration upon its digestibility, upon ruminal function, and upon the ruminal population. Aust. J. Agric. Res. 8: 253-265.

Mosimanyana, B.M. & Mowat, D.N., 1992. Rumen protection of heat-treated soybean proteins. Can. J. Anim. Sci. 72: 71-81.

National Research Council (NRC), 2001. Nutrient Requirements of Dairy Cattle. 7th Rev. ed. National Academy Press. Washington, D. C.

National Research Council (NRC), 1985. Ruminant Nitrogen Usage. National Academy Press. Washington D.C.

Nocek, J.E. & Russell, J.B., 1988. Protein and energy as an integrated system. relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J. Dairy Sci. 71(8): 2070-2107.

Nocek, J.E. & Tamminga, S., 1991. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. J. Dairy Sci. 74(10): 3598-3629.

Nungent, J.H.A. & Mangan, J.L., 1978. Rumen proteolysis of fraction I leaf protein, casein and bovine serum albumin. Proceedings of the Nutrition Society.

Ørskov, E.R., Reid, G.W. & McDonald, I., 1981. The effects of protein degradability and food intake on milk yield and composition in cows in early lactation. Br. J. Nutr. 45(03): 547-555.

Ørskov, E.R., Hine, R.S. & Grubb, D.A., 1978. The effect of urea on digestion and voluntary intake by sheep of diets supplemented with fat. Anim. Prod. 27(03): 241-245.

Ørskov, E.R., Hovell, F.D. & Mould, F., 1980. The use of the nylon bag technique for the evaluation of feedstuffs. Tropical Animal Production. 5(3): 195-213.

Ørskov, E.R. & McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92(02): 499-503.

Prigge, E.C., Galyean, M.L., Owens, F.N., Wagner, D.G. & Johnson, R.R., 1978. Microbial protein synthesis in steers fed processed corn rations. J. Anim. Sci. 46(1): 249.

Rooke, J.A., Brookes, I.M. & Armstrong, D.G., 1983. The digestion of untreated and formaldehyde-treated soyabean and rapeseed meals by cattle fed a basal silage diet. J. Agric. Sci. 100: 329-342.

Russell, J.B. & Hespell, R.B., 1981. Microbial rumen fermentation. J. Dairy Sci. 64(6): 1153-1169.

Russell, J.B., O'connor, J.D., Fox, D.G., Van Soest, P.J. & Sniffen, C.J., 1992. A net carbohydrate and protein system for evaluating cattle diets: I. ruminal fermentation. J. Anim. Sci. 70(11): 3551.

Russell, J.B., Sharp, W.M. & Baldwin, R.L., 1979. The effect of pH on maximum bacterial growth rate and its possible role as a determinant of bacterial competition in the rumen. J. Anim. Sci. 48(2): 251.

Satter, L.D., 1986. Protein supply from undegraded dietary protein. J. Dairy Sci. 69(10): 2734-2749.

Schroeder, G.E., Erasmus, L.J., Leeuw, K.J. & Meissner, H.H., 1995. Effect of roasting on ruminal degradation, intestinal digestibility and absorbable amino acid profile of cottonseed and soybean oilcake meals. S. Afr. J. Anim. Sci. 25(4): 109-117.

Schwab, C.G., Huhtanen, P., Hunt, C.W. & Hvelplund, T., 2005. Nitrogen Requirements of Cattle In: Nitrogen and Phosphorus Nutrition of Cattle: Reducing the Environmental Impact of Cattle Operations. Eds. Pfeffer, E., & Hristov, A.N.CABI Publishing, Oxfordshire, UK: 13-70.

Scott, T.A., Combs, D.K. & Grummer, R.R., 1991. Effects of roasting, extrusion, and particle size on the feeding value of soybeans for dairy cows. J. Dairy Sci. 74(8): 2555-2562.

Sniffen, C.J. & Robinson, P.H., 1987. Microbial growth and flow as influenced by dietary manipulations. J. Dairy Sci. 70(2): 425-441.

Stern, M.D., Bach, A. & Calsamiglia, S., 2006. New concepts in protein nutrition of ruminants. 21st annual southwest nutrition & management conference.

Stern, M.D. & Hoover, W.H., 1979. Methods for determining and factors affecting rumen microbial protein synthesis: A review. J. Anim. Sci. 49(6): 1590.

Stern, M.D. & Satter, L.D., 1984. Evaluation of nitrogen solubility and the dacron bag technique as methods for estimating protein degradation in the rumen. J. Anim. Sci. 58(3): 714.

Stern, M.D., Varga, G.A., Clark, J.H., Firkins, J.L., Huber, J.T. & Palmquist, D.L., 1994. Evaluation of chemical and physical properties of feeds that affect protein metabolism in the Rumen1. J. Dairy Sci. 77(9): 2762-2786.

Stern, M.O., Santos, K.A. & Satter, L.D., 1985. Protein degradation in rumen and amino acid absorption in small intestine of lactating dairy cattle fed heat-treated whole soybeans. J. Dairy Sci. 68(1): 45.

Tafaj, M., Zebeli, Q., Baes, C., Steingass, H. & Drochner, W., 2007. A meta-analysis examining effects of particle size of total mixed rations on intake, rumen digestion and milk production in high-yielding dairy cows in early lactation. Anim. Feed Sci. Technol. 138(2): 137-161.

Tamminga, S., 1979. Protein degradation in the forestomachs of ruminants. J. Anim. Sci. 49(6): 1615.

Tice, E.M., Eastridge, M.L. & Firkins, J.L., 1993. Raw soybeans and roasted soybeans of different particle sizes. 1. Digestibility and utilization by lactating cows. J. Dairy Sci. 76: 224-224.

Tremere, A.W., Merrill, W.G. & Loosli, J.K., 1968. Adaptation to high concentrate feeding as related to acidosis and digestive disturbances in dairy heifers. J. Dairy Sci. 51(7): 1065-1072.

Van der Merwe, F.J. & Smith, W. A., 1991. *Dierevoeding*. 2nd ed. Anim Sci Pty Ltd. Pinelands.

Van Keuren, R.W. & Heinemann, W.W., 1962. Study of a nylon bag technique for in vivo estimation of forage digestibility. J. Anim. Sci. 21(2): 340.

Van Straalen, W.M. & Tamminga, S., 1990. Protein Degradation of Ruminant Diets. In: Feedstuff Evaluation. Ed. Wiseman, J. and Cole, D.J.A. 1st ed. Butterworths, London. pp. 55-72.

Vanzant, E.S., Cochran, R.C. & Titgemeyer, E.C., 1998. Standardization of *in situ* techniques for ruminant feedstuff evaluation. J. Anim. Sci. 76(10): 2717.

Waldo, D.R., Smith, L.W. & Cox, E.L., 1972. Model of cellulose disappearance from the rumen. J. Dairy Sci. 55(1): 125-129.

Weakley, D.C., Stern, M.D. & Satter, L.D., 1983. Factors affecting disappearance of feedstuffs from bags suspended in the rumen. J. Anim. Sci. 56(2): 493.

CHAPTER 3

Effect of fine particle removal on chemical composition and protein solubility of protein sources for dairy cattle diets

Abstract

In preparation of in sacco digestibility trials, the NRC recommends the grinding of feedstuffs through a 2 mm screen. In the current study, five protein feedstuffs, viz. soybean oilcake, sunflower oilcake, maize gluten 20, maize gluten 60 and fish meal, were ground according to NRC guidelines and subsequently sieved through either a 106 μm or a 150 μm mesh. The control treatment was not sieved after grinding. The objectives of the study were to determine the crude protein solubility of the feedstuffs, the amount of fine material lost through sieving, and the effect of sieving on the chemical composition of the feedstuffs. The crude protein solubility of soybean oilcake, sunflower oilcake, maize gluten 20, maize gluten 60 and fish meal was 25.5%, 42.2%, 67.0%, 10.0% and 19.1%, respectively. Sieving resulted in 20 to 60% of the ("as is") material being lost, with the 150 μm mesh sieve having the highest losses. Sieving had no effect on the chemical composition of the sieve residues, except for maize gluten 20 (both mesh sizes) and fish meal (150 μm mesh). Protein solubility was determined in our laboratory and at Nutrilab, University of Pretoria. The deviation that occurred between the two laboratories ranged from 2.7 % to 73.4%. It was concluded that samples should be sieved through a 106 μm mesh prior to in sacco incubation.

3.1. Introduction

Nutrition plays an important role in the profitability of a dairy operation. Therefore, the available nutrients should be used efficiently and cost-effectively. This would only be possible if a well balanced diet is fed, ensuring that cattle could reach and maintain maximal milk production. To enable nutritionists to formulate a balanced diet, an accurate determination of the chemical composition and digestive properties of feedstuffs is required (Van der Merwe & Smith, 1991).

Protein is one of the most important nutrients affecting milk production (Clark & Davis, 1980). In Chapter 2, various factors that affect crude protein degradability were discussed. Two of these were the particle size of the feedstuffs and the solubility of the protein. Although protein solubility is not the same as protein degradability, it could be used as a measure to compare the degradability of different samples of the same feedstuff (Stern et al., 1994). It is an important factor determining the susceptibility of protein to microbial proteases and thus affects the degradability of protein (Bach et al., 2005).

In preparation of *in sacco* trials to determine the protein degradability of feedstuffs, samples are usually milled through a 2 mm screen, according to NRC recommendations (NRC, 2001). Such milling would result in various amounts of fine particles that could potentially escape through the pores of dacron bags. Although the mean pore size of dacron bags is usually provided by suppliers, large variations may occur. According to Cruywagen (2007; unpublished data), who did an image analysis on dacron material of bags (designated mean pore size = 53 µm) provided by Bar Diamond (Parma, ID, USA), the pore sizes ranged from 31 to 99 µm, with a mean of 63 µm. Thus, small particles (<100 µm) in ground feed samples may escape from the bags without being degraded and may lead to an overestimation of the zero-hours degradation values. A series of trials were performed to investigate the effect of fine particle removal on various evaluation parameters. Fine particles were removed by sieving ground samples through screens with different mesh sizes.

The objectives of the current study were the following:

- To determine the amount of material that is lost after sieving through different mesh sizes.

- To determine the chemical composition of the feedstuff residues remaining on top of the different screens, to see how it compares with that of the original ground samples.

- To compare the CP-A fraction (or a-value obtained from the *in sacco* procedure) of the respective sieve residues with the CP solubility values of the original samples.

3.2. Materials and methods

3.2.1. Treatments

Five different protein feedstuffs were used in this study, namely; soybean oilcake, sunflower oilcake, maize gluten 20, maize gluten 60, and fish meal. Samples of all the feedstuffs were milled through a 2 mm screen with a Scientec Hammer mill (Scientec, Cape Town, RSA). Three treatments were used to determine the effect of fine particle removal on the chemical composition of the feedstuffs.

Treatments were as follows:
1. Ground (2 mm), with no subsequent sieving.
2. Ground (2 mm), followed by sieving through a 106 µm screen.
3. Ground (2 mm), followed by sieving through a 150 µm screen.

The samples were sieved in portions of more or less 20 grams at a time. Samples were sieved with the aid of a vibrating sieve shaker (Retsch AS 200 basic, supplied by Wirsam Scientific, Cape Town) at an amplitude of more or less 80, for 10 minutes. Following this procedure, the residues were gently brushed with a soft brush, to ensure that the remainder of the fine particles were brushed through the sieve. The top and bottom sieved fractions were weighed to determine the amount of fine particles that were removed from the samples. Samples from the top fraction (residue), as well as from the original ground feedstuffs were taken for chemical

analysis and for the *in sacco* trial. All the samples were sealed in plastic containers and stored in a cold room at 4 ºC.

3.2.2. Bone-and-scales (BS) fraction

The bone-and-scales (BS) fraction in pelagic fish meal usually varies between 14 and 24%. The BS fraction was determined for all three treatments of the fish meal used in this study. It was done by adding 30 ml of chloroform to approximately two grams of sample in 50 ml porcelain evaporating dishes. After stirring, the BS fraction, which is the heavy fraction, was allowed to settle. Then the rest of the material was removed by carefully decanting the dishes and removing the light fraction. The dishes containing the BS residues were placed in an oven at 55 ºC for 15 minutes to allow any remaining chloroform to evaporate. The BS residues were then weighed and expressed as a percentage of the original sample on an air-dry basis.

3.2.3. Dacron bag *a*-values

The a-values obtained in *in sacco* studies contain soluble and rapidly degradable protein (Ørskov et al., 1980; Freer & Dove, 1984; NRC, 2001). Samples (8 g) were weighed into 200 mm x 100 mm dacron bags (Bar Diamond, Parma, ID, USA). The open ends of the bags were double folded and each bag was closed with two cable ties.

For the a-values (zero hours of incubation), the bags were not incubated in rumen liquor, but were washed in water using a twin-tub washing machine. The bags were washed repeatedly in one minute cycles (each cycle in clean water) until the drained water appeared clear. Between each cycle, the bags were spin dried for 15 seconds. Following the final cycle, bags were spin dried for one minute. After washing, the bags were dried at 60 ºC to constant weight (72 hours). Bags were weighed and stored at 4 ºC in a cool room until further analysis.

3.2.4. Chemical analyses

All the samples were analysed according to the methods described by the Association of Official Analytical Chemists (AOAC):

- Moisture (AOAC, 2002. Loss on drying (moisture) at 95 – 100 °C for feeds. AOAC Official Method 934.041).
- Ash (AOAC, 2002. Ash of Animal Feeds. AOAC Official Method 942.05).
- Crude Fibre (AOAC, 2002. Fibre (crude) in Animal Feed and Pet Food. AOAC Official Method 962.09).
- Crude Fat (AOAC, 2002. Fat (crude) or Ether Extract in Animal Feed. AOAC Official Method 920.39).
- Crude Protein, with the aid of the Leco protein analyser (LECO FP-528) (AOAC, 2002. Crude protein in meat and meat products including pet foods. Combustion method. AOAC Official Method 990.03).

The soluble protein content of the un-sieved fractions of all the feedstuffs was determined in two laboratories, viz. the Animal Sciences Laboratory at Stellenbosch University (SU) and Nutrilab at the University of Pretoria (UP).

The method for protein solubility used at SU was according to that described by Licitra *et al* (1996). The borate-phosphate buffer that was used was prepared as described in Table 3.1.

Table 3.2 Borate-phosphate buffer (pH 6.7 – 6.8) used in the determination of protein solubility.

Monosodium phosphate ($NaH_2PO_4.H_2O$)	12.20 g/L
Sodium tetraborate ($Na_2B_4O_7.10H_2O$)	8.91 g/L
Tertiary butyl alcohol	100 ml/L

Samples were milled through a 2 mm screen. Sub-samples of 0.5 g were weighed into 125 ml Erlenmeyer flasks and 50 ml of borate-phosphate buffer was added to each flask. Then, 1 ml of freshly prepared 10% sodium azide solution was added to each flask. The flasks were left to stand on the desk for 3 hours at room temperature.

The filter papers used for the analyses were Whatman #54, which were previously dried, and the weights were recorded. The contents of the Erlenmeyer flasks were filtered through the filter paper with the aid of a mild vacuum. The residues were washed with 250 ml of cold distilled water. The filter papers with the residues were then placed in an oven at 100 ℃ for 24 hours to dry, and the weights of the filter papers with the residues were recorded.

The N content of the residues was determined with the aid of a Leco (LECO FP-528). Just prior to the Leco analyses, the residues on the filter papers were weighed again to determine the moisture content so that the N content could be expressed on a DM basis. The protein solubility was calculated as follows:

$$Y = aP_1 - bP_2 / aP_1 \times 100$$

where Y = CP solubility (%)

       a = sample weight (g DM)

       b = residue weight (g DM)

       P_1 = CP content of sample (%)

       P_2 = CP content of residue (%).

The method for protein solubility used at UP was according to that described by Faichney and White (1983). Samples were milled through a 1 mm screen, and sub-samples of 0.5 g were accurately weighed into test tubes (200 mm x 40 mm). The test tubes were placed in a water bath at 39 ℃ and 50 ml of McDougall's artificial saliva (McDougall, 1948), which was previously warmed to 39 ℃, was added to each test tube. The contents of the test tubes were mixed carefully and swirled every hour during incubation. Test tubes were incubated for three hours in the water bath. The contents were filtered through Whatman # 2 filter paper and washed three times with distilled water, heated to 39 ℃. The N content of the original samples, and that of

41

the residues, was determined by the Kjeldahl method (AOAC, 1995. Total Nitrogen. AOAC Official Method 955.04).

The percentage of soluble protein was calculated as follows:

%SP = (N content in original sample – N content in residue) / N content of original sample x 100

3.2.5. Data analysis

The mean and standard error for the material lost through sieving and the CP content of the different sieving treatments for all the feedstuffs were calculated. The BS fractions in the fish meal were analysed with a one-way analysis of variance (ANOVA) using Statistica 10 (2011). The means were separated with a Bonferonni test, and significance was declared at $P<0.05$.

3.3. Results and discussion

3.3.1. The effect of sieve mesh size on particle loss

The percentage of material that was lost when protein sources were sieved with two different mesh sizes (106 µm and 150 µm) are presented in Table 3.2.

Table 3.3 The amount (%) of material lost (mean ± SE) when protein sources were sieved through two different mesh sizes and the 0h DM disappearance values.

Feedstuff	106 µm %ML	150 µm %ML	0h DM disappearance (%)
Soybean oilcake	28.2 ± 0.1	36.7 ± 0.1	45.0 ± 0.214
Sunflower oilcake	23.7 ± 0.1	33.0 ± 0.1	42.4 ± 0.793
Maize gluten 20	33.7 ± 0.7	45.7 ± 0.9	54.2 ± 0.313
Maize gluten 60	20.7 ± 0.40	33.7 ± 0.2	21.6 ± 0.29
Fish meal	40.2 ± 0.4	57.8 ± 0.7	42.3 ± 0.629

ML = Material lost; SE = standard error (n = 5)

As can be seen from Table 3.2, the losses were variable between the different feedstuffs and ranged from as low as 20% for Maize gluten 60 to as high as almost 60% for fish meal on the 150 µm sieve. This implies that only 40 to 80% residue would remain on top of the screen after sieving. The material that was lost was determined to standardise the procedure. Although these values might not be of much practical value, they will enable researchers to sieve sufficient amounts of material for trials or analyses. They could also give a rough indication of the possible DM disappearance of ground samples that still contain fine particles. It is interesting to note that, for the feedstuffs that contained a significant amount of fibre (oilcakes and gluten 20), the 0h DM disappearance values were closer to the 150 µm sieve values than to the 106 µm sieve values. For the other feedstuffs (gluten 60 and fish meal), the opposite was observed. This observation is, however, not a suggestion that the washing procedure should be replaced by sieving to obtain or estimate 0h DM disappearance values.

3.3.2. Chemical composition of raw materials

The chemical composition of the different fractions of all the protein sources used in this study can be seen in Table 3.3.

The practice of sieving samples to remove fine particles would be of little value if such sieving resulted in the chemical composition being altered. It was thus important to compare the chemical composition of the sieved and un-sieved samples.

Table 3.4 Chemical composition (g/kg) of the different fractions of protein sources used in this trial. All values (except DM) expressed on a DM basis.

Item	DM	OM	CP	EE	CF
Soybean oilcake					
Un-sieved	895.3	924.2	533.0	23.1	45.1
106 µm	899.2	923.4	540.1	18.0	45.6
150 µm	899.2	921.3	541.8	17.1	48.7
Sunflower oilcake					
Un-sieved	911.1	922.4	364.9	14.6	232.5
106 µm	912.3	923.0	353.8	13.0	258.8
150 µm	912.6	924.9	354.7	12.6	244.1
Maize gluten 20					
Un-sieved	930.2	920.8	226.1	21.2	107.0
106 µm	923.2	925.5	210.9	14.4	114.7
150 µm	923.2	928.2	203.1	12.8	120.5
Maize gluten 60					
Un-sieved	946.4	982.8	576.5	5.4	13.5
106 µm	941.0	984.4	577.2	4.2	9.9
150 µm	940.9	984.4	578.6	3.8	10.7
Fish meal					
Un-sieved	867.9	860.7	748.9	159.2	7.7
106 µm	892.1	888.3	767.1	153.4	3.2
150 µm	889.9	894.1	769.1	159.2	5.6

DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; CF = crude fibre

Analyses were done in duplicate on sub-samples to determine the chemical composition resulting from the different treatments. Feedstuffs were not sourced from different batches or locations, therefore no statistical analyses were performed. It would appear from Table 3.3 that there was a good agreement between the top fraction of the sieved and the un-sieved samples, with the exception of the EE and CF fractions of almost all the feedstuffs and the CP content of fish meal and maize gluten 20. There was apparently no difference between treatments in the EE of the fish meal and since the fat content of the rest of the protein sources were low, the differences between the different treatments were of little concern. The CF content of

44

maize gluten 60 and fish meal did not differ much between treatments, but due to their low CF content, it is not of practical concern. The CF content of the oilcakes and maize gluten 20 appeared to have increased after sieving. One might have expected the CF content of the 150 µm sieve residues to be the highest, but this was not the case for sunflower oilcake where the fibre content of the 150 µm sieve residue was lower than that of the 106 µm sieve.

Since the main objective of the study was to look at the protein degradability, CP content measurements of the two different sieve treatments were repeated and mean values are presented in Table 3.4. The un-sieved fraction was also re-analysed because these analyses were done 4 months later than the original CP determinations. One could also expect small differences between Leco values on different analysis days, because the instrument has to be calibrated at the beginning of each day. For both soybean and sunflower oilcake and maize gluten 60, the CP content did not appear to differ between treatments. However, the CP content of fish meal and maize gluten 20 differed between treatments. The CP content of the un-sieved maize gluten 20 was the highest, while that of the 150 µm residue was the lowest. Contradictory to this, for fish meal, the un-sieved fraction had the lowest CP content while the residue from the 150 µm had the highest CP content. This makes sense as fibre and ash were lost, therefore remaining nutrients were "diluted" less. However, the CP content of the un-sieved fraction and the 106 µm fraction seems to be similar for fish meal.

The differences of the CP content that occurred in maize gluten 20 could be partly explained by the fibrous matter present in maize gluten 20. One would expect the fibrous matter to be part of the larger particles and therefore these particles would remain on top of the sieves. Thus, the CP content of the residue from the 150 µm sieve was the lowest.

Table 3.5 Crude protein content (g/kg) of ground un-sieved protein sources and their residues after sieving through screens with different mesh sizes.

Feedstuff	Treatment		
	Un-sieved	106 µm[1]	150 µm[1]
Soybean oilcake	52.25	51.89 ± 0.20	51.87 ± 0.20
Sunflower oilcake	36.60	36.51 ± 0.35	36.28 ± 0.86
Maize gluten 20	23.12	21.87±0.05	20.99±0.07
Maize gluten 60	55.80	56.38 ± 0.16	56.73 ± 0.05
Fish meal	72.37	72.30 ± 0.24	74.38 ± 0.17

1= Mean ± SE

The CP content differences observed in fish meal can be partly explained by the distribution of bone in the fish meal. From Table 3.5 it is clear that the 150 µm fraction, which had the highest CP content, also had the lowest bone content ($P<0.05$). This was probably caused by the brittleness of the bone that caused the bone to be part of the fine matter and dust that passed through the sieve. Therefore, more meat and other components were present in this fraction, hence the higher CP content. Generally, the CP values of the 106 µm sieve residues appeared to have been closer to those of the original ground samples than those of the 150 µm sieves. The 106 µm mesh would thus be the preferred sieve for removing fine particles.

Table 3.6 The percentage of bone remaining in the different treatments of fish meal.

Feedstuff	% Bone			SE	*P*
	Un-sieved	106 µm	150 µm		
Fish meal	9.22[a]	9.30[a]	7.47[b]	0.169	<0.001

SE = Standard error

[a, b] Means with different superscripts, differed significantly

46

3.3.3. Protein solubility

Protein solubility values, as determined in our laboratory (SU) and that of the University of Pretoria (UP), are presented in Table 3.6. As can be seen, solubility values differed between the two laboratories. The best agreement between laboratories was for maize gluten 20 (only 2.7% deviation). The mean deviation in CP solubility between the two laboratories was 31.7%, ranging from 2.7% to as high as 73.4% in the case of fish meal. The variation could probably be explained by the fact that the two laboratories used different methods to determine protein solubility, as is explained in the methods section. However, these differences support the fact that protein nutrition is complex and that it is very difficult to determine the different N fractions accurately (VandeHaar & St-Pierre, 2006). It remains a concern that agreement between the two laboratories regarding CP solubility was so poor.

The protein solubility values of the CPM Dairy Feed Dictionary (CPM Dairy version 3.10, 2011) are also presented in Table 3.6. If one compares these values with the values presented in Table 3.6 that were obtained in the two laboratories, those for soybean oilcake and fish meal seem to correlate fairly well with the values obtained at SU, while those for sunflower oilcake and maize gluten 60 seem to correlate better with the values obtained at UP. For maize gluten 20, the protein solubility given by CPM Dairy was much lower than those of both SU and UP.

Soluble crude protein content is a function of both the crude protein content of the feedstuff, and protein solubility. Although differences can be expected between our values and those of the CPM Dairy Feed Dictionary (CPM Dairy version 3.10, 2011), a cause for concern is the differences in values obtained between the SU and UP laboratories. The only value that really differed markedly between the sources was the value obtained by the UP laboratory for fish meal, which is much lower than those of the other two sources.

47

Table 3.7 Crude protein (CP) content and CP solubility of protein sources commonly used in dairy cow diets.

Feedstuff	CP content g/kg DM[1]	Solubility			Soluble CP content		
		% (SU[2])	% (UP[2])	% (CPM[3])	g/kg DM (SU[4])	g/kg DM (UP[4])	g/kg DM (CPM[5])
Soybean oilcake	522.5	25.5	31.7	20.0	133.0	165.4	110.0
Sunflower oilcake	366.0	42.2	36.8	33.8	154.5	134.5	135.9
Maize gluten 20	231.2	67.0	68.8	54.0	154.9	159.1	129.6
Maize gluten 60	558.0	10.0	5.5	6.4	55.9	30.4	41.9
Fish meal	723.7	19.1	5.1	21.0	138.2	36.8	142.6

[1]CP values determined in our lab on the original un-sieved feedstuffs used in the current trial.

[2]CP solubility determined in the respective laboratories of Stellenbosch University (SU) and the University of Pretoria (UP).

[3]CP solubility as indicated in the Feed Library of the CPM Dairy Model version 3.10 (2010).

[4]Soluble CP content calculations based on solubility values determined by the respective laboratories and applied to the CP content of the feedstuffs used in the current trial.

[5]Soluble CP content calculations based on solubility and CP values of feedstuffs in the CPM Dairy Feed Library

3.3.3.1. *In sacco* a-values

The 0-hour, or a-values, as determined by washing the samples in water only, are presented in Table 3.7. Sieving had a significant effect on the a-values of all the feedstuffs. Fine particle removal resulted in much lower a-values, regardless of sieve mesh size. It appears that the grinding of feed samples, without sieving to remove fine particles, would result in an over-estimation of protein disappearance at 0 hours. The a-value is usually accepted to give an indication of soluble protein (NRC, 2001). However, because the pore sizes of the dacron bags used in the current trial varied between 31 and 99 μm (Cruywagen, 2007; unpublished data), it follows that particles smaller than 99 μm may escape through the dacron bag pores without being solubilised. Such fine particles were removed by the two sieving treatments in the current trial, resulting in the lower a-values observed in these treatments.

Erasmus et al. (1988), reported the following a-values: soybean oilcake = 10%, sunflower oilcake = 46%, maize gluten 20 = 62%, maize gluten 60 = 10 %, and fish meal = 29%. Sunflower oilcake and fish meal values were in agreement with the un-sieved values obtained in the current study. In contradiction of the hypothesis, the a-values reported by Erasmus et al., (1988) for soybean oilcake, and both maize gluten 20 and 60 were quite similar to the sieved fractions, and not to the un-sieved fractions of the current study. Some of these variations could be explained by the differences that are expected to occur between different batches and different processing methods (Tamminga, 1979).

According to the hypothesis of the current study, it was expected that the soluble CP values (Table 3.6) would correlate with the a-values obtained with the two sieved fractions (Table 3.7.). For soybean oilcake, maize gluten 60 and fish meal, the values of the sieved fractions appeared to correlate very well with the soluble CP content as determined by SU. However, for sunflower oilcake and maize gluten 20, the soluble CP content determined by both laboratories (SU and UP) was much lower than the sieved fraction a-values for these two feedstuffs. It is thus possible that for these feedstuffs, which also had the highest fibre content, the passage of particles through the dacron pores could still be higher than anticipated. A further

explanation might be that fibre particles retained on the sieves because of their length and not their thickness might have passed through the dacron pores where thickness, and not length, allowed it. This could have resulted in an over-estimation of DM a-values and consequently CP a-values. Nevertheless, for all the feedstuffs, the CP a-values of the sieved treatments were significantly lower than those of the un-sieved samples.

Table 3.8 Effect of fine particle removal on CP a-values (%) (for 0 hours incubation) as determined for the *in sacco* trial.

Feedstuff	Treatment			SE	*P*
	Un-sieved	106 µm	150 µm		
Soybean oilcake	27.4	11.5	11.6	0.221	<0.001
Sunflower oilcake	34.6	27.4	28.3	1.013	<0.001
Maize gluten 20	72.4	60.8	62.0	0.905	<0.001
Maize gluten 60	19.5	5.2	6.2	0.431	<0.001
Fish meal	37.0	14.1	13.4	0.929	<0.001

3.4. Conclusion

It is concluded that fine particle losses due to washout from dacron bags results in an overestimation of CP a-values. Sieving through a screen with a mesh size of 106 µm resulted in more realistic a-values. *In sacco* dacron bag procedures should therefore include a preparation step where ground samples are sieved through screens with a mesh size of at least 100 µm.

3.5. References

Association of Official Analytic Chemists International (AOAC), 2002. *Official methods of analysis of AOAC Internaitonal*. 17th ed. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Association of Official Analytic Chemists International (AOAC), 1995. *Official methods of analysis of AOAC Internaitonal*. 16th ed. Association of Official Analytical Chemists, Maryland, USA.

Bach, A., Calsamiglia, S. & Stern, M.D., 2005. Nitrogen metabolism in the rumen. J. Dairy Sci. 88: E9-E21.

Clark, J.H. & Davis, C.L., 1980. Some aspects of feeding high producing dairy Cows1. J. Dairy Sci. 63(6): 873-885.

Erasmus, L.J., Prinsloo, J. & Meissner, H.H., 1988. The establishment of a protein degradability data base for dairy cattle using the nylon bag technique. 1. Protein sources. S. Afr. J. Anim. Sci. 18: 23-29.

Faichney, G.J. & White, G.A., 1983. *Methods for the analysis of feeds eaten by ruminants*. Division of Animal Production, Ian Clunies Ross Animal Research Laboratory.

Freer, M. & Dove, H., 1984. Rumen degradation of protein in sunflower meal, rapeseed meal and lupin seed placed in nylon bags. Anim. Feed Sci. Technol. 11(2): 87-101.

Licitra, G., Hernandez T.M. & Van Soest P.J. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57(4): 347-358.

McDougall, E.I., 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. Biochem. J. 43(1): 99.

National Research Council (NRC), 2001. *Nutrient Requirements of Dairy Cattle*. 7th Rev. ed. National Academy Press. Washington, D. C.

Ørskov, E.R., Hovell, F.D. & Mould, F., 1980. The use of the nylon bag technique for the evaluation of feedstuffs. Tropical Animal Production. 5(3): 195-213.

Statistica 10, 2011. StatSoft Inc., USA.

Stern, M.D., Varga, G.A., Clark, J.H., Firkins, J.L., Huber, J.T., & Palmquist, D.L., 1994. Evaluation of chemical and physical properties of feeds that affect protein metabolism in the Rumen1. J. Dairy Sci. 77(9): 2762-2786.

Tamminga, S., 1979. Protein degradation in the forestomachs of ruminants. J. Anim. Sci. 49(6): 1615.

Van der Merwe, F.J. & Smith, W. A., 1991. *Dierevoeding*. 2nd ed. Anim Sci Pty Ltd. Pinelands.

Van de Haar, M.J. & St-Pierre, N. 2006. Major advances in nutrition: Relevance to the sustainability of the dairy industry. J. Dairy Sci. 89(4): 1280-1291.

CHAPTER 4

The effect of fine particle removal on *in situ* dry matter and crude protein disappearance of various protein feedstuffs in dairy cattle

Abstract

The objectives of this study were to determine the effect of fine particle removal on in sacco DM and CP degradability and the effective CP degradability values of protein feedstuffs commonly used in dairy cow diets. Feedstuffs were soybean oilcake, sunflower oilcake, maize gluten 20, maize gluten 60 and fish meal. Samples were ground through a 2 mm screen, followed by sieving through sieves with mesh sizes of either 106 µm or 150 µm. For the control treatment, samples were not sieved after grinding. Three lactating Holstein cows, fitted with rumen cannulae, were used in the trial. Cows received a commercial lactating cow semi-complete feed and oat hay ad libitum. Samples were incubated in dacron bags in the rumen and the incubation times were 0; 2; 4; 8; 12; 24 or 48 hours. Dry matter (DM) and CP disappearances were determined and the values were used to estimate degradability parameters. For all the feedstuffs, the a-values were significantly higher for the un-sieved treatments than for the sieved ones. On average, the DM a-values were 39.4% higher and the CP a-values 40.3% higher for the un-sieved treatment compared to the sieved ones. Effective CP degradability was also higher (23.2% on average) for the un-sieved treatment in comparison with the sieved treatments. It was concluded that sieving samples through a 106 µm mesh prior to in sacco incubation would result in more realistic estimations of protein degradation.

4.1. Introduction

Ingested dietary protein is broken down by ruminal micro-organisms into amino acids (AA), which are absorbed in the small intestine (Stern *et al.*, 1994). The main form of protein that reaches the small intestine of ruminants is of microbial origin, produced by the micro-organisms in the rumen (Storm & Ørskov, 1983). This is usually sufficient for ruminants under extensive conditions, but when these animals are used for intensive production (e.g. dairy cattle) microbial protein alone would result in a deficiency of limiting essential AA, and therefore, additional UDP is often required (Clark et al., 1992). Dairy cattle have a high requirement for UDP, especially during early lactation when intake is lower than required for high milk production levels (Stern et al., 1994).

The disappearance of feedstuffs from the rumen is caused by two activities that occur at the same time, namely, degradation and passage from the rumen (NRC, 2001). Protein degradation in the rumen has a definite influence on the way in which the animal utilises protein (Ha & Kennelly, 1984). Accurate determination of protein degradation taking place in the rumen is required to ensure that optimal amounts of UDP are supplied by formulated diets. As *in vivo* measurements are expensive and subject to errors, an alternative method is needed to determine protein degradation (Van der Walt & Meyer, 1988). At present, one of the best alternatives is the *in sacco* technique (Erasmus et al., 1990; Michalet-Doreau & Ould-Bah, 1992; Stern et al., 1994; Vanzant et al., 1998). Standard procedures (NRC, 2001) recommend that feedstuffs be ground through a 2 mm screen in preparation for *in sacco* trials. Such samples, however, contain a significant proportion of fine material (Chapter 3) that can escape through the dacron bag pores without being solubilised. This may result in an over-estimation of the soluble fraction, typically referred to as the a-fraction.

The current study was executed to determine the effect of fine particle removal from ground samples on rumen degradation parameters. The following hypotheses were tested:

54

H_0: Fine particle removal will not affect rumen degradation parameters.

H_1: Fine particle removal will affect rumen degradation parameters.

4.2. Materials and methods

4.2.1 Animals and diets

The trial was conducted on Welgevallen Experimental Farm, the research facility of Stellenbosch University, Western Cape Province, South Africa. Three lactating Holstein cows fitted with ruminal cannulae were used. Ethical clearance was obtained from the Stellenbosch University's Animal Ethics Committee (Reference # 10NP CRU01). The mean body mass of the cows was 594.3 ± 17.3 (SE) kg, and the mean milk production was 24.9 ± 4.0 (SE) litres per day.

The cows were kept in a small camp with grass pastures and were fed a semi-complete diet daily at 07h00 and 16h00. Each cow received 25 kg of a commercial semi-complete diet for lactating dairy cattle, supplied by Afgri Animal Feeds (Klipheuwel, South Africa), and oat hay was provided *ad libitum*. The chemical composition of the semi-complete diet and the oat hay is presented in Table 4.1.

Table 4.9 Chemical composition (g/kg) of the diet that the cannulated cows consumed during the *in sacco* trial.

Composition	Ingredients	
	Semi-complete diet[2]	Oat hay
DM[1]	883	900.4
Ash	83	59.9
CP[1]	165	56.6
NDF[1]	283	686.4

[1] DM = dry matter, CP = crude protein, CF = crude fibre, NDF = neutral detergent fibre, EE = ether extract
[2] Values provided by feed company

4.2.2. Experimental design

A completely randomised block design was used. The trial was done in two periods, and each period served as a block. All treatments were included in each of the three cows in each period.

As the capacity of the rumen limits the number of nylon bags that could be incubated at a time, the trial was done in three separate runs. In run one, all the treatments of the soybean and sunflower oilcakes were incubated; in run two, all the treatments of maize gluten 20 and maize gluten 60 were incubated; and in run three, all the treatments of the fish meal were incubated. The entire trial was repeated in a second period. This resulted in six observations for each treatment of all the feedstuffs for each incubation period.

4.2.3. Treatments

Five protein feedstuffs were used in this study: soybean oilcake, sunflower oilcake, maize gluten 20, maize gluten 60 and fish meal. Samples were milled and sieved as explained in Chapter 3. Three different fractions of each feedstuff served as the different treatments.

Treatments were the following:
1. Ground (2 mm), with no subsequent sieving.
2. Ground (2 mm), followed by sieving through a 106 µm screen.
3. Ground (2 mm), followed by sieving through a 150 µm screen.

4.2.4. *In sacco* technique

The feedstuffs were incubated in dacron bags of 100 x 200 mm (Bar Diamond, Parma, ID, USA) with a designated pore size of 53 µm. Bags were marked and then dried for 24

hours at 60 ºC. The bags were transferred to desiccators for 30 minutes to cool down and were weighed. Samples of 8 g were weighed into each bag. The open ends of the bags were folded, and the bags were closed securely with two cable ties.

The bags were placed in opaque women's stockings according to the method described by Cruywagen (2006). A marble was placed in the toe of each stocking to serve as a weight. Six bags were placed into each stocking and bags were separated with cable ties. As there were six removal times, each stocking contained one treatment of a feedstuff, and hence, there were three stockings for every feedstuff. Each stocking was tied to the rumen lid with a 'catcher' stocking. Only six stockings could be incubated at a time due to the limited space in the rumen. Two blank bags were included per run per cow to correct for any microbial contamination that occurred.

The stockings with the bags were pre-soaked for 15 minutes in water with a temperature of 39 ºC and then incubated in the rumen (Lykos & Varga, 1995). Incubation started at 07:00 on the first day of each run. The incubation times were 0, 2, 4, 8, 12, 24 or 48 hours (NRC, 2001). The two blanks were removed after 24 and 48 hours respectively. For 0 hours, the bags were not incubated in the rumen but were washed with water. Six bags were used for each fraction to determine 0 hours.

Upon removal, the bags were washed under running water until the water that was squeezed out was clear. The bags were stored in airtight-bags at -18 ºC until analysed. After the trial had been completed, the bags were taken out of the freezer and allowed to thaw over night. The following day, bags were washed in a twin-tub washing machine, as described in Chapter 3, to obtain zero hour incubation values.

4.2.5. Chemical analyses

Dried bags were transferred to desiccators and allowed to cool for 30 minutes. Weights were recorded and the dry matter (DM) disappearance was calculated for each bag. DM

disappearance was expressed as a percentage of the original DM that was weighed into the bags.

The original ground samples, as well as the residue in each bag were used to determine the nitrogen (N) content, with the aid of a Leco protein analyser (LECO FP-528, AOAC Official Method 992.15). The crude protein content was determined by multiplying the % N obtained from the Leco by 6.25. On the day of analyses, the moisture content was determined so that the CP content could be expressed on a dry-matter basis.

4.2.6. Data analyses

An iterative least-square procedure was used (Solveer function of Excel) to fit the DM and CP disappearance data to the following model (Ørskov & McDonald, 1979) to determine degradability parameters:

$$Y = a + b (1 - e^{-ct})$$

where Y = degradation at time t

   a = soluble and rapidly degradable fraction

   b = fraction that will potentially be degraded over time

   c = rate of degradation of fraction b

The effective degradability of CP was determined as follow:

$$D_{eff} = a + (bc / c + k)$$

where D_{eff} = effective degradability

   a,b and c = degradability parameters determined with model

   k = passage rate (8% per hour).

58

Data regarding the non-linear parameters a, b and c; the effective degradability; and the degradability at specific time points were also analysed. The data were analysed with a main effects analysis of variance (ANOVA), using Statistica 10 (2011). The main effects were treatment, cow and period. The means were separated with a Bonferonni test, and significance was declared at P<0.05.

4.3. Results and discussion

4.3.1. Non-linear parameters

4.3.1.1. *In sacco* DM degradability

The effect of the removal of the small particles on *in sacco* DM degradation is presented in Table 4.2 and Figure 4.1.

Effect of fine particle removal on the a-values for DM degradability

It is clear from Table 4.2 that the rapidly soluble fractions (a-values) of the un-sieved fraction were significantly higher than those of the sieved samples for all the protein feedstuffs used in this trial (P<0.05). There were no differences in the a-values between the different sieves used in this study, except for gluten 20 where the two screens differed significantly from one another. However, if one looks at the mean values of the two sieved fractions, the differences are not that big. These results support the hypothesis that the standard method for the *in sacco* determination of degradability, as described by the NRC (2001), overestimates the soluble fraction.

Table 4.10 The effect of fine particle removal of protein sources on the non-linear parameters for DM degradation, as determined *in sacco* in ruminally cannulated lactating Holstein cows.

Item	Treatment			SE	*P*
	Un-sieved	106 µm	150 µm		
Soybean oilcake					
a	46.1[a]	34.4[b]	34.0[b]	0.445	<0.001
b	54.8[a]	66.2[b]	65.7[b]	0.912	<0.001
c	0.068	0.078	0.078	0.005	0.233
Sunflower oilcake					
a	42.9[a]	33.0[b]	31.9[b]	0.435	<0.001
b	36.9[a]	39.8[b]	39.8[b]	0.733	<0.05
c	0.130	0.148	0.135	0.007	0.208
Maize gluten 20					
a	54.1[a]	37.2[b]	35.2[c]	0.245	<0.001
b	23.3[a]	35.5[b]	45.3[c]	2.408	<0.001
c	0.039[a]	0.033[a]	0.019[b]	0.003	<0.01
Maize gluten 60					
a	23.2[a]	9.6[b]	9.9[b]	0.351	<0.001
b	40.8	46.6	44.4	1.915	0.140
c	0.048	0.059	0.058	0.004	0.098
Fish meal					
a	41.9[a]	18.9[b]	18.1[b]	0.238	<0.001
b	160.5	219.5	289.7	38.841	0.104
c	0.003	0.003	0.002	0.001	0.622

SE = Standard error

[a, b, c] Means in the same row with different superscripts differed significantly.

a = rapidly soluble fraction; b = potentially degradable fraction; c = rate at which b is degraded

60
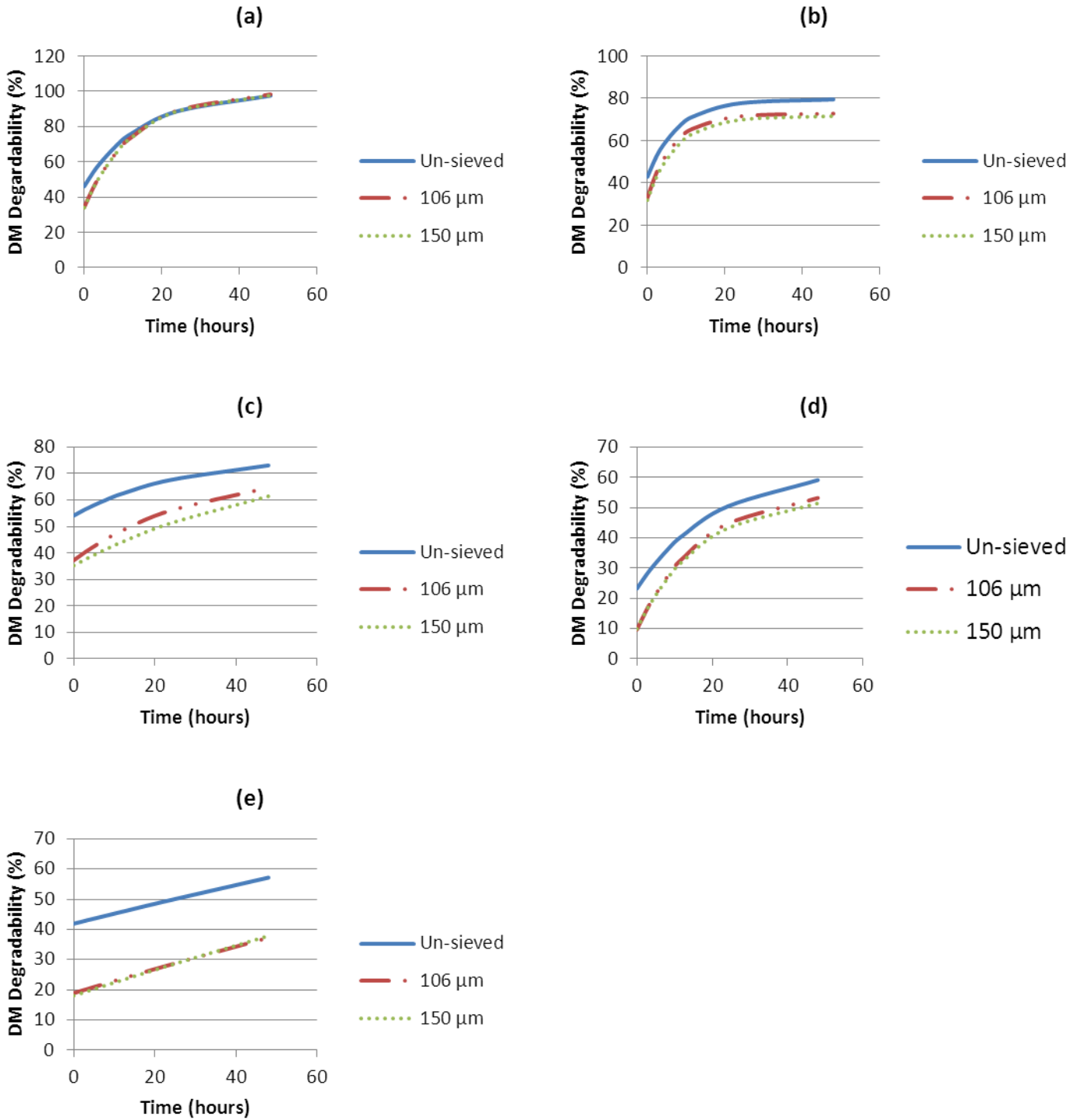
Figure 4.3 The effect of fine particle removal on DM degradability

Where (a) = soybean oilcake, (b) = sunflower oilcake, (c) = maize gluten 20, (d) = maize gluten 60, (e) = fish meal.

The un-sieved fraction is expected to have the smallest particle sizes on average, as the fine matter would form a large part of this fraction. The higher DM disappearance of the un-sieved fractions correlates with previous research, as DM disappearance was found to decrease with an increase in particle size (Figroid et al., 1972). These authors, however, did not remove fine particles. In a study done with dry rolled corn, it was also found that DM disappearance increased from 5.0% for particles of 6000 µm to 18.4% for particles of 750 µm (Galyean et al., 1981). Although DM disappearance was expected to be higher in the un-sieved fractions, the sieved fractions might constitute a better representation of material that is actually being degraded.

Ehle et al. (1982) performed a trial where they looked at particle size distribution. Soybean oilcake was sieved through four different screens: 1180, 600, 300 and 150 µm, and an un-sieved sample was also incubated. When these samples were incubated in distilled water for 30 minutes, no differences were found for DM or CP disappearance. However, these screens are much larger than the screens that were used in the current study and they are also not representative of the dacron bags, as the pore sizes range from 33 to 99 µm.

In contrast with the a-values for sunflower oilcake and fish meal in the current study, Alexandrov (1998), also using the *in sacco* method, reported lower a-values for these two feedstuffs. That of sunflower was 28% and that of fish meal 15%. These a-values are much lower than the values obtained in the current study. They are even lower than the values for the sieved treatments, contrary to what one would have expected. For the sunflower oilcake, the differences could be explained by the differences in the extraction process used for the sunflower seeds, and one could also expect differences between different batches of sunflower oilcake. Similarly, the processing of fish meal would influence degradability; different batches of fish meal could also be expected to differ.

Effect of fine particle removal on the b-values for DM degradability

The b-values of soybean and sunflower oilcake, and those of maize gluten 20 differed significantly ($P<0.05$) between treatments. For both oilcakes, the different sieve-

treatments did not differ from one another. For maize gluten 20, however, there was a marked difference between the two sieved treatments. This could be expected as the model declares that the value of a plus b should be 100 (Ørskov et al., 1980). Therefore, if the a-values were significantly higher, one could expect the b-values to be significantly lower in the un-sieved fractions. However, the b-values of maize gluten 60 and fish meal did not seem to differ among the three different treatments.

Although the b-values of maize gluten 60 appeared to be normal, those of fish meal were greater than 100 and therefore unrealistic. If one looks at the figures for maize gluten 60, and especially the one for fish meal (Figure 4.1 (d) and (e)), it can be seen that degradation had not reached an asymptote at the end of the incubation period and that the graph lines were still increasing. For soybean oilcake, the value of a plus b is also slightly greater than 100. In the graph in Figure 4.1 (a), it appears as though degradation was still increasing slightly at the end of the incubation period of the current study. It might have been better if a 72-hour incubation period had been included, but it was initially decided to use the NRC-recommended procedures.

Effect of fine particle removal on the rate of degradation (c-values) for DM degradability
There were no differences in the rate at which the b-fraction was degraded, except in the case of maize gluten 20. For maize gluten 20, the un-sieved fraction and the residue of the 106 µm sieve were degraded significantly faster than the residue of the 150 µm sieve ($P<0.05$).

4.3.1.2. *In sacco* CP degradability

The CP degradability parameters are presented in Table 4.3 and Figure 4.2.

Table 4.11 The effect of fine particle removal of protein sources on the non-linear parameters for CP degradation as determined *in sacco* in ruminally cannulated lactating Holstein cows.

Item	Treatment			SE	P
	Un-sieved	106 µm	150 µm		
Soybean oilcake					
a	30.47[a]	16.28[b]	16.09[b]	1.026	<0.001
b	71.06[a]	87.96[b]	87.14[b]	1.607	<0.001
c	0.061	0.067	0.069	0.006	0.554
Sunflower oilcake					
a	37.27[a]	30.51[b]	31.14[b]	0.634	<0.001
b	55.92[a]	62.31[b]	61.28[b]	1.172	<0.005
c	0.161	0.165	0.161	0.034	0.977
Maize gluten 20					
a	73.9[a]	62.7[b]	63.4[b]	0.285	<0.001
b	18.7[a]	26.9[b]	23.9[b]	0.791	<0.001
c	0.072	0.060	0.077	0.014	0.687
Maize gluten 60					
a	21.4[a]	7.4[b]	8.1[b]	0.298	<0.001
b	33.2	104.5	105.9	35.08	0.283
c	0.017	0.012	0.006	0.003	0.116
Fish meal					
a	37.51[a]	15.62[b]	15.24[b]	0.273	<0.001
b	211.8	81.7	255.9	100.055	0.464
c	0.004[a]	0.016[b]	0.008[ab]	0.003	<0.05

SE = Standard error

[a, b, c] Means in the same row with different superscripts, differed significantly.

a = rapidly soluble fraction; b = potentially degradable fraction; c = rate at which b is degraded
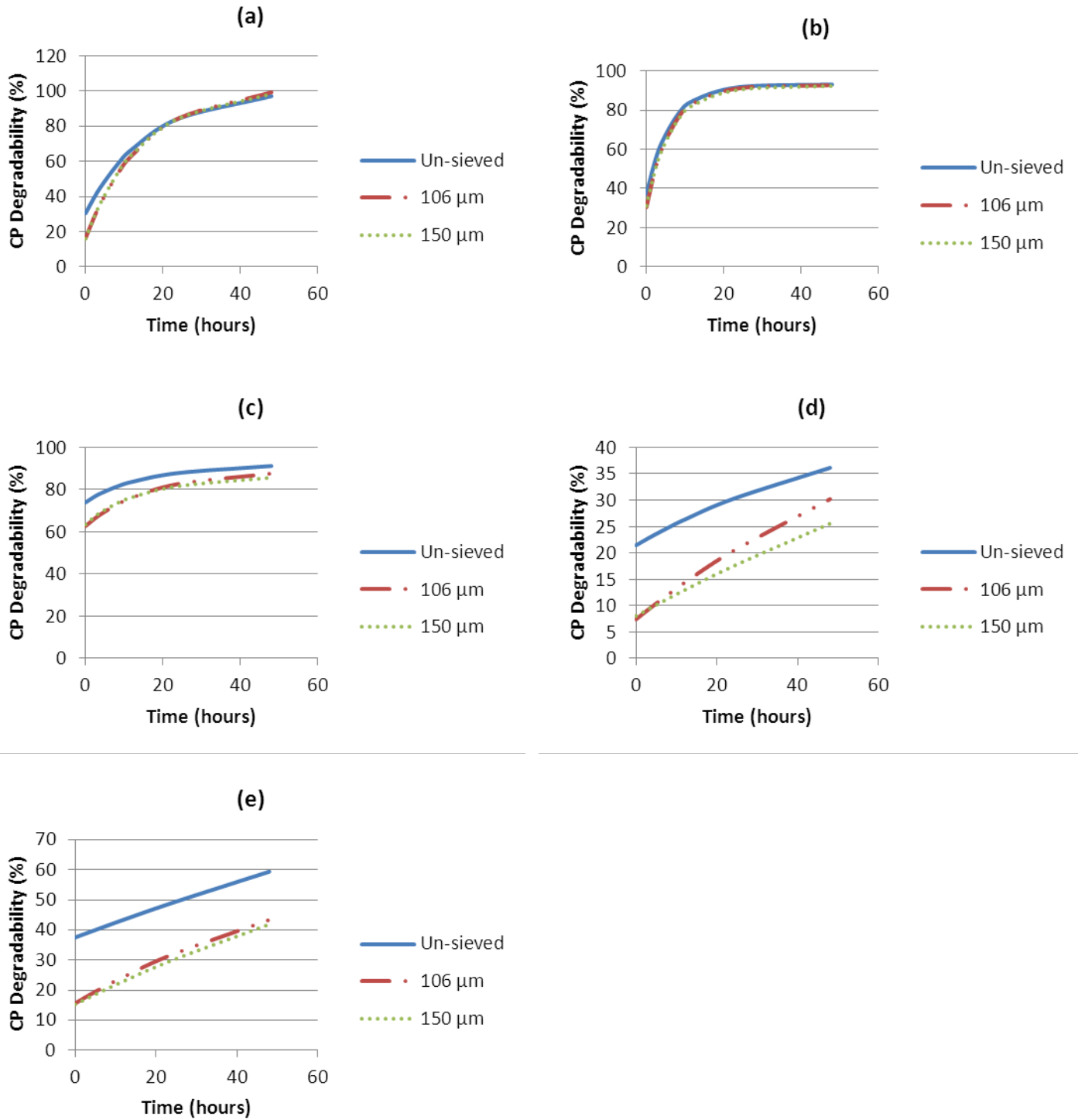
Figure 4.4 The effect of fine particle removal on CP degradability.

Where (a) = soybean oilcake, (b) = sunflower oilcake, (c) = maize gluten 20, (d) = maize gluten 60, (e) = fish meal.

Effect of fine particle removal on the a-values for CP degradability

From Table 4.3 it can be seen that the soluble and rapidly degradable fractions (a-values) were significantly higher in the un-sieved fractions of all the protein feedstuffs used in the current study (P<0.05). The two sieved fractions did not differ from one another for any of the feedstuffs. As in the case of DM degradability, these observations support the hypothesis that the standard method for the *in sacco* determination of degradation as recommended by the NRC (2001) overestimates degradability. The small particles that are present in the un-sieved fraction could be washed out of the dacron bags without necessarily being solubilised.

In a study where omasal sampling was used to determine degradation, Broderick et al. (2010) have found that 7 to 9% of the protein predicted by the NRC (2001) to be degraded in the rumen would pass undegraded from the rumen. Thus, they concluded that the UDP fraction is underestimated with the method described by the NRC (2001). This correlates with the results in the current study, for if protein degradation was overestimated, it would result in an underestimation of UDP.

Decreasing particle size results in an increase in the surface area per unit weight of substrate that is exposed to micro-organisms (Weakley et al., 1983). Crude protein degradation would therefore decrease with an increase in particle size. Thus, the un-sieved fractions were expected to have the highest CP degradation, as these would include fine material with the smallest particle sizes. This correlates with the results of the current study.

Michalet-Doreau & Cerneau (1991) did a trial where they compared soybean meal that was ground through a 2.5 mm screen with soybean meal that was ground through a 0.8 mm screen. The a-value for the 2.5 mm screen was 15.8% and it increased to 22.4% for the 0.8 mm screen. This correlates with the values obtained in the current study where the un-sieved fraction contained fine particles, and therefore, it was expected that it would have the highest a-values. The values for the 0.8 mm screen were similar to the

results found in the current study. These were lower for the un-sieved treatment, but they were still higher than for both sieved treatments.

In an *in sacco* trial done by Susmel et al. (1993), solvent extracted soybean meal was studied, and an a-value of 18.4% was recorded. This a-value was also lower than the value obtained for the un-sieved treatment in the current study, but it was still higher than for both sieved treatments, supporting the hypothesis that the un-sieved treatment overestimates degradation. In contrast to this low value, Schroeder et al. (1995) reported an a-value of 29.7% for soybean oilcake, which was very similar to the a-value of the un-sieved treatment in the current study.

An a-value of 30.3% was reported when solvent-extracted sunflower meal was studied *in sacco* (Susmel et al., 1993). This a-value was lower than the a-value for un-sieved sunflower oilcake observed in the current study. Likewise, Alexandrov (1998) found the a-value of sunflower meal to be 29%, which is somewhat lower than that for the current study. Processing of the sunflower seed before the oilcake is produced, and the differences among different batches of sunflower seed would have contributed to the differences between studies.

Susmel et al. (1993) reported an a-value of 7.4% for maize gluten 60. This was lower than expected and agreed with the a-value for the 106 µm sieve used in the current study. For fish meal, Susmel et al. (1993) found the a-value to be 34.7%, which was similar to the value observed for the un-sieved fraction of fish meal in the current study, and higher than those of the two sieve treatments. Thus, it supports the hypothesis that degradation may be overestimated in the un-sieved fractions.

Effect of fine particle removal on the b-values for CP degradability

Similar to the results for DM degradability, the b-values for both oilcakes and maize gluten 20 differed among treatments. The two sieve treatments did not differ for any of the feedstuffs. The b-values for maize gluten 60 and fish meal did not differ between treatments. As the values of a plus b are supposed to add up to 100 (Ørskov et al.,

1980), it could be expected that the b-values of the un-sieved treatments would be significantly lower, as the a-values of the un-sieved treatments were significantly higher.

Similar to the DM degradability, the b-values of soybean oilcake, maize gluten 60 and fish meal were unrealistically high. If one looks at the graphs for these three feedstuffs depicted in Figure 4.2, it is clear that an asymptote was not reached at the end of the incubation period. Therefore, degradation was probably still occurring at the end of the incubation period.

For soybean meal, the b-values reported in the literature are also relatively high and correspond with the b-values obtained in the current study: 81.6% (Susmel et al., 1993), 71.2% (Schroeder et al., 1995), and 77.6 and 84.2% for soybean meal ground through 0.8 mm and 2.5 mm screens, respectively (Michalet-Doreau & Cerneau, 1991). Ha & Kennely (1984) reported that these high values could be explained by the high protein disappearance during the first 24 hours of incubation.

Similar to the high b-values of the sieved treatments of maize gluten 60, Susmel et al. (1993) reported a high b-value for this feedstuff (92.6%). However, the b-value that these authors observed for fish meal was 48.9%, which is much lower than the values of the current study, and their incubation period was only 24 hours. This difference could be due to the processing of the fish meal or differences between different batches of fish meal.

Effect of fine particle removal on the rate of degradation (c-values) for CP degradability

The rate of degradation differed only between the three treatments for fish meal. The un-sieved treatment was degraded significantly slower than the residue of the 106 µm sieve, but no difference occurred between the 150 µm sieve and either the un-sieved treatment or the 106 µm treatment.

For the other four feedstuffs used in this study, there were no differences between treatments regarding the rates of degradation. This correlates with the data of Michalet-Doreau & Cerneau (1991). These authors ground different feedstuffs through three screen sizes (0.8 mm, 3.0 mm and 6.0 mm) and also found no differences between the degradation rates of the different fractions.

4.3.2. Disappearance at specific time points

Two incubation times, viz. 0 hours and 12 hours were looked at in more depth, for the following reasons: i) The 0 hours values were obtained from samples that were not incubated in the rumen, and they represent the soluble and rapidly degradable fraction. The 0-hour value is also the intercept value on the Y-axis of the degradation curves. ii) The 12-hour value would closely resemble the mean retention time of feeds that pass from the rumen at a rate of 8% per hour. The average passage rate for high-producing dairy cows is generally estimated to be 8%. If the passage rate is 8%, then the mean retention time will be 12.5 hours, as the mean retention time equals the invert of the passage rate (Pienaar et al., 1989). Disappearance values at this incubation time should therefore theoretically be comparable to effective degradability values in dairy cows. Also, the overestimation of fermentability is mainly a problem at shorter incubation times, which is representative of the short retention times of high-producing dairy cattle (Dewhurst et al., 1995).

4.3.2.1. *In sacco* DM disappearance at 0 and 12 hours

The DM degradability values of protein sources at 0 and 12 hours of incubation are presented in Table 4.4. Disappearance was significantly higher in the un-sieved treatments than in the sieved treatments for all the feeds ($P<0.05$). This corresponds with the results of the non-linear degradation parameters (Table 4.2) and indicates that DM degradability is most likely overestimated in the un-sieved treatments.

For most of the protein sources, there was no difference between the two sieve treatments used in the trial. The exceptions were for gluten 20, where all the treatments differed from one another and for soybean oilcake at 12 hours, where un-sieved and the 106 µm sieve treatment differed from one another, but the 150 µm sieve did not differ from the other two treatments.

Table 4.12 The effect of fine particle removal on *in sacco* DM disappearance (%) at 0 hours and after 12 hours incubation in the rumen of lactating cannulated Holstein cows.

Item	Treatment			SE	*P*
	Un-sieved	106 µm	150 µm		
Soybean oilcake					
0 h	45.0[a]	32.1[b]	32.0[b]	0.214	<0.001
12 h	74.1[a]	70.4[b]	72.0[ab]	0.674	<0.01
Sunflower oilcake					
0 h	42.4[a]	31.2[b]	30.8[b]	0.793	<0.001
12 h	71.3[a]	64.9[b]	63.3[b]	1.0269	<0.002
Maize gluten 20					
0 h	54.2[a]	36.0[b]	33.9[c]	0.313	<0.001
12 h	63.6[a]	49.1[b]	45.2[c]	0.498	<0.001
Maize gluten 60					
0 h	21.6[a]	7.8[b]	7.9[b]	0.29	<0.001
12 h	40.5[a]	32.5[b]	31.5[b]	0.652	<0.001
Fish meal					
0 h	42.3[a]	18.8[b]	18.0[b]	0.629	<0.001
12 h	45.4[a]	23.2[b]	22.2[b]	0.465	<0.001

SE = Standard error

[a, b, c] Means in the same row with different superscripts, differed significantly

4.3.2.2. *In sacco* CP disappearance at 0 and 12 hours

In Table 4.5, the CP degradability of protein sources are presented at 0 and 12 hours of incubation. Degradation was significantly higher (*P*<0.05) in the un-sieved treatments of

all the feeds, and at both incubation times, indicating that CP degradability is most likely overestimated in the un-sieved treatments. Sunflower oilcake is an exception, as there was no difference between the three treatments at 12 hours of incubation.

For most of the protein sources, there was no difference between the two sieve treatments used in the trial, except for soybean oilcake at 12 hours, where the un-sieved treatment differed from the 106 µm sieve, but the 150 µm sieve did not differ from the other two treatments.

Table 4.13 The effect of fine particle removal on *in sacco* CP disappearance (%) at 0 h and after 12 hours' incubation in the rumen of lactating cannulated Holstein cows

	Treatment				
Item	Un-sieved	106 µm	150 µm	SE	*P*
Soybean oilcake					
0 h	27.4[a]	11.5[b]	11.6[b]	0.221	<0.001
12 h	65.3[a]	56.6[b]	59.9[ab]	1.534	<0.01
Sunflower oilcake					
0 h	34.6[a]	27.4[b]	28.3[b]	1.013	<0.001
12 h	81.5	81.3	80.5	0.644	0.495
Maize gluten 20					
0 h	72.4[a]	60.8[b]	62.0[b]	0.905	<0.001
12 h	83.5[a]	75.9[b]	76.6[b]	0.97	<0.001
Maize gluten 60					
0 h	19.5[a]	5.2[b]	6.2[b]	0.431	<0.001
12 h	26.1[a]	14.0[b]	12.7[b]	0.674	<0.001
Fish meal					
0 h	37.0[a]	14.1[b]	13.4[b]	0.929	<0.001
12 h	42.8[a]	24.4[b]	21.7[b]	0.672	<0.001

SE = Standard error

[a, b, c] Means in the same row with different superscripts, differed significantly

4.3.3. Effective CP degradability

Effective degradability refers to the amount of protein that would actually be degraded in the rumen (Ha & Kennelly, 1984). The effective degradability of crude protein determined with the model (Ørskov & McDonald, 1979) is depicted in Table 4.6. For all the feedstuffs used in the trial, the degradability was significantly higher in the un-sieved treatment than in the two sieved treatments ($P<0.05$). For most of the feedstuffs, degradation of the two sieve fractions did not differ, except for fish meal. Thus, the removal of fine particles resulted in a decrease in the estimation of effective degradability of CP.

Table 4.14 The effect of fine particle removal on effective degradability (%) of crude protein at a passage rate of 8%/h, as determined *in sacco* in ruminally cannulated lactating Holstein cows

Feedstuff	Treatment			SE	P
	Un-sieved	106 µm	150 µm		
Soybean oilcake	60.8[a]	55.3[b]	55.5[b]	0.793	<0.001
Sunflower oilcake	74.4[a]	72.0[b]	71.5[b]	0.698	<0.05
Maize gluten 20	82.0[a]	74.0[b]	74.1[b]	0.247	<0.001
Maize gluten 60	26.1[a]	14.2[b]	12.9[b]	0.435	<0.001
Fish meal	43.5[a]	24.1[b]	22.9[c]	0.272	<0.001

SE = Standard error

[a, b, c] Means in the same row with different superscripts, differed significantly

The effective CP degradability is presented in the bar graphs in Figure 4.3. The model values were determined with the given equation (section 4.1.6) with an outflow rate of 8% per hour (k = 0.08). The *in sacco* values are those that were observed after 12 hours of incubation.

Soybean oilcake

The effective degradability of soybean oilcake is presented in Figure 4.3(a). For all three treatments (un-sieved, 106 μm and 150 μm), it appeared that the values determined with the model, did not differ from the 12-hour values observed during the *in sacco* trial. The un-sieved treatment did however, have a larger effective degradability than the sieved treatments, which supports the hypothesis that degradability is overestimated when the fine particles are not removed from the samples.

Erasmus et al. (1988) reported the effective degradability of CP in soybean oilcake to be 51.4% (k = 0.08). This value is lower than the values obtained for the un-sieved fraction with the model and *in sacco*. Susmel et al. (1993) also found the effective degradability of solvent-extracted soybean meal to be lower than the values obtained in the current study. They reported effective degradability to be 52% at a passage rate of 7% per hour. Contrary to these findings, Schroeder et al. (1995) reported the effective degradability to be 73.3%, which is higher than the un-sieved values for the current study. These differences could be due to the extraction process of the oil possibly differing, and also due to differences that would occur between different batches of soybean oilcake.

Sunflower oilcake

The effective degradability of the CP of sunflower oilcake is presented in Figure 4.3 (b). For all three treatments, the values observed after 12 hours of *in sacco* incubation were higher than the values calculated from the model. The *in sacco* values did not differ between the different treatments, but model values did. The effective CP degradability of the un-sieved treatment was higher than that of the other two treatments with the model, which again supports the hypothesis that degradability is overestimated with the standard *in sacco* procedure. Erasmus et al. (1988) reported the CP degradability of sunflower oilcake to be 80.9%, which is similar to the value observed with the *in sacco* method for the un-sieved treatment. Susmel et al. (1993) found the CP degradability to be 75.6% at a passage rate of 7% per hour, while Alexandrov (1998) reported it to be

73.9% at a passage rate of 6% per hour. These two values seemed to correlate with the values obtained for the un-sieved fraction with the model in the current study.
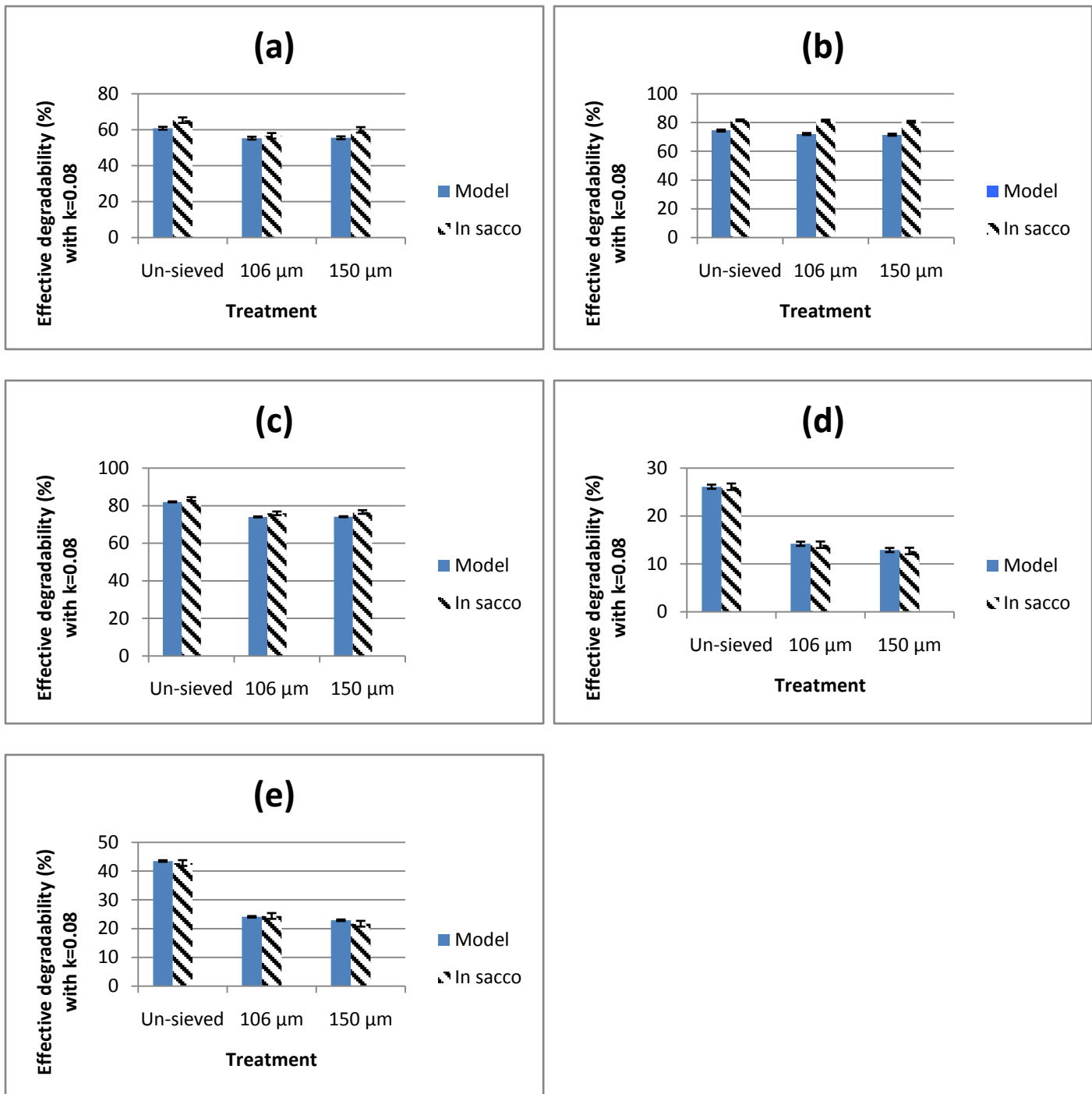
Maize gluten 20

The effective CP degradability of maize gluten 20 is presented in Figrure 4.3(c). The values observed with the *in sacco* trial were higher than those calculated with the model for all three the treatments. For both the *in sacco* values and those of the model, the un-sieved treatments had a higher effective degradability than the two sieved treatments. This again supports the hypothesis that degradability is overestimated when fine particles are not removed prior to incubation in the rumen. Erasmus et al. (1988) reported the effective CP degradability to be 78.9%. Thus, it is lower than both values obtained in the current study for the un-sieved fraction. This could be partially explained by the differences that could occur between different batches of maize. However, this value is still higher than for both sieved fractions and it therefore supports the hypothesis.

Maize gluten 60

The effective CP degradability of maize gluten 60 is presented in Figure 4.3(d). The values obtained with the model and those observed with the *in sacco* trial did not appear to differ between the different treatments. However, the values for the un-sieved treatments seem to be much higher than for both sieved treatments, which again supports the hypothesis that degradability is overestimated in the standard method for *in sacco* trials. Erasmus et al. (1988) found that the effective degradability of maize gluten 60 was equal to 24.5%, which is lower than the values obtained in the current study for the un-sieved treatments. In contrast, Susmel et al. (1993) found the effective CP degradability to be 29.6% at a passage rate of 7% per hour, which was higher than the values obtained in the current study. Both of these values were still higher than the values obtained with the two sieve treatments.

Fish meal

The effective CP degradability of fish meal is presented in Figure 4.3(e). As with maize gluten 60, there was no real difference between the values calculated with the model and those obtained with the *in sacco* study for all three of the treatments. Again, the values for the un-sieved treatments were higher than for both sieved treatments, supporting the hypothesis of the current study. Erasmus et al. (1988) reported the effective degradability of CP to be 36.4%, which is lower than the values for the un-sieved treatment of fish meal in the current study, but still higher than both sieved treatments. Similarly, Alexandrov (1998) reported the effective CP degradability to be 31.3%, but this was at a passage rate of 6% per hour. Contrary to this, Susmel et al. (1993) found the effective CP degradability to be 46.0% at a passage rate of 7% per hour. This value was even higher than the values for the un-sieved fractions that were obtained in the current study. Again, processing and batch effects should be kept in mind.

Figure 4.5 Effective degradability of CP in various protein feedstuffs

Where (a) = soybean oilcake, (b) = sunflower oilcake, (c) = maize gluten 20, (d) = maize gluten 60, (e) = fish meal.

Model = Effective degradability determined with k = 0.08; *In sacco* = values observed after 12h of incubation.

4.4 Some practical implications

To further illustrate the effect of fine particle removal on *in sacco* results and consequently on ration formulation, two examples will be discussed. In these examples, the control treatment and 106 µm sieve results of soybean oilcake (SBOC) and fish meal (FM) were used in the CPM Dairy Model. One of these two protein sources were used at a time in the CPM Dairy Model and *in sacco* results were used to calculate their RDP and UDP values (as % of CP) as follows (Orskov & MacDonald, 1979):

RDP = a + b*c/(c+k)
UDP = 100 - RDP

The respective RDP and UDP values were thus:

	<u>RDP</u>	<u>UDP</u>
SBOC 1, un-sieved	61.2	38.8
SBOC 2, 106 µm sieve	56.4	43.6
FM 1, un-sieved	47.6	52.4
FM 2, 106 µm sieve	23.0	76.9

Four CPM Dairy simulation runs were done, using the 45 kg cow model example. The feeds in the model example were used, but in each run, the respective soybean oilcake and fish meal sources of the current trial were used. The following animal information applied for lactating Holstein cows:

Body weight:	640 kg
Age:	50 months
Lactation #:	3
Milk production:	45 kg/d
Milk fat:	3.5%

For the rest, the CPM Dairy default values were used. In each run, the aim was to keep the metabolizable protein balance positive, but as close as possible to 0 g.

To maintain the milk production at 45 kg/d, the effect of fine particle removal on UDP estimations had the following results regarding the amount of SBOC and FM required:

	Amount required (kg)
SBOC 1:	2.95
SBOC 2:	2.51
FM 1:	0.64
FM 2:	0.59

It is clear that the over-prediction of CP degradability in the un-sieved samples resulted in a higher estimation of SBOC and FM required to maintain milk production. For SBOC this resulted in an 18% difference and for FM only an 8% difference. However, in a thousand cow unit, this would result in 1.5 tonnes of FM that could have been excluded from the diet. At an average price of R8500 per tonne this would have resulted in an extra cost of almost R12 800 per month.

4.5. Conclusion

The removal of fine particles from protein feedstuffs ground through a 2 mm screen resulted in a reduction of *in sacco* DM and CP disappearance values. Fine particle removal also reduced effective CP degradability estimations. It was concluded that the currently recommended *in sacco* procedures result in an overestimation of CP degradability because of fine particle losses through dacron bag pores. This may have significant implications in the formulation of dairy cow diets. An additional step should be included in the preparation of feed samples for *in sacco* trials, whereby fine particles are removed by sieving. A sieve with a mesh size of 106 μm is recommended.

78

4.5. References

Alexandrov, A.N., 1998. Effect of ruminal exposure and subsequent microbial contamination on dry matter and protein degradability of various feedstuffs. Anim. Feed Sci. Technol. 71(1-2): 99-107.

Broderick, G., Huhtanen P., Ahvenjarvi S., Reynal S., & Shingfield K., 2010. Quantifying ruminal nitrogen metabolism using the omasal sampling technique in cattle--A meta-analysis1. J. Dairy Sci. 93(7): 3216-3230.

Clark, J.H., Klusmeyer, T.H. & Cameron, M.R., 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy Cows1. J. Dairy Sci. 75(8): 2304-2323.

Cruywagen, C. W., 2006. Technical note: A method to facilitate retrieval of polyester bags used in in sacco trials in ruminants. J. Dairy Sci. 89(3): 1028-1030.

Dewhurst, R.J., Hepper, D. & Webster, A.J.F., 1995. Comparison of *in sacco* and *in vitro* techniques for estimating the rate and extent of rumen fermentation of a range of dietary ingredients. Anim. Feed Sci. Technol. 51(3-4): 211-229.

Ehle, F.R., Murphy M.R. & Clark J.H., 1982. In situ particle size reduction and the effect of particle size on degradation of crude protein and dry matter in the rumen of dairy steers. J. Dairy Sci. 65(6): 963.

Erasmus, L.J., Prinsloo, J., Botha, P.M. & Meissner, H.H., 1990. Establishment of a ruminal protein degradation data base for dairy cattle using the *in situ* polyester bag technique. 2 energy sources. S. Afr. J. Anim. Sci. 20: 124-129.

Erasmus, L.J., Prinsloo, J. & Meissner, H.H., 1988. The establishment of a protein degradability data base for dairy cattle using the nylon bag technique. 1. Protein sources. S. Afr. J. Anim. Sci. 18: 23-29.

Figroid, W., Hale, W.H. & Theurer, B., 1972. An evaluation of the nylon bag technique for estimating rumen utilization of grains. J. Anim. Sci. 35(1): 113.

Galyean, M.L., Wagner, D.G. & Owens, F.N., 1981. Dry matter and starch disappearance of corn and sorghum as influenced by particle size and processing. J. Dairy Sci. 64(9): 1804-1812.

Ha, J.K. & Kennelly, J.J., 1984. *In situ* dry matter and protein degradation of various protein sources in dairy cattle. Canadian Journal of Animal Science. 64(2): 443-452.

Lykos, T. & Varga, G.A., 1995. Effects of processing method on degradation characteristics of protein and carbohydrate sources *in situ*. J. Dairy Sci. 78(8): 1789-1801.

Michalet-Doreau, B. & Cerneau, P., 1991. Influence of foodstuff particle size on *in situ* degradation of nitrogen in the rumen. Anim. Feed Sci. Technol. 35(1-2): 69-81.

Michalet-Doreau, B. & Ould-Bah, M.Y., 1992. *In vitro* and *in sacco* methods for the estimation of dietary nitrogen degradability in the rumen: A review. Anim. Feed Sci. Technol. 40(1): 57-86.

National Research Council (NRC), 2001. *Nutrient Requirements of Dairy Cattle*. 7th Rev. ed. National Academy Press. Washington, D. C.

Ørskov, E.R., Hovell, F.D. & Mould, F., 1980. The use of the nylon bag technique for the evaluation of feedstuffs. Tropical Animal Production. 5(3): 195-213.

Ørskov, E.R. & McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92(02): 499-503.

Pienaar, J.P., Roux, C.Z. & Cronjé, P.B., 1989. Comparison of in vivo and *in sacco* methods to estimate mean retention time of fermentable organic matter in the rumen. S. Afr. J. Anim. Sci. 19(2): 71-75.

Schroeder, G.E., Erasmus, L.J., Leeuw, K.J. & Meissner, H.H., 1995. Effect of roasting on ruminal degradation, intestinal digestibility and absorbable amino acid profile of cottonseed and soybean oilcake meals. S. Afr. J. Anim. Sci. 25(4): 109-117.

Statistica 10, 2011. StatSoft Inc., USA.

Stern, M.D., Varga, G.A., Clark, J.H., Firkins, J.L., Huber, J.T. & Palmquist, D.L., 1994. Evaluation of chemical and physical properties of feeds that affect protein metabolism in the Rumen1. J. Dairy Sci. 77(9): 2762-2786.

Storm, E. & Ørskov, E.R., 1983. The nutritive value of rumen micro-organisms in ruminants. Br. J. Nutr. 50(02): 463-470.

Susmel, P., Mills, C.R., Colitti, M. & Stefanon, B., 1993. *In vitro* solubility and degradability of nitrogen in concentrate ruminant feeds. Anim. Feed Sci. Technol. 42(1-2): 1-13.

Van der Walt, J.G. & Meyer, J.H.F., 1988. Protein digestion in ruminants. S. Afr. J. Anim. Sci. 18(1): 30-41.

Vanzant, E.S., Cochran, R.C. & Titgemeyer, E.C., 1998. Standardization of *in situ* techniques for ruminant feedstuff evaluation. J. Anim. Sci. 76(10): 2717.

Weakley, D.C., Stern, M.D. & Satter, L.D., 1983. Factors affecting disappearance of feedstuffs from bags suspended in the rumen. J. Anim. Sci. 56(2): 493.

CHAPTER 5

General conclusion

Dairy farmers are continually challenged to increase production and profitability. This necessitates highly specialised, well balanced diets. To enable nutritionists to formulate these diets, accurate determinations of the nutritional value of feedstuffs are needed.

The NRC recommends that feedstuffs should be ground through a 2 mm screen prior to *in sacco* incubation. This result in fine particles that could be washed out of the dacron bags because of the variation in bag pore sizes. The objective of this study was to determine whether this fine material, resulting from the grinding of feedstuffs, would lead to an overestimation of protein degradation occurring in the rumen. The protein feedstuffs that were used were feedstuffs that are generally used in the dairy industry in South Africa.

Sieving resulted in a substantial amount of material passing through the sieve and being lost (between 20 and 60%). The 106 μm mesh appeared to be the best for the removal of fine particles without changing the chemical composition, except for maize gluten 20, where all three treatments differed and for which an optimal mesh size could not be found.

The un-sieved treatments resulted in the highest a-values for DM and CP degradability and also the highest effective CP degradability for all the feedstuffs. The a-values and effective CP degradability of the un-sieved treatments were significantly higher than for both sieved treatments.

More research is needed to find a suitable mesh size for maize gluten 20, as the chemical composition of the un-sieved samples differed from the sieved ones in the current study. It could be beneficial to use a longer incubation period for maize gluten 60 and fish meal, as an asymptote was not reached at the end of the incubation period of the current study.

To conclude, it appears that sieving enables one to more accurately determine RDP, and that this will allow a more accurate calculation of the amount of UDP that reaches the small intestine. An additional sieving step is thus needed for the preparation of samples for *in sacco* degradation trials. The recommended mesh size for sieving is 106 μm.