

**THE EFFECT OF EXTRUSION ON THE DEGRADABILITY
PARAMETERS OF VARIOUS VEGETABLE PROTEIN SOURCES**

By

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**Thesis submitted in partial fulfillment of the
requirements for the degree of Masters
(Agricultural Sciences)**



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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my original work and that I have not previously in its entirety or in part submitted it at any university for a degree.



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ABSTRACT

THE EFFECT OF EXTRUSION ON THE DEGRADABILITY PARAMETERS OF VARIOUS VEGETABLE PROTEIN SOURCES

The objective of this study was to determine the effect of extrusion, as a method of heat treatment, on the dry matter (DM) and crude protein (CP) degradability parameters of various vegetable protein sources commonly used in the Western Cape Province, South Africa. The feedstuffs used were lupins (LUP), full fat soybeans (SB), full fat canola seeds (FCS), soybean meal (SBM), canola meal (CM) and sunflower meal (SFM).

In the first trial, the degradability parameters were determined according to an *in sacco* degradability procedure. Four non-lactating Holstein cows, fitted with rumen cannulae, were used in the trial and all cows received the same basal lactation diet. The samples were incubated in dacron bags and bags were removed at intervals of 0, 2, 4, 8, 16, 24 and 48 hours. Dry matter and CP disappearance values were determined and fitted to a one-compartment model by means of an iterative least-square procedure in order to determine the DM and CP degradability parameters. Results indicated that extrusion significantly lowered the effective degradability of the DM-fraction (20.1% on average) of all the feedstuffs, except LUP, and the effective degradability of CP in all the raw materials (27% on average).

The second trial was an *in vitro* degradability trial that ran parallel with the *in sacco* degradability trial and was done with the aid of a Daisy^{II} Incubator (ANKOM Technology Corp., Fairport, NY). The same feedstuffs were tested in both trials. A composited sample of rumen liquor from two of the cows used in the *in sacco* trial was used for *in vitro* incubation of the samples. The data obtained in this trial were analyzed in a similar way to that of the *in sacco* trial. Due to a limited amount of residue left after incubation, CP disappearance could not be calculated at each time interval for SB and SBM in the *in vitro* trial. In this case, actual disappearance values after 8h were used to compare treatments. Extrusion significantly lowered the effective degradability (as determined *in vitro*) of DM in all the feedstuffs tested

(16.8% on average), as well as the effective degradability of CP in LUP, FCS, CM and SFM (21.8% on average). A comparison of the actual disappearance values after 8 hours incubation indicated that extrusion also lowered the rate of CP disappearance for SB and SBM.

The values obtained in the *in vitro* trial and those from the *in sacco* trial, for the same feedstuffs, were compared. It appeared as if the *in vitro* determined values were over-estimations of the *in sacco* determined values. A regression analysis showed a high correlation between the actual *in vitro* CP disappearance values after 8h incubation and *in sacco* determined effective degradability values.

The third part of this study was a set of chemical analysis to determine the effect of extrusion on certain nitrogen fractions of the feedstuffs tested in the above mentioned trials. Solubility in a mineral buffer solution was determined to estimate the potential rumen degradability of the protein. The buffer insoluble nitrogen (BIN) fraction of all the feedstuffs, except FCS, was significantly increased by extrusion. Extrusion lowered the acid detergent insoluble nitrogen (ADIN) content of all feedstuffs, except FCS, which could imply that the temperature reached during extrusion (115°C - 120°C) was not high enough to cause damage to the protein. The neutral detergent insoluble nitrogen (NDIN) fraction of extruded SB, SBM, CM and SFM was significantly higher than that of the raw feedstuffs. Extrusion left the NDIN-fraction of FCS and LUP unaltered. Comparison of the NDIN : ADIN ratio of extruded with that of the raw feedstuffs provided reason to believe that extrusion had a positive effect on all feedstuffs (except FCS).

Extrusion appears to be a useful method to decrease rumen degradation of vegetable protein sources, without causing heat damage. Furthermore, this means that protein sources of which the use have been limited due to its high rumen degradable protein (RDP) content, could be included in diets at higher levels following extrusion. The protein sources mentioned are also good sources of energy and the combination of energy and rumen undegradable protein (RUP) in the diet of the high-producing dairy cow could only be beneficial.

SAMEVATTING

DIE EFFEK VAN EKSTRUSIE OP DIE DEGRADERINGSPARAMETERS VAN VERSKEIE PLANTAARDIGE PROTEÏEN BRONNE

Die doel van hierdie studie was om die effek van ekstrusie op die droëmateriaal (DM) en ru-proteïen (RP)-degradeerbaarheidsparameters van verskeie plantaardige proteïenbronne wat algemeen in die Wes-Kaap (RSA), gebruik word, te bepaal. Ekstrusie is 'n metode van hitteprosessering wat algemeen gebruik word deur plaaslike en internasionale veevoervervaardigers. Die volgende grondstowwe is geëvalueer: lupiene, volvet sojabone, volvet canolasaad, sojaboon-oliekoekmeel, canola-oliekoekmeel en sonneblom-oliekoekmeel.

In die eerste proef is die degradeerbaarheidsparameters met behulp van 'n *in sacco* studie bepaal. Vier droë Holstein koeie met rumen kannulas is in die studie gebruik en al vier koeie het dieselfde basale dieet ontvang. Monsters is in dacronsakkies geïnkubeer en die sakkies is uit die rumen verwyder na onderskeidelik 0, 2, 4, 8, 16, 24 en 48 uur intervalle. Die waardes van DM- en RP- verdwyning is bereken en dan met 'n iteratiewe kleinste kwadraat prosedure op 'n een-kompartement model gepas om die *in sacco* DM- en RP-degradeerbaarheidsparameters te bepaal. Die resultate van die studie het getoon dat ekstrusie die effektiewe degradeerbaarheid van die DM-fraksie van al die grondstowwe, behalwe lupiene, betekenisvol verlaag het (met gemiddeld 20.1%), asook die effektiewe degradeerbaarheid van die RP-fraksie van al die grondstowwe (met gemiddeld 27%).

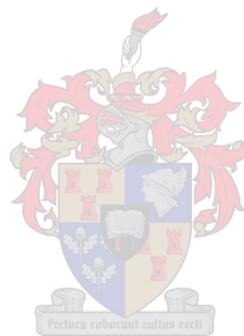
Die tweede proef was 'n *in vitro*-degradeerbaarheidsstudie wat met behulp van 'n ANKOM Daisy^{II} Inkubeerder uitgevoer is en wat parallel met die *in sacco*-studie gedoen is. Dieselfde grondstowwe is in beide proewe geëvalueer. 'n Saamgestelde monster van die rumenvloeistof van twee van die koeie wat vir die *in sacco*-studie gebruik is, is gebruik vir die *in vitro*-inkubasie van die monsters. Data-verwerking is op 'n soortgelyke wyse as dié van die *in sacco*-studie uitgevoer. As gevolg van 'n beperkte hoeveelheid residu na afloop van die inkubasies, kon die RP-verdwyning vir volvet sojabone en sojaboon oliekoekmeel nie bereken

word nie. In hierdie geval is waargenome verdwyningswaardes na 8h gebruik om behandelings te vergelyk. Hierdie studie het getoon dat ekstrusie die effektiewe degradeerbaarheid van DM (soos *in vitro* bepaal) in al die getoetste grondstowwe betekenisvol verlaag het (met gemiddeld 16.8%). Die effektiewe degradeerbaarheid van RP in lupiene, volvet canola saad, canola oliekoekmeel en sonneblom oliekoekmeel is ook betekenisvol verlaag (met gemiddeld 21.8%). 'n Vergelyking van die oorspronklike verdwyningswaardes van volvet sojabone en sojaboon oliekoekmeel na 'n inkubasieperiode van 8 ure het ook getoon dat ekstrusie die tempo van RP-verdwyning uit die rumen vertraag het.

Die *in sacco*- en *in vitro*-bepaalde waardes vir elke grondstof is vergelyk en dit kom voor asof die *in vitro*-waardes oorskattings van die *in sacco*-waardes is. 'n Regressie-analise het verder getoon dat daar 'n hoë korrelasie was tussen die waargenome *in vitro* RP-verdwyningswaardes na 8 ure inkubasie en die beraamde effektiewe degradeerbaarheid, soos *in sacco* bepaal.

Die derde deel van die studie was 'n stel chemiese analyses wat uitgevoer is om die effek van ekstrusie op sekere stikstof (N)-fraksies van die grondstowwe, wat in bogenoemde proewe gebruik is, te bepaal. Die oplosbaarheid van N in 'n mineraal-bufferoplossing kan gebruik word as aanduiding van die potensiële rumendegradearbaarheid van die proteïen. Die buffer-onoplosbare N-fraksie van al die grondstowwe (behalwe volvet canolasaad) is betekenisvol verlaag deur ekstrusie. Ekstrusie het ook die suur-onoplosbare N-fraksie (ADIN) van al die grondstowwe (behalwe volvet canolasaad) betekenisvol verlaag. Dit kan moontlik daarop dui dat die temperatuur wat tydens ekstrusie (115°C - 120°C) bereik is, nie hoog genoeg was om die proteïen in die grondstowwe te beskadig nie. Ekstrusie het die N-fraksie wat onoplosbaar was in 'n neutrale oplossing (NDIN) betekenisvol verhoog in volvet sojabone, sojaboon-oliekoekmeel, canola-oliekoekmeel en sonneblom-oliekoekmeel en dit onveranderd gelaat in lupiene en volvet canolasaad). Die verhouding van NDIN : ADIN van die geëkstrueerde grondstowwe is vergelyk met dié van die rou grondstowwe. Dit blyk dat ekstrusie wel 'n positiewe effek op al die grondstowwe (behalwe volvet canolasaad) gehad het.

Dit wil dus voorkom asof ekstrusie wel aangewend kan word om die rumen-degradeerbaarheid van plantaardige proteïenbronne te verlaag sonder om die proteïen te beskadig. Dit kan daartoe lei dat proteïenbronne waarvan die gebruik voorheen beperk was as gevolg van die hoë rumen-degradeerbare proteïen-inhoud daarvan nou wel in rantsoene ingesluit kan word na die ekstrusie daarvan. Die proteïenbronne, soos genoem, is ook redelike bronne van energie en die kombinasie van energie en rumen nie-degradeerbare proteïen in die rantsoen van die hoog-produiserende melkkoei kan slegs voordelig wees.



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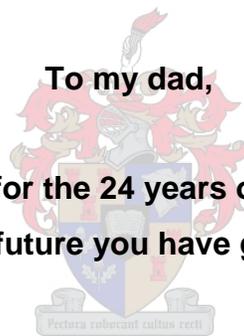
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**To my dad,
Thank you for the 24 years of education,
and the future you have given me.**



LIST OF ABBREVIATIONS

a	Soluble fraction
AA	Amino acids
ADF	Acid detergent fibre
ADIN	Acid detergent insoluble nitrogen
b	Potential degradable fraction
c	Rate of degradation
CM	Canola meal
CP	Crude protein
D _{eff}	Effective degradability
DM	Dry matter
EAA	Essential amino acids
FCS	Full fat canola seeds
k _p	Rate of passage
LUP	Lupins
N	Nitrogen
NDF	Neutral detergent fibre
NDIN	Neutral detergent insoluble nitrogen
NPN	Non-protein nitrogen
NH ₃	Ammonia
RDP	Rumen degradable protein
RUP	Rumen undegradable protein
SB	Full fat soybeans
SBM	Soybean oilcake meal
SFM	Sunflower oilcake meal
SNF	Solids-not-fat

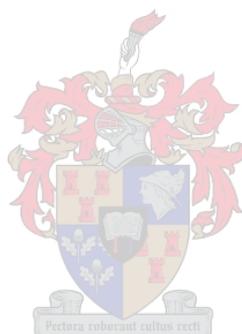


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CHAPTER 1

LITERATURE REVIEW AND PROBLEM STATEMENT

GENERAL INTRODUCTION

For many years, since as early as 1927, scientists have been investigating the possibility of manipulating milk composition by means of altered feeding strategies (Sutton, 1989). Today, this method receives much attention from animal scientists and nutritionists.

Over the years, the consumer's demands in terms of the required milk components have changed remarkably, this mainly being due to better knowledge of the role of food and its components in human health. Unfortunately, consumer demands can change quite fast and therefore an alternative means of manipulation other than genetic methods have to be investigated as genetic manipulation is suitable for long-term (>5 years), but not for short-term (<5 years) alterations. Providing the substrates necessary for the synthesis of the desired milk components in the diet is a possible means of short-term manipulation of the milk composition (Kennely & Glimm, 1998).

Furthermore, the alteration of milk composition also has economic implications. When looking at milk pricing schemes, which reflects the market value of the milk components, it is clear that the price of milk depends on the protein content, the fat content and the quality of the milk. Most buyers pay for the quantity of milk delivered, whilst the percentage composition determines whether the producer qualifies for a premium or not (Chase, 1990). The main milk buyers in South Africa use a multifactor pricing system to determine the price of the milk per litre. According to this system, more emphasis is placed on the milk protein rather than the milk fat content of milk. The price ratio is more or less 60 : 40 in favour of protein (Erasmus, 2001).

Unfortunately, feeding does not give such a drastic response when compared to genetic manipulation in terms of change in the protein content of milk, but it still is an integral part of management that can be altered quickly and therefore deserves some attention. Though changes in milk protein concentration can be brought about by dietary manipulations, compared with the alterations possible in fat concentration, the scope for these changes is far smaller. According to Sutton (1989) the reasons being:

- Smaller possible natural variation
- Important dietary factors are less well identified
- Milk protein concentration is only recently becoming a factor affecting the unit price of milk so the subject has attracted less attention
- Until recently the results of many experiments were reported in terms of fats and solids-not-fat (**SNF**)
- The basic factors affecting milk protein synthesis and concentration are relatively poorly understood

The protein value of feeds for ruminants depend on an estimate of the protein quantity absorbed from the small intestine. Dietary proteins that escape degradation in the rumen are therefore a significant factor in determining the protein value of feeds (Aufrère *et al.*, 2001). More specifically, the value of the protein source is determined by the potential thereof to provide limiting essential amino acids (**EAA**) to the small intestine where absorption can take place (Cros *et al.*, 1992).

Decreasing the rate and extent of digestion of dietary protein in the rumen has been shown to increase the milk protein percentage although a greater positive effect is seen on milk yield and milk protein yield (Robinson *et al.*, 1991). Various studies, such as those by DePeters & Cant (1992) also suggest that the quality and therefore the degradability of dietary protein can influence the protein content of milk. Thus, for nutritionists it is of great importance to find a way of altering the degradation of protein.

Extrusion of soybean meal (**SBM**) has been shown to reduce nitrogen (**N**) solubility and rumen degradable protein (**RDP**) (Sahlu *et al.*, 1984, Schingoethe *et al.*, 1988; Waltz &

Stern, 1989). Extruded SBM fed to early lactating cows led to greater milk production compared to raw SBM, probably because of greater amino acid (**AA**) flow from crude protein (**CP**) that was more resistant to microbial degradation. Including extruded SBM in the diet thus poses a way of increasing the rumen undegradable protein (**RUP**) concentration without the use of by-product feedstuffs (Casper *et al.*, 1999). Furthermore, Schroeder (1998) suggested that oilcakes could be used more efficiently when heat processing is applied. Normally, due to the high ruminal degradability of oilcakes used in South African dairy cow diets, their inclusion levels are limited.

The purpose then of this project is to determine whether extrusion, as a heat treatment process, can significantly increase the RUP fraction of some oilcakes and oilseeds commonly used as protein sources in South African dairy cow diets.

PROTEIN DEGRADATION AND DIGESTION

Dietary protein also referred to as CP or dietary CP can be defined as the N content of the feedstuff multiplied by 6.25 which is a factor derived from the average % N in vegetable protein. The main purpose of this dietary protein is to provide the AA which is the main building blocks for protein synthesis in the cow (Webster, 1987).

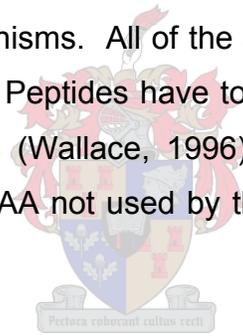
Crude protein can basically be divided into 3 fractions, namely true protein, non-protein nitrogen (**NPN**) and the acid detergent insoluble nitrogen (**ADIN**) fraction (Webster, 1987).

Acid detergent insoluble nitrogen refers to nitrogenous compounds that are bound up in the lignified, totally indigestible portion of the cell wall and thus unavailable for degradation in the rumen or subsequent acid digestion (Van Soest, 1982, cited by Webster, 1987). This fraction is excreted as N in the faeces.

The NPN fraction is either absorbed by the animal, and then recycled or retained in the tissues and milk, or it is excreted in the faeces and urine. In addition, it can be used by certain rumen microbes.

The true protein can be subdivided into the rumen degradable protein (**RDP**) fraction and the rumen undegradable protein (**RUP**) fraction. These two fractions of dietary CP have distinct different functions. The RDP is the fraction that is broken down by rumen microbes into ammonia, energy and carbon fragments. These products are to provide in the needs of the microbes, which then in turn supply ruminally synthesized microbial protein, which provide most of the AA passing into the small intestine.

The pool of potentially fermentable protein not only consists of the dietary proteins, but also includes the endogenous proteins of the saliva, sloughed epithelial cells and the remains of lysed rumen microorganisms. All of the enzymatic activity of ruminal protein degradation is of microbial origin. Peptides have to be broken down to AA before they can be used by certain microbes (Wallace, 1996). Thus, the peptides that escape ruminal degradation, and the free AA not used by the microbes will flow through to the abomasum (NRC, 2001).



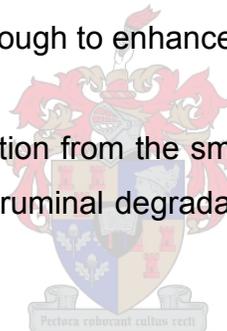
The main microorganisms involved in ruminal degradation, and also the most abundant microorganisms in the rumen, are bacteria. The initial step in protein degradation by ruminal bacteria is adsorption of soluble proteins by bacteria (Nugent & Mangan, 1981; Wallace, 1985) or the adsorption of bacteria to insoluble proteins (Broderick *et al.*, 1991, cited by the NRC, 2001). Bacteria cannot distinguish between sources of N for protein synthesis (Kung & Huber, 1983).

The RUP fraction is the smaller fragment of protein which passes to the abomasum intact. Here one can thus find a combination of dietary and microbial protein which is available for digestion that starts in the abomasum with acid-pepsin digestion and is completed in the small intestine with pancreatic and intestinal proteases (Stern *et al.*, 1997). Microbial protein supplies approximately two-thirds of the ruminant's AA

requirement, while dietary protein sources accounts for the most of the remainder (Satter, 1986). Digestion finally yields free AA. These AA then continue towards the small intestine where it can be absorbed (Chamberlain & Wilkinson, 2002) for metabolism in the different tissues of the animal, including the mammary gland, where milk protein is but one of the final products to be formed (AFRC, 1998). RUP is the second most important source of absorbable AA to the animal (NRC, 2001).

From this it is clear that the rate and extent of degradation not only affects microbial proteins synthesis, but also determines the amount of undegradable protein reaching the small intestine (Erasmus *et al.*, 1988). Thus for the lactating dairy cow to reach her genetic potential for production, the diet needs to provide sufficient RDP to supply in the needs of the rumen microbial population, and sufficient RUP to escape rumen fermentation to supply additional AA to the small intestine. Only once this is achieved will protein reserves be elevated enough to enhance milk production (Crish *et al.*, 1986).

Amino acid passage to and absorption from the small intestine depends on the amount of protein consumed, the extent of ruminal degradation of the protein and the synthesis of microbial protein (AFRC, 1998).



At first it has been assumed that the degradability of a given feed is constant. This however is not the case due to the fact that high-producing cows have a higher intake, which means that feed passes through the rumen faster, resulting in shorter retention time in the rumen and accordingly the time that the protein is exposed to the rumen microbial population. As a smaller fraction of the protein is exposed to the rumen microbes, a smaller fraction of the protein is thus degraded. For some feed, such as SBM, the retention time of the feed in the rumen alters the degradability of protein considerably. Therefore, in high-producing dairy cows which have a short retention time for feed in the rumen, the degradability of a feed such as SBM will be lower than in low-producing cows where degradability is higher due to increased rumen retention time and effectively more time for the microbes to act on the protein.

The extent of protein degradation is also dependant on microbial activity and access to the protein. Access by proteolytic enzymes is influenced by the 3-dimensional structure of the protein molecule. Proteins with extensive cross-linking are relatively resistant to degradation (Nugent & Mangan, 1978, cited by Satter, 1986). Feed processing methods such as extrusion may generate enough heat to alter the protein structure.

Soluble proteins tend to be more rapidly or completely degraded than insoluble proteins (Henderickz & Martin, 1963, cited by Satter, 1986). Access to protein by proteases is greater if the protein is in solution. Unfortunately, protein solubility as a measure of protein degradation can lead to serious error when applied across a variety of feeds but it is reasonable to expect protein solubility to predict differences in protein degradation more accurately when applied to a group of similar feeds than when used across a diverse group of feeds differing in physical and chemical properties. Variation in protein degradation within a feed can be large, for example due to differences in processing conditions which can affect protein degradation. Variation from one supplier to another can be significant (Satter, 1986).

Many factors thus influence the degradability of any protein source. Effective degradability of a given feed depends on the level of production in the animal, and hence the rate of outflow, as well as the specific degradability pattern for that feed (Chamberlain & Wilkinson, 2002).

THE SYNTHESIS OF MILK PROTEIN

The synthesis of milk fat, proteins and lactose all occur in the alveolar cells of the mammary gland. Amino acids in the arterial blood are the principal precursors of their corresponding residues in milk protein (Mephram, 1982). Small peptides also contribute to this (Backwell *et al.*, 1994, cited by AFRC, 1998). Amino acids are transferred to the milk quantitatively (Metcalf *et al.*, 1996), catabolized, especially the branched chain AA, or synthesized within the mammary gland (AFRC, 1998).

Although the arterial concentration of AA may vary, mammary uptake remains relatively constant, suggesting that mammary cells have a range over which they are able to adjust the efficiency of AA uptake to match the requirements for milk protein synthesis. Thus, the mammary gland can obtain adequate amounts of AA over a wide range of blood concentrations (Griinari *et al.*, 1996).

The osmotic concentration of milk is necessarily the same as that of plasma. The ratio of lactose to water is fixed within very narrow limits. The synthesis of milk protein is metabolically linked rather closely to lactose synthesis within any one genotype and is therefore indirectly but closely related to water secretion. Thus, when feeding to produce more milk protein, one must be prepared to accept more lactose and water as accompanists (Webster, 1987).

The main milk proteins are called caseins and are synthesized only in the mammary gland from single AA carried to the gland in the arterial blood. Whey proteins in normal milk, lactalbumin and lactoglobulin, are synthesized for the most part in the alveolar epithelium. Immunoglobulins, the immune proteins containing the antibodies against infectious agents and other antigens with which the cow may come in contact, are synthesized by specialized plasma cells in the mammary gland and elsewhere and are then transported in the blood plasma to and through the alveolar epithelium and into the milk, especially the colostrum (Webster, 1987).

PROTEIN REQUIREMENTS OF THE LACTATING DAIRY COW

The protein requirement of the dairy cow is based on the factorial determination of her requirements for maintenance, which includes urinary endogenous N, scurf N (skin, skin secretions and hair) and metabolic faecal N, and her requirements for production, which can be sub-divided into her needs for conceptus gain, growth and lactation. For the purpose of this study we are only interested in the protein requirements as for lactation. This is based on the amount of protein secreted in the milk. The efficiency of use of metabolizable protein for lactation is assumed to be 0.67 (NRC, 2001).

Dado *et al.* (1993) used models of the biochemical reactions of milk synthesis to estimate the absorbed protein requirements for lactation. According to Dado *et al.* (1993) most protein systems assume that milk protein is the only component requiring absorbed true protein, but this may not be the case as protein could also be needed for milk lactose or fat synthesis, should an absolute requirement for this exist.

Requirements for RDP and microbial protein synthesis is linked to energy intake, which means that the amount of microbial protein produced may fail to meet the requirements for net tissue protein if an animal is producing large quantities of high protein products such as milk (Chamberlain & Wilkinson, 2002). If the energy requirement for milk yield is met, feed intake will increase with milk yield, causing a higher outflow rate and consequently more dietary protein will be available (Ørskov *et al.*, 1980). Peak milk production occurs in the first six to nine weeks of lactation, whereas peak feed intake only follows at around 10 to 13 weeks. Thus, nutritionists need to focus on providing enough protein and energy in the ration to prevent a deficit (Satter & Roffler, 1974; Hutjens, 1980, cited by Van Dijk *et al.*, 1983).

With the start of lactation, the cow's protein requirement increases drastically to levels which cannot be met by the microbial protein supply. The need for protein additional to microbial protein increases with an increase in milk yield (Ørskov *et al.*, 1980). In this case, the diet must contain a digestible source of RUP (Chamberlain & Wilkinson, 2002), as mobilization of body protein is minimal and consequently depletion of mobilizable protein is rapid (Kung & Huber, 1983). Ultimately, digestible RUP is necessary for maximum expression of the cows genetically determined production potential. This holds true as long as the AA profile of the RUP supplement is of high quality, thus supplying the required EAA (NRC, 2001). Digestibility of RUP, variation in AA profile of the RUP and protein degradability are all taken into account in diet formulation for high producing dairy cows. Production can benefit from an increase in RUP (Kung & Huber, 1983; Faldet & Satter, 1991; Wohlt *et al.*, 1991). Chamberlain & Wilkinson (2002) also noted that sources of RUP had a positive response on production in situations where dietary CP and metabolizable protein supplies are adequate.

The metabolizable protein requirement is met primarily by rumen microbial protein and dietary protein that escapes undegraded. Both the Agricultural Research Council (**ARC**) and National Research Council (**NRC**) recognize that dietary CP can be divided into the degradable part which supports microbial growth and the undegradable part which supplements rumen microbial protein for use by the cow (Robinson *et al.*, 1991). The goal of protein nutrition of ruminants can thus be divided into two main areas. Firstly one has to ensure adequate, but not excessive, RDP supply to fulfill the nitrogen needs of the rumen microbes in order to ensure maximal synthesis of microbial CP. Secondly, one has to include adequate amounts of digestible RUP that will optimize the profile and amounts of absorbed AA (NRC, 2001). Nevertheless, the specific requirement for RUP is unknown as it is supplemental to microbial protein, which is almost always insufficient to supply in the metabolizable protein requirement of the cow (ARC, 1984, and NRC, 1989, cited by Robinson *et al.*, 1991).

THE LACTATIONAL RESPONSE TO PROTEIN IN THE DIET

Milk protein concentration and composition are influenced by many factors and there are limits to the extent to which milk components can be altered. The magnitude of change is less for milk protein than that observed for milk fat content. For milk protein an average of 0.6% units is susceptible for change (Erasmus, 2001).

In a review by Emery (1978), he found that for every 1% increase in the dietary CP, as long as it is not urea, there is an increase of approximately 0.02% in milk protein. He calculated the correlation between milk protein yield and dietary CP to be 0.37. Furthermore, he calculated that dietary CP and milk protein content are positively correlated ($r = 0.25$). Dietary protein affects total milk production much more than it affects the concentration of protein in the milk (Kirchgessner *et al.*, 1967, cited by Emery, 1978). Differences in the rate and extent of degradation of dairy cow diets may influence milk yield and major components of milk (Khorasani *et al.*, 1994).

Various authors (Clark, 1975; Clark *et al.*, 1977; Rogers *et al.*, 1984) found that the post-ruminal infusion of protein or AA stimulates milk and milk protein production. These results show that the digestibility of the protein is a determining factor regarding not only the response, but also the efficiency with which dietary N is used for milk production (Hof *et al.*, 1994). The source of starch in the diet could have a limited effect on milk yield and the N fractions in the milk (Khorasani *et al.*, 1994). Burgess & Nicholson (1984) found that increasing the level of dietary protein from deficient to more adequate levels (10 vs. 13 and 16% dietary CP) would result in an increase in milk protein percentage. According to the NRC (2001) the response to a change in the CP content of the diet depends on the change in the relative concentrations of the RDP and RUP. Though dietary CP is poorly correlated with milk protein yield ($r = 0.14$) and not at all with milk protein percent, the general assumption is that milk yield increases with an increase in dietary CP.

Robinson *et al.* (1991) noted that feed intake and milk yield was not influenced by substitution of rapidly degraded protein sources low in estimated RUP with more slowly degraded protein sources with higher estimated RUP.

Milk protein yield appears to increase linearly with increasing dietary RUP. In a review by Santos *et al.* (1998) the effects of replacing SBM with various RUP sources on protein metabolism and production was investigated. It was found that RUP supplementation increased milk production in only a few of the studies and heat-treated SBM or fish meal were the most likely RUP supplements to cause increased milk production (NRC, 2001). When a higher percentage RUP (as percentage of CP) was fed, high-producing cows tended to have a higher milk yield and greater efficiency of feed conversion. Thus, for lactating cows, the response to RUP appears to be related to milk yield (Santos *et al.*, 1998). Furthermore, Ferguson *et al.* (1994, cited by Santos *et al.*, 1998) found early lactating cows and high producing cows to be more responsive to protein sources with low degradability.

For protein sources to be resistant to microbial degradation does not necessarily mean that they will support high milk production. The quality of the protein source still depends on its AA profile and adequate attention must be given to this and also to the protein status of the animal (Satter, 1986).

VARIOUS TREATMENTS OF PROTEIN SOURCES

Animal feeds, generally protein-containing feeds that have been treated or processed in ways to decrease ruminal protein degradability and thereby increasing the content of digestible RUP, are frequently called “rumen protected”. The Association of American Feed Control Officials (Noel, 2000, cited by the NRC, 2001) has defined “rumen protected” as “a nutrient(s) fed in such a form that it provides an increase in the flow of that nutrient(s) unchanged, to the abomasum, yet so that it is available to the animal in the intestine”.

Rumen protected proteins are important in dairy cow nutrition, especially for high-producing cows where the basal diet mostly contains adequate or more than adequate amounts of RDP, but is deficient in RUP (NRC, 2001). Furthermore, protection of protein against rumen degradation would mean that there will be more AA available in the small intestine. This would imply a higher ratio of absorbable AA per unit absorbable energy, and ultimately a positive response in production, should the cow have a requirement for, or is able to use more AA (Chalupa, 1975). It is important to note that the fact that protein passes through the rumen doesn't necessarily mean that it can be digested efficiently or that it has the correct AA profile (Ørskov *et al.*, 1980).

One of the major advantages of feeding protected protein is the greater opportunity for utilization of NPN for microbial protein synthesis in the rumen and the economy inherent with NPN use (Satter, 1986). Less degradation of protein in the rumen could also reduce the production of ammonia (NH_3) and the cost of urea synthesis (Cros *et al.*, 1992).

Attempts to protect protein against rumen degradation have not been uniformly successful and are further complicated by the need to maintain ruminal NH_3 concentration capable of supporting optimal microbial protein synthesis (Emery, 1978).

In an attempt by Erasmus *et al.* (1988) to establish a protein degradability data base for protein sources generally used in South Africa, the results showed that heat-treated protein sources had a lower soluble N fraction than did unheated protein sources. Heat treatment thus poses to be a method of protecting protein against rumen degradation. Various authors (Nishimuta *et al.*, 1974; Schingoethe & Ahrar, 1979; Mielke & Schingoethe, 1981; Robinson & Tamminga, 1984, cited by Deacon *et al.* 1988) used heat treatment of protein sources to increase the protein fraction escaping rumen degradation.

Although various methods have been investigated, most methods involve treatment with heat and or chemicals. In the USA, heat treatment is the favoured method of processing. These methods are based on the principle of decreasing rumen protein degradability by denaturation of proteins and by formation of protein-carbohydrate and protein-protein cross-linkages. Various authors (Chalupa, 1975; Schingoethe & Ahrar, 1979) found that subjecting certain feeds to controlled high heat decreases N solubility by coagulation or denaturization of the protein. According to Broderick & Craig (1980) heat treatment can decrease the solubility and degradability of feed protein in the rumen. Modest amounts of heat damage can be beneficial. Commercial methods of heat treatment include cooker-expeller processing of oilseeds, additional heat treatment of solvent extracted oilseeds meals, roasting, extrusion, pressure toasting, micronization of legume seeds and expander treatments of cereal grains and protein supplements (NRC, 2001). Both roasting and extrusion increase the RUP fraction of oilseed proteins (Meyer *et al.*, 2001) and could therefore render a way of including higher levels of these feedstuffs in dairy diets (Schroeder *et al.*, 1995a).

In an article by Satter (1986) he explains that as the heat input increases, the amount of undegraded protein increases, and with that, the amount of unavailable protein, but

initially the quantity of unavailable protein formed is less than the amount of protein protected from degradation. The maximum amount of protein available for digestion in the small intestine will most likely occur when there is a modest amount of heat damage to the protein. There could be a temperature threshold above which protein needs to be heated before there is any significant protection.

Great care has to be taken not to under- or over-heat the feedstuffs. Under-heating will only result in minimal increases in the digestible RUP, while over-heating of feeds can reduce the intestinal digestibility of RUP through the formation of indigestible Maillard products and protein complexes and also increased losses of certain susceptible AA (Van Soest, 1994, cited by NRC, 2001). Over-heating could cause extensive denaturation and thereby defeat the purpose of protection. Excessive treatment of feedstuffs can render the undegradable protein indigestible in the small intestine. This protein will then be passed out in the faeces. Heat-damaged protein is estimated by measuring the amount of nitrogen in the acid detergent fibre (**ADF**) fraction, which is then referred to as the acid detergent insoluble nitrogen (ADIN) fraction (Goering *et al.*, 1972; Chamberlain & Wilkinson, 2002), which reflects the formation of indigestible N-containing Maillard products resulting from heat (Schroeder *et al.*, 1995b). In contrast to this, Van Soest (1989, cited by McKinnon *et al.*, 1995) proposed that the ADIN fraction might be an indicator of the heat damage that took place, but it might not quantitatively represent the amount of Maillard products. True absorption of N from the undegradable protein fraction of feeds is closely but inversely related to the ADIN fraction (Webster *et al.*, 1986, cited by Schroeder *et al.*, 1995b). Schroeder *et al.* (1995a) also found high correlations between intestinal digestibility of protein and % ADIN, and between total tract CP disappearance and % ADIN. Amongst others, they found % ADIN therefore to be a potential indicator of heat damage to protein sources. An increase in the % ADIN reflects a decrease in the nutritional value of the protein source (Krishnamoorthy *et al.*, 1982; Merchen, 1990, cited by Schroeder *et al.*, 1995b).

Van Soest (1989), cited by McKinnon *et al.* (1995), summarized this very well. According to him, the purpose of any treatment, such as heating in this case, of a

protein supplement is to change it chemically, and thereby decreasing the rumen degradability of the CP. The ideal heat treatment would then reduce the soluble N, increase the N associated with the neutral detergent fibre (**NDIN**), but minimize the increase in the ADIN fraction. The NDIN fraction represents the slowly degradable protein fraction in the rumen. The ratio of NDIN : ADIN is also indicative of the effectiveness of the increase in slowly degradable protein in the rumen. A wide ratio will indicate an effective increase, whereas a narrow ratio shows an unacceptable increase in heat damaged protein (McKinnon *et al.*, 1995).

Trials by Scott *et al.* (1991) showed that milk protein percentage was depressed by heat processing of protein sources regardless of level of production. This is in agreement with results of other trials such as those by Mielke & Schingoethe (1981) and Schingoethe *et al.* (1988) but in disagreement with results by Block *et al.* (1981) who found an increase in milk protein percentage but no change in daily yield of milk protein in early lactating cows.

For the current study, extrusion was selected as the method of heat treatment.

Extrusion and the effect on degradability

Extrusion, a process where the feedstuff is forced through a set of dies under high pressure, is commonly used as a method of heat processing of oilseeds. Steam is usually added in the extrusion process. The net result of extrusion is the generation of considerable amounts of heat. Heat treatment is said to decrease ruminal breakdown of CP and increase the digestibility of dietary protein entering the small intestine. Satter (1986) suggested that feed processing methods such as extrusion may generate enough heat to alter protein structure.

Extrusion of seeds rich in protein generally results in decreased ruminal degradation of protein, and increased duodenal flow of protein (Benchaar *et al.*, 1994), which can result in higher supply of AA for the mammary gland, particularly methionine that is abundant

in canola proteins (Bayourthe *et al.*, 2000). Stern *et al.* (1985) found that the total amount of AA flowing to the small intestine was greater in cows fed extruded soybeans compared to raw soybeans.

In experiments by Scott *et al.* (1991) with extruded and raw soybeans, they found that the propionate concentrations and acetate : propionate ratios were higher for extruded soybeans than raw soybeans. This indicates that ruminal fermentation was not impaired. A further indication of minimal alteration of ruminal fermentation was found by Casper *et al.* (1999) whose data indicated that pool sizes and ruminal outflows were not affected by substituting extruded SBM for raw SBM. Processing also had very little effect on lactation performance.

Deacon *et al.* (1988) found extrusion not to have a significant influence on the rumen degradability of canola and soybeans. They suggested that this might indicate that temperature reached during extrusion is not high enough and the duration of exposure to this temperature not long enough. The exposure to steam during the extrusion process could also affect the effectiveness of the heat treatment.

PROTEIN SOURCES

A number of vegetable protein sources have been selected for the current study. These are common oilseeds and oilcake meals available for use in dairy cow diets in South Africa.

The following is a short review of literature on these protein sources, and if available, literature on the effect of extrusion of these sources on milk production and milk composition.

Oilseeds generally have high protein and fat contents. The protein is highly degradable in the rumen and the fat is a good source of energy. Inclusion of oilseeds in rations for early lactating cows can be beneficial as it can provide in the large amounts of protein

required to balance the calories mobilized from the body when the cow experiences a negative energy balance (Mohamed *et al.*, 1988). Oilcake meals also have relatively high contents of highly degradable protein, but are low in fat as they have previously been extracted. Oilcake meals can be well used in diets for high-producing dairy cows as they can supply both energy and protein, but as is the case with oilseeds, use thereof is limited mainly due to the high RDP fraction of the available protein.

Lupins

Lupins are an acceptable supplemental protein source for cows (May *et al.*, 1993), but unfortunately this protein is highly degradable in the rumen. Extrusion of whole lupin seeds has shown to reduce the solubility of proteins, thus lowering the susceptibility to ruminal degradation (Cross *et al.*, 1991a, cited by Bayourthe *et al.*, 1998). Higher disappearance of AA from the intestine has been noted. Aufrère *et al.* (2001) found that heat treatment reduces degradation of the CP without altering the intestinal digestibility. Benchaar *et al.* (1994) found heat treatment to have no significant effect on the CP content of lupin seeds, but that it could reduce the CP solubility by 75%.

According to Cros *et al.* (1992) extrusion did not alter the AA profile of white lupin seeds, but the AA composition of the protein that escaped rumen degradation differed from that of the original source. After extrusion, the RUP fraction had a higher protein value.

In a trial by Bayourthe *et al.* (1998) milk protein was significantly lower for cows fed extruded lupins. Though extrusion of lupins resulted in slightly increased milk yield and lowered milk fat percentage, which is preferred by the dairy industry (Bayourthe *et al.*, 1998), while the lowered milk protein percentage is unfavourable.

Full fat soybeans and soybean meal

Full fat soybeans are a popular high energy supplement in dairy cow diets (Scott *et al.*, 1991). Soybean meal is an excellent source of lysine (Satter, 1986) and is a rapidly

degradable protein source (Robinson *et al.*, 1991; Bayourthe *et al.*, 1998). It is an excellent protein source and it can also contribute fat to the diet, which can provide energy (Van Dijk *et al.*, 1983).

It has been proved to be beneficial to submit soybeans to heat treatment. Roasting and extrusion of soybeans depressed the fractional rate of CP disappearance relative to raw soybeans (Scott *et al.*, 1991). This is in contrast to trials by Deacon *et al.* (1988) who found extrusion not to alter the dry matter (**DM**) or CP disappearance from the rumen or the effective degradability thereof. Research done by Stern *et al.* (1985) showed that raw soybeans are extensively degraded in the rumen, which means less total AA to reach the duodenum and thus less absorption of AA. Extrusion of whole soybeans on the other hand increased the availability of the total essential AA in the small intestine and higher absorption from the small intestine when compared to raw whole soybeans and soybean meal. Extruded soybean meal resulted in higher milk production than conventional soybean meal in trials by Stehr (1984, cited by Satter, 1986). This could be attributable to high levels of polyunsaturated fat made accessible to the rumen due to processing (Meyer *et al.*, 2001). In a study by Faldet & Satter (1991), feeding of heat treated soybeans compared to soybean meal and raw soybeans, increased milk and milk protein yield, left milk fat unaltered and milk protein percentage was lower than for soybean meal. Mohamed *et al.* (1988) suggested that extrusion ruptures the fat micelles within soybeans, which may allow a more rapid release of oil into the rumen, resulting in milk fat depression.

In growth assays using laboratory mice, Schingoethe & Ahrar (1979) found that heat-treatment did not alter the growth rate of mice when they were fed soybean meal, raw or extruded, or sunflower meal, raw or extruded. This would suggest that, while the solubility of the protein was decreased, it was still digestible and absorbable in the intestines.

Heat treatment is believed to have the greatest potential for safe and economical treatment of soybeans (Faldet & Satter, 1991). This will not only increase the RUP

fraction, but could possibly maximize the available lysine passing to the small intestine too. Additional benefits from heating soybeans include the destruction of trypsin inhibitors and reduced lipase activity, resulting in better storage qualities (Mielke & Schingoethe, 1981) and fewer problems generally associated with the feeding of large amounts of soybeans.

Canola seeds and canola meal

Canola is widely used as a protein supplement due to its high CP content, but the inclusion rate in rations is limited due to the high degradability of the CP fraction in the rumen (McKinnon *et al.*, 1991; Bayourthe *et al.*, 1998 ; Von Keyserlingk *et al.*, 2000). Whole canola seeds had higher rumen degradability than canola meal in a study done by Deacon *et al.* (1988).

Mustafa *et al.* (2000) conducted a study to determine the effects of stage of processing of canola seeds on chemical characteristics and *in vitro* CP degradability of canola products. They found that major changes in protein composition and degradability took place as a result of heating in a desolventizer-toaster. Temperatures in the desolventizer-toaster were between 103°C and 107°C. It has been shown that heat treatment reduces protein solubility and increases rumen undegradable protein of canola seed (Deacon *et al.*, 1988) and canola meal (McKinnon *et al.*, 1995). A study by McKinnon *et al.* (1995) compared the effect of heating canola meal to 125°C and 145°C, and found that heating to 145°C reduced intestinal CP disappearance and reduced the ruminal and total tract availability of the DM and CP fractions. Heating to 125°C reduced rumen disappearance of the DM and CP fractions, but did not significantly reduce the disappearance of CP over the total tract of the ruminant. This indicates that short duration dry heat treatment of canola meal to 125° can increase the RUP content of the canola meal without compromising the digestibility thereof.

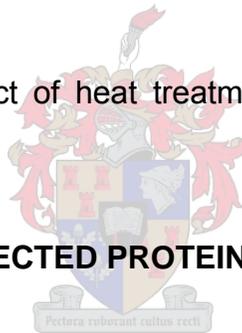
Because of its high fat content, extrusion of pure canola seeds is difficult and can result in important loss of fat. In experiments using blends of canola seeds and other protein

sources, extrusion has been found to decrease ruminal degradation of CP and DM (Chapoutot & Sauvant, 1997).

Sunflower oilcake meal

In South Africa, sunflower oilcake meal is a prominent plant protein source in animal feeds. Inclusion levels are unfortunately limited due to its high rumen degradability (Erasmus *et al.*, 1988). Shroeder *et al.* (1995b) proposed that with efficient heat processing, the RUP levels could be increased, and with that the inclusion levels of sunflower meal in ruminant diets. They found heat processing to decrease the effective protein degradability and increase the availability of total essential amino acids. This quality increase should have a positive effect when inclusion levels of sunflower meal are increased.

Very little information on the effect of heat treatment on sunflower meal is currently available.



STRATEGIES FOR USING PROTECTED PROTEINS IN DAIRY COW DIETS

According to Satter (1986) there are at least 3 different strategies for using protected proteins in dairy cow diets:

1. Substitution of a conventional protein supplement with an equal amount of protein from a relatively resistant protein source. This is in anticipation of higher milk production. The amount of additional milk expected from the substitution might be minimal, therefore the obvious question the dairy farmer has to ask himself is whether the increase in milk production from feeding the protected protein will pay for the additional cost of such a protein source.
2. Replacement of the conventional protein supplement with a smaller quantity of the relatively resistant protein. Milk production should be unaffected, but the cost of protein supplementation might be lowered, because less total protein will be used.

3. Combining a low cost NPN source with a resistant protein supplement and an energy source to give a low cost mixture that may substitute for the conventional protein supplement. Change in milk production would not be expected, but the cost of supplementation would be reduced.

PROBLEM STATEMENT AND MOTIVATION FOR THIS RESEARCH

The feed dictionaries of dynamic models and programs, such as the CPM Dairy and the NRC tables, are widely used as standard references for feed formulations by nutritionists. We have found that these sources lack data on the effect of heat treatment, especially extrusion, on the degradability and nutritional value of oilseeds and oilcake meals with the exception of full fat soybeans and soybean meal.

The aim of the current study was therefore to provide the industry with usable information in this regard. Extrusion is a method of heat treatment commonly used by many commercial feed manufacturers in South Africa. We hope to prove that extrusion can successfully be implemented to decrease the ruminal degradability of oilseeds and other protein sources as this could have a positive impact on both milk production and milk composition.

Rumen degradability was determined by means of an *in sacco* degradability trial and an adapted *in vitro* method. We found a lack of data comparing the results of these two methods. Thus, results were compared to determine whether the proposed *in vitro* method is a reliable alternative to the *in sacco* method that is generally used.

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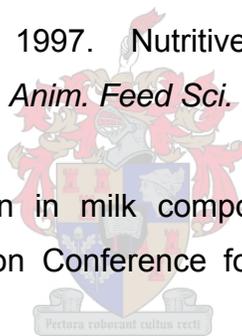
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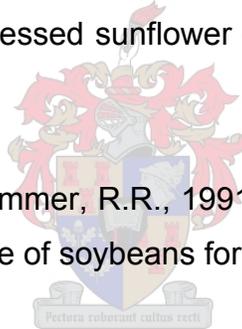
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CHAPTER 2

THE EFFECT OF EXTRUSION ON *IN SACCO* DRY MATTER AND CRUDE PROTEIN DEGRADABILITY OF VARIOUS PROTEIN SOURCES

ABSTRACT

The dry matter (DM) and crude protein (CP) degradability of six protein sources, raw and extruded, were determined in sacco. Feedstuffs used were lupins (LUP), full fat soybeans (SB), full fat canola seeds (FCS), soybean oilcake meal (SBM), canola meal (CM) and sunflower oilcake meal (SFM). Four non-lactating Holstein cows, fitted with rumen cannulae, were used in the trial and all received the same basal lactation diet. The samples were incubated in the rumen in polyester dacron bags and bags were removed at intervals of 0, 2, 4, 8, 16, 24 and 48 hours. The disappearance of DM and CP were determined and were then used to estimate the in sacco DM and CP degradability parameters. Extrusion significantly lowered the effective degradability of the DM-fraction of SB, FCS, SBM, CM and SFM (20.1% on average). The effective degradability of CP in all the raw materials was significantly lowered (27% on average) by extrusion.

Key words: dry matter, crude protein, degradability, *in sacco*, Holstein cows, extrusion.

INTRODUCTION

Protein nutrition of dairy cows enjoys increasingly more attention. This is attributable to various reasons, mainly being the special dietary requirements of the modern, high-producing dairy cow which has a great demand for digestible protein for which the microbial protein produced is generally accepted to be insufficient (ARC, 1984, and NRC, 1989, cited by Robinson *et al.*, 1991). The genetic potential of these cows can only be reached if they are fed specialized diets, providing in their specific needs.

Due to increased consumer awareness of health issues, there is also an economical influence. Lately an increase in the protein content of milk could mean a higher income for the producer.

Requirements for rumen degradable protein (**RDP**) and microbial protein synthesis is linked to energy intake, which means that the microbial protein produced might not meet the net tissue protein requirements when the cow is producing high protein products such as milk (Chamberlain & Wilkinson, 2002). According to Ørskov *et al.* (1980) feed intake will increase with milk yield if the energy requirement for milk yield is met. This increased intake will lead to a higher passage rate from the rumen and consequently more dietary protein should be available.

With peak milk production occurring in the first six to nine weeks post-partum, and peak feed intake only between 10 and 13 weeks after the onset of lactation, nutritionists need to focus on providing enough protein and energy in the ration to prevent a deficit (Satter & Roffler, 1974; Hutjens, 1980, cited by Van Dijk *et al.*, 1983). With the onset of lactation, the cow's protein requirement increases above levels that can be met by microbial protein supply. This additional protein requirement increases with an increase in milk yield (Ørskov *et al.* 1980). As the mobilization of body protein is minimal and depletion of mobilizable protein is rapid (Kung & Huber, 1983), inclusion of a digestible source of rumen undegradable protein (**RUP**) in the diet is necessary (Chamberlain & Wilkinson, 2002). Inclusion of a high quality RUP supplement will help the cow towards

maximum expression of her genetically determined production potential (NRC, 2001). Protein quality depends on an estimation of the protein quantity that can be absorbed from the small intestine and furthermore by the potential of this protein to provide essential amino acids (**EAA**) (Cros *et al.*, 1992; Aufrère *et al.*, 2001). Various authors (Kung & Huber, 1983; Faldet & Satter, 1991; Wohlt *et al.*, 1991) have found production to benefit from an increase in RUP.

The ideal ration for a dairy cow should thus contain adequate amounts of RDP to fulfill the nitrogen (**N**) needs of the rumen microbes and to ensure maximal synthesis of microbial CP, as well as adequate amounts of digestible RUP to optimize the absorbed amino acid (**AA**) profile (NRC, 2001). The exact requirement for RUP is unknown as it is supplemental to RDP and microbial protein synthesized in the rumen.

The feeding of oilseeds and oilcake meals to high-producing dairy cows has become common practice. Oilseeds generally have a relatively high fat content and could provide energy in a diet. Both oilseeds and oilcake meals have high protein content, but utilization in ruminant feeds is often limited due to the relatively high rumen degradability thereof. Methods to decrease dietary protein degradability without altering the intestinal digestibility thereof have been the focus of many research trials over the last two decades.

Heat treatment has been used as a method to decrease the soluble N fraction of feedstuffs (Nishimuta *et al.*, 1974; Schingoethe & Ahrar, 1979; Mielke & Schingoethe, 1981; Robinson & Tamminga, 1984, cited by Deacon *et al.* 1988). Satter (1986) suggested that extrusion would generate enough heat to alter protein, but this is in contrast with reports by Deacon *et al.* (1988) who found extrusion not to have a significant influence on the rumen degradability of canola and soybeans. Benchaar *et al.* (1994) found extrusion of protein-rich seeds to decrease ruminal degradation and increase duodenal flow of protein. Stern *et al.* (1985) reported that the total amount of AA flowing to the small intestine was greater in cows fed extruded rather than raw soybeans.

Extrusion is a method of heat processing that is currently used by many feed manufacturers in South Africa and abroad. The aim of the current study was to determine whether extrusion can sufficiently lower the ruminal degradation of protein sources frequently used in South Africa.

The protocol used in the current study is based on a summary of methods proposed by various authors (Cronjé, 1983; Nocek, 1988; AFRC, 1992; Michalet-Doreau & Ould-Bah, 1992; Vanzant *et al.*, 1998).

MATERIALS AND METHODS

Animals and diets

Four non-lactating Holstein cows, previously fitted with rumen cannulae, were used in the trial. The cows were housed in pairs in semi-enclosed stalls on the Welgevallen Experimental Farm of the Stellenbosch University, Western Cape Province, South Africa.

All cows received 3 kg of a commercial semi-complete feed for lactating dairy cows and 1.5 kg lucern hay twice daily at approximately 07h00 and 16h00. The concentrate level in this diet was much higher than that normally fed to dry cows. This was done in an attempt to simulate the rumen environment and passage rate of lactating cows. The chemical composition of these components is presented in Table 1. Cows were adapted to the diet for three weeks before the *in sacco* trial started.

Treatments

The following feedstuffs were evaluated in an *in sacco* degradability trial: lupins (**LUP**), full fat soybeans (**SB**), full fat canola seeds (**FCS**), soybean oilcake meal (**SBM**), canola meal (**CM**) and sunflower oilcake meal (**SFM**). These feedstuffs were chosen based on their availability and use in the Western Cape Province, South Africa. Oilseeds are

good sources of fat and can thus provide energy in the diet. The raw materials used have moderate to high protein values. All of these protein sources were sampled before and after extrusion.

Table 1: Chemical composition of the semi-complete cubes and lucern hay fed to the cows during the trial on an “as is” basis.

Composition	Ingredients	
	Semi-complete cubes ⁵	Lucern hay
Protein	16.0 %	19.4 %
UDP ¹	33.0 %	35.0 %
ME ²	9.65 MJ / kg	8.0 MJ/ kg
Fat	Min. 2.5 %	3.3 %
Fibre	Min. 14 %	-
ADF ³	-	33.0 %
NDF ⁴	-	44.8 %
Starch	18 %	-
Calcium	0.9 %	1.5 %
Total Phosphate	0.55 %	0.22 %
Sodium	0.65 %	0.9 %
Ash	6.6 %	12.2 %
Dry matter	-	89.0 %

¹UDP = rumen undegradable protein, ²ME = metabolizable protein, ³ADF = acid detergent fibre, ⁴NDF = neutral detergent fibre, ⁵Values as provided by the feed company.

The raw materials were extruded individually by a commercial feed mill (Equifeeds, Durbanville) with temperatures reaching a maximum of between 115°C and 120°C. At the time, full fat soybeans were only commercially available in an extruded form. The maximum temperature reached during the extrusion thereof was 135°C.

Samples of all the raw materials were milled through a 2mm screen using a Scientec hammer mill (Scientec, RSA). They were then sieved through a 124µ screen to remove

dust and extremely fine particles. The residue left on the screen was used for chemical analysis and the *in sacco* trial.

For the *in sacco* trial, 8g samples were weighed into each of a series of polyester dacron bags (10 x 20 cm) with pore size 53 μ that were marked for easy identification, dried in a forced draught oven for a minimum of 48h at 55°C and weighed beforehand. The bags were closed and secured with cable ties and placed in tandem into weighted opaque ladies stockings which were tied to the lid of the rumen cannula via a catcher stocking, according to the method described by Cruywagen (2004). This method allows for easy retrieval of bags from the rumen. Bags were removed from the rumen after specified time intervals, being 0h, 2h, 4h, 8h, 16h, 24h and 48h. A 48h incubation series started at 08h00 in the morning. Each cow served as a repetition of the trial. Thus, all 12 samples were evaluated in all 4 cows.

Following extraction from the rumen, bags were washed under running tap water until water squeezed from it was clear and then frozen in airtight bags. Due to too great a number of bags to be incubated all at once, the trials were done in three separate runs, using the same four cows in all three runs. After all the bags have been incubated, washed and frozen, the bags were allowed to thaw overnight and were then washed in a washing machine for 5 cycles of 1 minute each or until the water was clear. Bags were allowed to dry to constant weight in a forced draught oven at 55°C.

Chemical analysis

The cable ties were removed from the dried bags which were then weighed to determine the DM residue. The nitrogen content (%N) of the residue was determined using the Dumas method (AOAC Official Method 968.06; AOAC, 2000) with the aid of a LECO FP-528. The CP content of the dry matter was determined by multiplying the %N with 6.25.

The Weende analysis was performed on all the raw materials, using the AOAC official methods (AOAC, 2000) to determine the composition thereof (Table 2).

Data analysis

Dry matter and CP disappearances were expressed as percentages of incubated samples. An iterative least-square procedure was used to fit the data to the following one-compartment model (Ørskov & McDonald, 1979) to determine DM and CP degradability parameters:

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

where p = degradation at time t

a = rapidly soluble fraction

b = the fraction that will degrade over time

c = the rate of degradation of the b -fraction

Because ruminal retention time affects the extent of degradation, a fractional outflow rate of undegraded protein from the rumen (k_p) was taken into account when the effective percentage degradation (D_{eff}) was calculated as $D_{\text{eff}} = a + bc / (c + k_p)$. Chosen values were $k_p = 0.08$ and $k_p = 0.0625$. In this study, actual disappearance values obtained after 16h incubation were also compared. The latter value was chosen to represent 16 hours of ruminal incubation.

The non-linear parameters a , b and c , as well as the effective degradability values (D_{eff}), were submitted to a oneway ANOVA with the aid of SAS PROG ANOVA (SAS, 2000). Significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

Composition of the raw materials

The chemical composition of the raw materials used in this study is presented in Table 2.

Table 2: Chemical composition of protein sources used in the trial. All values (except DM) expressed on a DM basis.

	LUP	ELUP	SB	ESB	FCS	EFCS	SBM	ESBM	CM	ECM	SFM	ESFM
DM	85.1	88.9	90.8	92.5	93.5	91.8	87.9	94.2	89.8	90.8	88.2	82.0
CP	40.9	42.0	41.7	40.5	22.5	23.6	53.7	54.6	36.9	36.3	40.5	40.9
Fat	3.8	3.3	17.0	17.3	35.7	44.2	3.0	2.1	13.7	13.1	2.3	1.7
Fibre	19.6	17.0	11.3	11.0	28.6	28.3	4.7	4.3	10.7	12.3	16.0	16.3
Ash	4.2	4.3	5.6	5.2	4.3	4.4	8.0	8.2	6.9	6.6	9.4	8.9

LUP = lupins, ELUP = extruded lupins, SB = full fat soybeans, ESB = extruded full fat soybeans, FCS = full fat canola seeds, EFCS = extruded full fat canola seeds, SBM = soybean oilcake meal, ESBM = extruded soybean oilcake meal, CM = canola meal, ECM = extruded canola meal, SFM = sunflower oilcake meal, ESFM = extruded sunflower oilcake meal

As can be seen from Table 2, extrusion appeared to have no effect on the chemical composition of the feedstuffs, except for the DM content. With the exception of FCS and SFM, the extruded feedstuffs appeared to be somewhat dryer than the raw ones. Furthermore, there was a rather large difference in fat content between FCS and extruded FCS. The latter had a much higher fat content. This cannot be readily explained, but it could be an artifact of the process.

In sacco DM disappearance

The *in sacco* DM disappearance parameters are summarized in Table 3. Extrusion significantly lowered the soluble fraction (**a**) of SB, FCS, SBM and CM. The a-fraction of SFM was unaltered and that of extruded LUP was significantly higher than that of raw

LUP. The latter phenomenon was unexpected and difficult to explain. The water soluble treatment was repeated and the effect confirmed. This disagrees with the results of Aufrère *et al.* (2001) who found the a-fraction of extruded LUP to be significantly lower than that of raw LUP.

The potential degradable fraction (**b**) was significantly higher for extruded SBM and SFM and significantly lower for extruded SB when compared to the raw forms of these materials.

Table 3: The effect of extrusion of vegetable protein sources on *in sacco* DM disappearance parameters

Feed-stuff ³	Non-linear parameter ¹											
	a				b				c			
	Treatment ²				Treatment				Treatment			
	Raw	Extr	SE _m	P	Raw	Extr	SE _m	P	Raw	Extr	SE _m	P
LUP	25.1	35.8	2.24	<.0001	77.9	96.6	5.06	0.1641	0.05	0.021	0.01	0.0003
SB	45.7	41.5	0.94	0.0012	85.4	49.3	9.6	0.0094	0.028	0.026	0	0.7328
FCS	43.9	27.4	3.15	<.0001	46.3	27	3.68	0.1503	0.071	0.097	0.01	0.0009
SBM	34.8	27.9	1.33	<.0001	80.9	135.5	14.51	0.0002	0.031	0.012	0	0.0119
CM	52.2	46.8	1.06	<.0001	33.3	52.9	4.79	0.1467	0.044	0.025	0.01	0.0119
SFM	22.8	23.8	0.54	0.3902	66.5	101.7	9.74	0.0112	0.044	0.016	0.01	0.0005

¹a = rapidly soluble fraction (%), b = fraction degradable over time (%), c = rate of degradation of b (% / h),

²Extr = extruded, ³LUP = lupins, SB = full fat soybeans, FCS = full fat canola seeds, SBM = soybean oilcake meal, CM = canola meal, SFM = sunflower oilcake meal.

Looking at all the feedstuffs, extrusion did not appear to have a consistent effect on the b-values, but in most feedstuffs the c-value (rate of degradation) was lowered by extrusion. This agrees with result by Aufrère *et al.* (2001) who found the c-value of extruded LUP to be significantly lower than that of raw LUP, while the b-value of extruded LUP was about twice as high as that of raw LUP. In a study by Chouinard *et*

al. (1997), no significant differences between the a-, b- or c-values for raw and extruded SB were found.

The effective degradability (D_{eff}) was calculated with the rate of passage (k_p) estimated at 8 % per hour which is generally used as an average value for high-producing cows. Extrusion significantly lowered the D_{eff} of all the raw materials, except LUP (Table 4).

Table 4: The effect of extrusion of vegetable protein sources on the effective degradability of dry matter

Feedstuffs ²	$D_{\text{eff}} (k_p = 0.08)^1$			
	Treatment ³		SE_m	P
	Raw	Extr		
LUP	54.9	54.6	0.57	0.8127
SB	65.5	53.2	2.34	< .0001
FCS	65.5	42.1	4.45	< .0001
SBM	56.7	43.3	2.62	< .0001
CM	63.7	58	1.28	< .0001
SFM	45.6	39.5	1.31	< .0001

¹ D_{eff} = effective degradability (%), ²LUP = lupins, SB = full fat soybeans, FCS = full fat canola seeds, SBM = soybean oilcake meal, CM = canola meal, SFM = sunflower oilcake meal, ³Extr = extruded,

***In sacco* CP disappearance**

The *in sacco* CP disappearance parameters are summarized in Table 5. The rapidly soluble fraction of CP was significantly lower for the extruded samples of SB, FCS, SBM and CM, while it was higher for extruded LUP and extruded SFM. As in the case of DM, this phenomenon was unexpected, but confirmed when repeated. Research by Schroeder *et al.* (1996) found the a-fraction of heat-treated SFM to be lower than that of the control.

The b-fraction of CP in all the raw materials (except SBM and SFM) was not significantly affected by extrusion. The b-fractions estimated by the model were extremely high for extruded SBM and extruded SFM. This is probably due to the fact that in both cases, the curve of degradation has not yet reached an asymptote within the 48 hours incubation period. Consequently, the predicted rates of CP degradation for the extruded SBM and SFM were extremely low. Furthermore, only the c-values for extruded LUP and SFM were significantly lower than that of the untreated raw materials. Schroeder *et al.* (1996) found the b-fraction and c-value of heat processed SFM to be lower than that of the control.

Table 5: The effect of extrusion of vegetable protein sources on *in sacco* crude protein disappearance parameters

Feed-stuff ²	Non-linear parameter ¹											
	a				b				c			
	Treatment ³				Treatment				Treatment			
	Raw	Extr	SE _m	P	Raw	Extr	SE _m	P	Raw	Extr	SE _m	P
LUP	59.5	61.5	0.6	0.0371	38.2	40.8	1.37	0.9891	0.164	0.043	0.03	<.0001
SB	57.3	28.4	5.49	<.0001	60.2	54.1	2.74	0.9743	0.033	0.022	0	0.5473
FCS	50.1	38.4	2.21	< 0001	41.7	25.9	3.06	0.933	0.129	0.101	0.01	0.1236
SBM	31.1	16.6	2.82	<.0001	96.8	1380.1	294.39	<.0001	0.025	0.001	0	0.1696
CM	69.9	53.1	3.2	<.0001	23.4	40.1	3.53	0.9292	0.079	0.047	0.01	0.0767
SFM	27.2	32.2	0.98	<.0001	78	1295.1	264.53	<.0001	0.044	0.0008	0.01	0.0184

¹a = rapidly soluble fraction (%), b = fraction degradable over time (%), c = rate of degradation of b (% / h),

²LUP = lupins, SB = full fat soybeans, FCS = full fat canola seeds, SBM = soybean oilcake meal, CM = canola meal, SFM = sunflower oilcake meal, ³Extr = extruded.

The effective degradability of CP was calculated using $k_p = 0.08$ and 0.0625 , representing incubation times of 12.5 and 16 hours, respectively (Table 6). Extrusion significantly lowered the D_{eff} of CP in both cases. Figure 1 shows the D_{eff} calculated with $k_p = 0.08$. The effect of extrusion on effective degradability is clearly visible. The values for LUP and SBM are in agreement with results of Erasmus *et al.* (1988).

Table 6: The effect of extrusion of vegetable protein sources on the effective degradability of crude protein

Feedstuffs ²	$D_{\text{eff}} (k_p = 0.08)^1$				$D_{\text{eff}} (k_p = 0.0625)$			
	Treatment ³				Treatment			
	Raw	Extr	SE _m	P	Raw	Extr	SE _m	P
LUP	84.4	75.3	1.88	< 0.0001	86.4	77.3	1.87	< 0.0001
SB	73.8	39.9	6.42	< 0.0001	76.9	42.7	6.47	< 0.0001
FCS	75.5	52.7	4.35	< 0.0001	77.9	54.2	4.5	< 0.0001
SBM	52.9	30.6	4.3	< 0.0001	57.3	34.5	4.4	< 0.0001
CM	81.4	67.1	2.82	< 0.0001	82.7	70	2.6	< 0.0001
SFM	53.8	45.7	1.73	< 0.0001	58.2	49.4	1.87	< 0.0001

¹ D_{eff} = effective degradability (%), ²LUP = lupins, SB = full fat soybeans, FCS = full fat canola seeds, SBM = soybean oilcake meal, CM = canola meal, SFM = sunflower oilcake meal, ³Extr = extruded

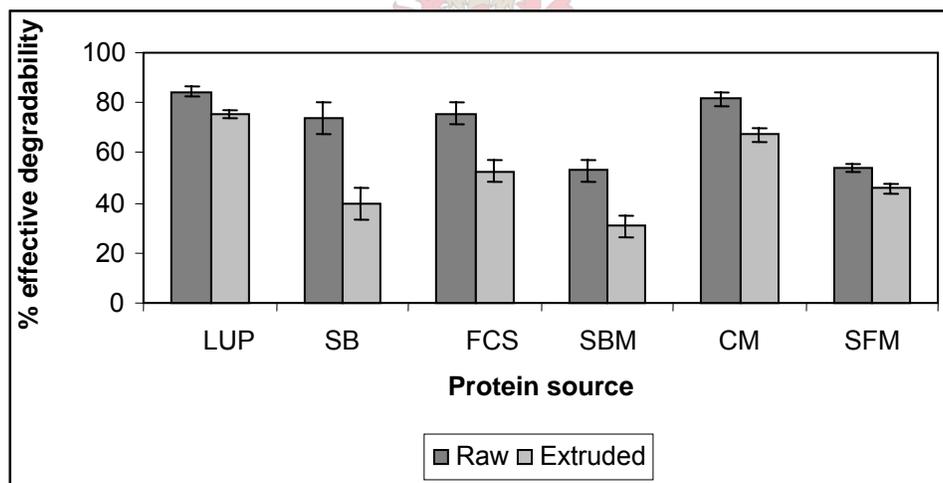


Figure 1: The effect of extrusion of vegetable protein sources on effective degradability at $k_p = 0.08$ (LUP = lupins, SB = full fat soybeans, FCS = full fat canola seeds, SBM = soybean oilcake meal, CM = canola meal, SFM = sunflower oilcake meal).

All the data obtained in this study did not exactly fit the model that was used. In some cases, especially for the extruded feedstuffs, an asymptote has also not been reached after 48h. For this reason, the actual disappearance values obtained after 16 hours,

equivalent to $k_p = 0.0625$, have also been compared (Table 7). The data in Table 7 clearly indicate that CP disappearance was lowered significantly by extrusion.

Table 7: Actual crude protein disappearance values at 16 hours of raw and extruded vegetable protein sources.

Feedstuffs ²	16h response			
	Treatment ¹		SE _m	P
	Raw	Extr		
LUP	91.7	78.5	2.64	< 0.0001
SB	79.6	43.1	7	< 0.0001
FCS	87.1	56.5	5.87	< 0.0001
SBM	63.2	32.9	5.82	< 0.0001
CM	87.2	69.9	3.44	< 0.0001
SFM	64.9	47.8	3.51	< 0.0001

¹Extr = extruded, ²LUP = lupins, SB = full fat soybeans, FCS = full fat canola seeds, SBM = soybean oilcake meal, CM = canola meal, SFM = sunflower oilcake meal

The predicted response curves of CP disappearance in raw and extruded vegetable protein sources are indicated in Figure 2. The effect of extrusion is clearly visible. The graphs of extruded full fat soybeans, soybean meal, sunflower meal and full fat canola seeds almost have a linear appearance. This is probably attributable to the effect of extrusion on, especially, the rate of degradation of these feedstuffs.

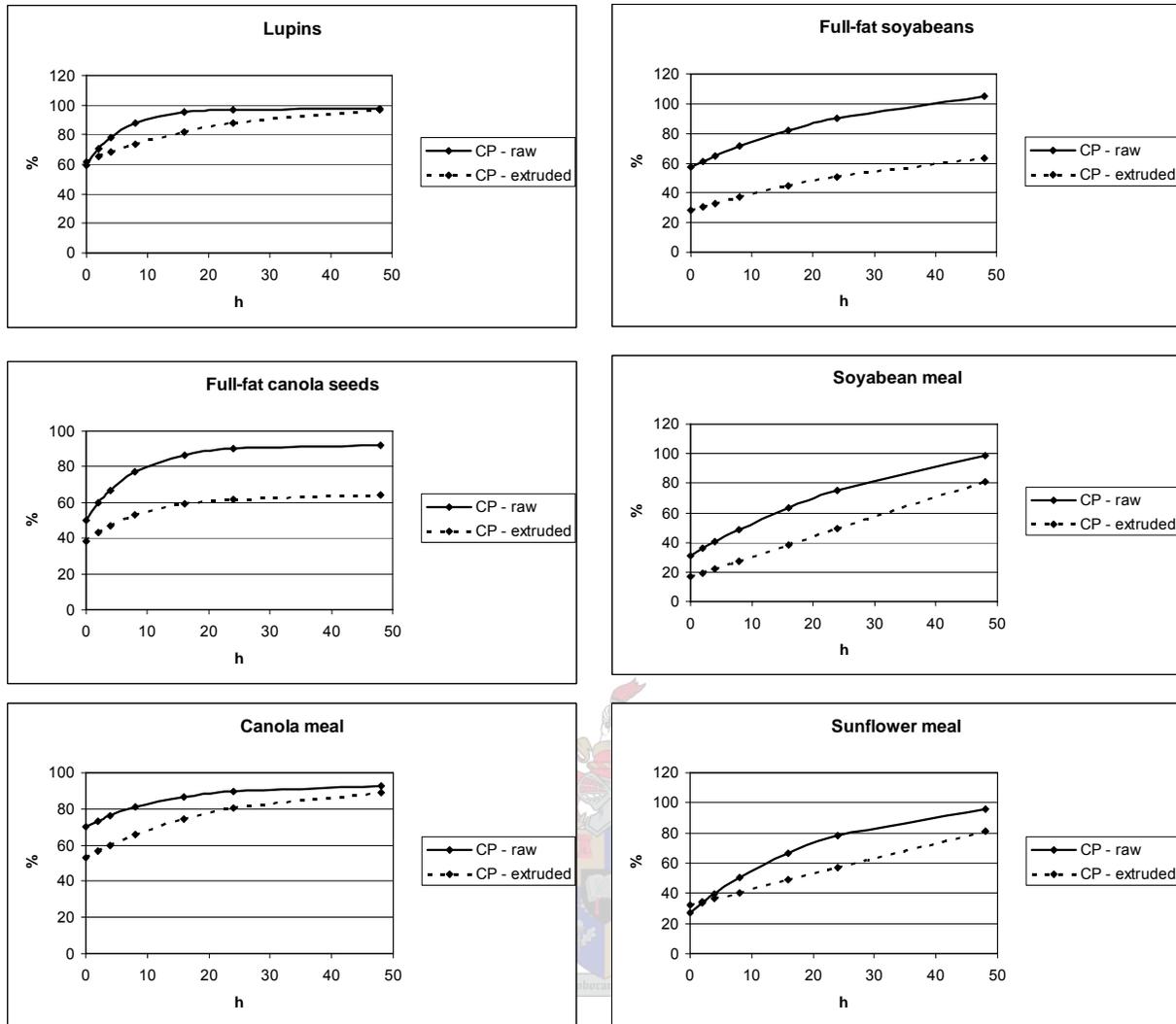


Figure 2: The effect of extrusion on *in sacco* CP disappearance

CONCLUSION

Extrusion appears to be a useful method to decrease rumen degradation of vegetable protein sources, thereby increasing the RUP fraction of these feedstuffs. Furthermore, this means that protein sources of which the use have been limited due to its high RDP content, could be included in diets at higher levels following extrusion. The protein sources mentioned are also good sources of energy and the combination of energy and RUP in the diet of the high-producing dairy cow could only be beneficial.

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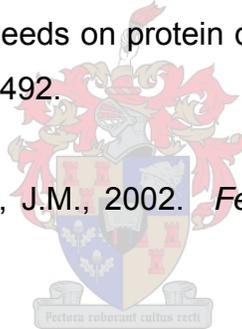
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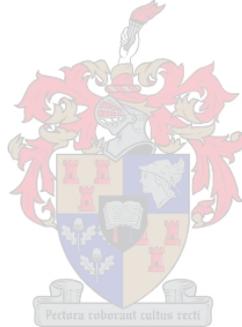
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CHAPTER 3

THE EFFECT OF EXTRUSION ON *IN VITRO* DRY MATTER AND CRUDE PROTEIN DEGRADABILITY OF VARIOUS PROTEIN SOURCES

ABSTRACT

The dry matter (**DM**) and crude protein (**CP**) degradability of six protein sources, raw and extruded, were determined *in vitro*. Feedstuffs used were lupins (**LUP**), full fat soybeans (**SB**), full fat canola seeds (**FCS**), soybean oilcake meal (**SBM**), canola meal (**CM**) and sunflower oilcake meal (**SFM**). These feedstuffs were incubated in an ANKOM Daisy^{II} Incubator and samples were removed after 0, 2, 4, 8, 16, 24 and 48h incubation. Results showed that extrusion decreased the effective degradability (**D_{eff}**) of DM in all the feedstuffs tested (16.8% on average). Crude protein disappearance could only be calculated for LUP, FCS, CM and SFM as there was too little residue left of SB and SBM for nitrogen (**N**)-determination. Extrusion lowered the **D_{eff}** of CP significantly for LUP, FCS, CM and SFM (21.8% on average). In the case of SB and SBM, the actual disappearance values after 8 hours were compared and indicated that extrusion lowered the rate of CP disappearance for these feedstuffs too. The values obtained from this study were compared to the *in sacco* degradability parameters for the same feedstuffs. Based on this comparison, it appears as if the *in vitro* determined values were over-estimations of the *in sacco* determined values. A regression analysis showed a high correlation between the *in vitro* CP disappearance values at 8h incubation and the *in sacco* determined effective degradability values.

Key words: dry matter, crude protein, degradability, *in vitro*, Holstein cows, extrusion.

INTRODUCTION

As peak feed intake lags behind peak milk production in the dairy cow, nutritionists need to focus on providing adequate amounts of protein and energy in the diet to ensure that the modern, high-producing dairy cow achieves her full genetic potential (Satter & Roffler, 1974; Hutjens, 1980, cited by Van Dijk *et al.*, 1983).

The microbial protein supply alone cannot meet the cow's increased protein requirements at the onset of lactation. This problem is aggravated by the fact that mobilization of body protein is minimal and depletion of mobilizable protein is rapid (Kung & Huber, 1983). The inclusion of a digestible source of rumen undegradable protein (**RUP**) in the diet is therefore necessary (Chamberlain & Wilkinson, 2002).

Various methods of decreasing ruminal, but not intestinal, digestibility of dietary protein have been investigated and heat treatment has been found to successfully lower the soluble nitrogen fraction of feedstuffs (Erasmus *et al.*, 1988).

The current study was executed to determine whether an *in vitro* method of estimating protein degradability would be an acceptable laboratory alternative to the traditional *in sacco* method.

In the current study, six protein sources were submitted to extrusion, a method of heat processing that is currently used by many feed manufacturers in South Africa and abroad. The chosen protein sources all have high protein contents and nutritional value, but inclusion thereof is limited by the relatively high degradability of their protein in the rumen.

MATERIALS AND METHODS

The *in vitro* degradability trial ran parallel with an *in sacco* degradability trial (see Chapter 2) in which the same protein sources were evaluated. A composited sample of

rumen liquor from 2 of the cows used in the *in sacco* trial was used for *in vitro* incubation of samples. These non-lactating Holstein cows, previously fitted with rumen cannulae, were housed in pairs in semi-enclosed stalls on the Welgevallen Experimental Farm of the Stellenbosch University, Western Cape Province, South Africa.

The cows received a lactation diet to simulate the rumen environment and passage rate of lactating cows. All cows received 3 kg of a commercial semi-complete feed for lactating dairy cows and 1.5 kg lucern hay (see Chapter 2, Table 1) twice daily at approximately 07h00 and 16h00. Cows were adapted to the diet for three weeks before the trials started.

Treatments

The following feedstuffs were evaluated in an *in vitro* degradability trial: lupins (**LUP**), full fat soybeans (**SB**), full fat canola seeds (**FCS**), soybean oilcake meal (**SBM**), canola meal (**CM**) and sunflower oilcake meal (**SFM**). These feedstuffs were chosen based on their availability and use in the Western Cape Province, South Africa. All of these protein sources were sampled before and after extrusion.



The raw materials were extruded individually by a commercial feed mill (Equifeeds, Durbanville) with temperatures reaching a maximum of between 115°C and 120°C. At the time, full fat soybeans were only commercially available in an extruded form. The maximum temperature reached during the extrusion thereof was 135°C.

Samples of all the raw materials were milled through a 2mm screen using a Scientec hammer mill (Scientec, RSA). They were then sieved through a 124µ screen to remove dust and extremely fine particles. The residue left on the screen was used for chemical analysis and the *in vitro* trial.

For the *in vitro* trial, 2g samples were weighed into each of a series of polyester dacron bags (5 x 10 cm) with pore size 53µ that were marked for easy identification, dried in a

forced draught oven for a minimum of 48h at 55°C and weighed beforehand. The bags were closed with a double heat-seal using an ANKOM Heat Sealer (ANKOM Technologies Corp., Fairport, NY).

Bags were incubated in duplicate in a mixture of rumen liquor and Goering / Van Soest (1970) buffer for *in vitro* dry matter digestibility studies with added cysteine sulfide reducing agent in an ANKOM Daisy^{II} Incubator (ANKOM Technologies Corp., Fairport, NY) at 39°C.

To enable comparison of *in vitro* and *in sacco* results, the two trials were executed at the same time. The rumen liquor used in the *in vitro* trial was extracted from the same cows in which the *in sacco* trials were performed. However, due to the much smaller size of the incubator flasks compared to the rumen, much less bags could be incubated in the flasks despite the fact that smaller bags and smaller sample sizes were used in the *in vitro* trial. For this reason, two flasks represented one cow in the *in vitro* trial. Due to the fact that the Daisy Incubator^{II} (ANKOM Technologies Corp., Fairport, NY) only contains four incubation flasks, only two cows could therefore be simulated in the Incubator. This limited the degrees of freedom in the statistical analysis. Due to too great a number of bags to be incubated all at once, the trial was done in three separate runs.

Bags were removed from the Incubator at specified time intervals, being 0h, 2h, 4h, 8h, 16h, 24h and 48h. Rumen liquor was collected at 08h00 in the morning and a 48h incubation series started as soon as possible after that, but not later than 09h00. Care was taken to keep the rumen liquor at 39°C and anaerobic (by gassing with CO₂).

Following extraction from the Daisy^{II} Incubator, bags were washed under running tap water until water squeezed from it was clear and then frozen in airtight bags. After all the bags have been incubated, washed and frozen, the bags were allowed to thaw overnight and were then hand-washed until the water remained clear. Bags were allowed to dry to constant weight in a forced draught oven at 55°C.

Chemical analysis

The dry bags were weighed to determine the DM residue. The nitrogen content of the residue was determined using the Dumas method (AOAC Official Method 968.06; AOAC, 2000) with the aid of a LECO FP-528. The CP content of the dry matter was calculated as $N \times 6.25$.

The Weende analysis was performed on all the raw materials, using the AOAC official methods (AOAC, 2000) to determine the composition thereof.

Data analysis

Dry matter and CP disappearances were expressed as percentages of incubated samples. An iterative least-square procedure was used to fit the data to the following one-compartment model (Ørskov & McDonald, 1979) to determine DM and CP degradability parameters:

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



where p = degradation at time t

a = rapidly soluble fraction

b = the fraction that will degrade over time

c = the rate of degradation of the b -fraction

Because ruminal retention time affects the extent of degradation, a fractional outflow rate of undegraded protein from the rumen (k_p) was taken into account when the effective percentage degradation (D_{eff}) was calculated as $D_{\text{eff}} = a + bc / (c + k_p)$.

The non-linear parameters a , b and c , as well as the values of D_{eff} were submitted to a oneway ANOVA with the aid of SAS PROG ANOVA (SAS, 2000). Significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

Composition of the diet and raw materials

The chemical composition of the diet of the animals and the raw materials used in the trial was reported previously.

In vitro DM disappearance

The *in vitro* DM disappearance parameters are summarized in Table 1.

Table 1: The effect of extrusion of vegetable protein sources on *in vitro* dry matter disappearance parameters

Feed-stuff ²	Non-linear parameter ¹											
	a				b				c			
	Treatment ³				Treatment				Treatment			
	Raw	Extr	SE _m	P	Raw	Extr	SE _m	P	Raw	Extr	SE _m	P
LUP	23.1	31.1	2.32	<.0001	76.7	65.3	3.29	0.0002	0.15	0.09	0.02	0.0008
SB	50.5	41.8	2.5	<.0001	53.2	62.6	2.81	0.0011	0.08	0.05	0.01	0.0211
FCS	46.5	27.8	5.42	<.0001	36.9	26.9	3.37	0.0007	0.14	0.15	0.01	0.8135
SBM	32.4	27.1	1.55	<.0001	69.7	79	2.83	0.0012	0.09	0.05	0.01	0.0063
CM	53.2	42.4	3.12	<.0001	30.3	43.3	3.74	<.0001	0.14	0.1	0.01	0.185
SFM	24	23.5	0.18	0.3649	56.7	57.7	0.66	0.6583	0.11	0.08	0.01	0.0648

¹a = rapidly soluble fraction (%), b = fraction degradable over time (%), c = rate of degradation of b (% / h),

²LUP = lupins, SB = full fat soybeans, FCS = full fat canola seeds, SBM = soybean oilcake meal, CM = canola meal, SFM = sunflower oilcake meal, ³Extr = extruded.

Extrusion significantly lowered the soluble fraction (a) of SB, FCS, SBM and CM. The a-fraction of SFM was unaltered and that of extruded LUP was significantly higher than that of raw LUP. Although this was unexpected, it confirmed a similar observation

reported in a previous trial (see Chapter 2). In a study reported by Aufrère *et al.* (2001), they found extrusion to lower the a-fraction of lupins.

The potentially degradable fraction (**b**) was significantly higher for extruded SB, SBM and CM. Extrusion did not have a significant effect on the b-fraction of SFM. For FCS and LUP, however, extrusion lowered the b-fraction significantly. These results differ from those of Aufrère *et al.* (2001) who found the b-fraction of extruded LUP to be about twice that of raw lupins.

Extrusion significantly lowered the rate of degradation (**c**) in LUP, SB and SBM. The difference between the c-values of the extruded and raw forms of FCS, CM and SFM was insignificant. This is in agreement with results of Aufrère *et al.* (2001).

The results of this study contradicts those of Chouinard *et al.* (1997) who found no significant difference in the a, b or c values of DM degradability of raw and extruded SB.

The effect of extrusion on *in vitro* effective degradability of DM is summarized in Table 2.

Table 2: The effect of extrusion of vegetable protein sources on *in vitro* effective degradability (D_{eff}) of dry matter

Feedstuffs ²	$D_{\text{eff}} (k_p = 0.08)^1$			
	Treatment ³		SE_m	P
	Raw	Extr		
LUP	73.1	64.8	2.45	0.0002
SB	77.5	64.2	3.87	<0.0001
FCS	70.2	45	7.34	<0.0001
SBM	69.9	55.6	4.2	<0.0001
CM	72.3	66.5	1.78	0.0029
SFM	56.4	51.9	1.32	0.0136

¹ D_{eff} = effective degradability, ²LUP = lupins, SB = full fat soybeans, FCS = full fat canola seeds, SBM = soybean oilcake meal, CM = canola meal, SFM = sunflower oilcake meal, ³Extr = extruded

The effective degradability (D_{eff}) was calculated with the rate of passage (k_p) estimated at 8 % per hour which is generally used as an average value for high-producing cows. Extrusion significantly lowered the D_{eff} of all the raw materials.

The results, with regards to D_{eff} , obtained in the current study is in agreement with studies by Aufrère *et al.* (2001), Chouinard *et al.* (1997) and Cros *et al.* (1992), but disagrees with the results of Deacon *et al.* (1988) who found extrusion to have no effect on the D_{eff} of FCS, CM and SBM.

***In vitro* CP disappearance**

The *in vitro* CP disappearance parameters are summarized in Table 3.

Table 3: The effect of extrusion on *in vitro* crude protein disappearance parameters

Feed-stuff ²	Non-linear parameter ¹											
	a				b				c			
	Treatment ³				Treatment				Treatment			
	Raw	Extr	SE _m	P	Raw	Extr	SE _m	P	Raw	Extr	SE _m	P
LUP	56.7	49.5	2.08	<.0001	38.6	35.2	1.06	0.06	0.71	0.23	0.14	<.0001
SB ⁴	-	-	-	-	-	-	-	-	-	-	-	-
FCS	52.3	33.4	5.44	<.0001	37.4	28.8	2.7	0.0005	0.28	0.13	0.04	0.0036
SBM ⁴	-	-	-	-	-	-	-	-	-	-	-	-
CM	69.9	48.4	6.21	<.0001	22.8	44.4	6.25	<.0001	0.3	0.11	0.06	0.0006
SFM	28.6	29.8	0.51	0.077	64.9	70.6	1.73	0.0058	0.13	0.05	0.03	0.0545

¹a = rapidly soluble fraction (%), b = fraction degradable over time (%), c = rate of degradation of b (% / h),

²LUP = lupins, SB = full fat soybeans, FCS = full fat canola seeds, SBM = soybean oilcake meal, CM = canola meal, SFM = sunflower oilcake meal, ³Extr = extruded, ⁴SB and SBM did not leave enough residue for N-analysis.

From Table 3 it can be seen that extrusion lowered the soluble CP of LUP, FCS and CM. Extrusion did not have an effect on the soluble protein fraction of SFM.

The potentially degradable fraction (**b**) of CP in FCS was significantly decreased by extrusion, while it was increased in the case of CM and SFM.

The rate of degradation of the b-fraction (**c**) was significantly lower for the extruded forms of LUP, FCS and CM. The lack of significance in the case of SFM is probably due to the limited degrees of freedom, as explained earlier.

Due to the small sample sizes used in the *in vitro* trial and the high disappearance rate of SB and SBM, there were not enough residues left for the determination of %N after the 48h incubation period. For these samples, CP disappearance parameters could therefore not be estimated by the model and actual responses after 8h incubation were compared (Table 4). As expected, the CP disappearance at 8h was lower for the extruded samples when compared to the raw ones.

Table 4: Actual crude protein disappearance values at 8 hours for raw and extruded vegetable protein source

Feedstuffs ²	8h response			
	Treatment ¹		SE _m	P
	Raw	Extr		
SB	76.7	46.5	8.82	<0.0001
SBM	67.9	36.4	9.25	<0.0001

¹Extr = extruded, ²SB = full fat soybeans, SBM = soybean oilcake meal

The effective degradability of CP (Table 5) was calculated with an estimated $k_p = 0.08$, representing a retention time of 12.5 hours. As depicted in Figure 1, extrusion significantly lowered the D_{eff} of CP in LUP, FCS, CM and SFM. This is in agreement with the work of Cros *et al.* (1992), Benchaar *et al.* (1994), McKinnon *et al.* (1995), Mustafa *et al.* (2000) and Aufrère *et al.* (2001), but in disagreement with the results of Deacon *et al.* (1988).

Table 5: The effect of extrusion on *in vitro* effective degradability (D_{eff}) of crude protein disappearance parameters

Feedstuffs ²	$D_{\text{eff}} (k_p = 0.08)^1$			
	Treatment ³		SE_m	P
	Raw	Extr		
LUP	91.4	75.6	4.57	0.0001
SB ⁴	-	-	-	-
FCS	81.3	51.5	8.64	<.0001
SBM ⁴	-	-	-	-
CM	88.0	73.7	4.13	<.0001
SFM	68.4	56.8	3.46	<0.0001

¹ D_{eff} = effective degradability (%), ²LUP = lupins, SB = full fat soybeans, FCS = full fat canola seeds, SBM = soybean oilcake meal, CM = canola meal, SFM = sunflower oilcake meal, ³Extr = extruded, ⁴SB and SBM did not leave enough residue for N-analysis.

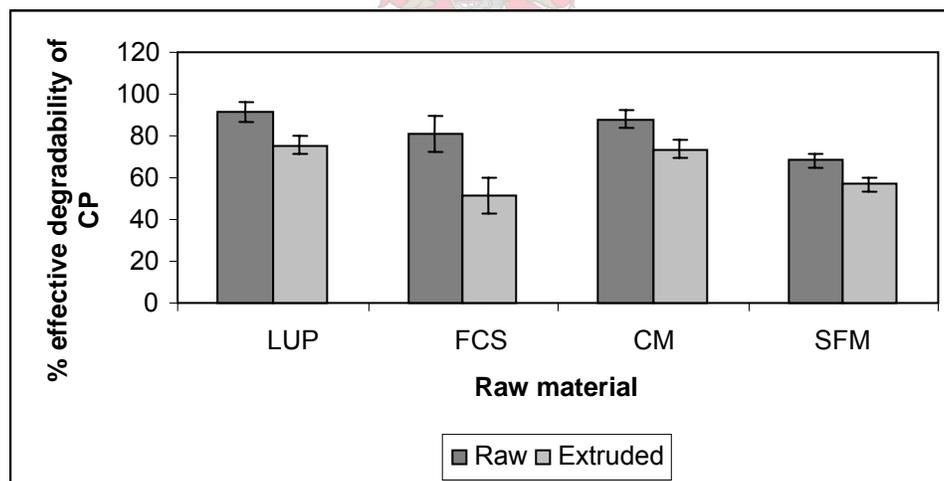


Figure 1: The effect of extrusion of vegetable protein sources on effective degradability at $k_p = 0.08$ (LUP = lupins, FCS = full fat canola seeds, CM = canola meal, SFM = sunflower oilcake meal).

The predicted response curves of CP disappearance in raw and extruded vegetable protein sources are indicated in Figure 2.

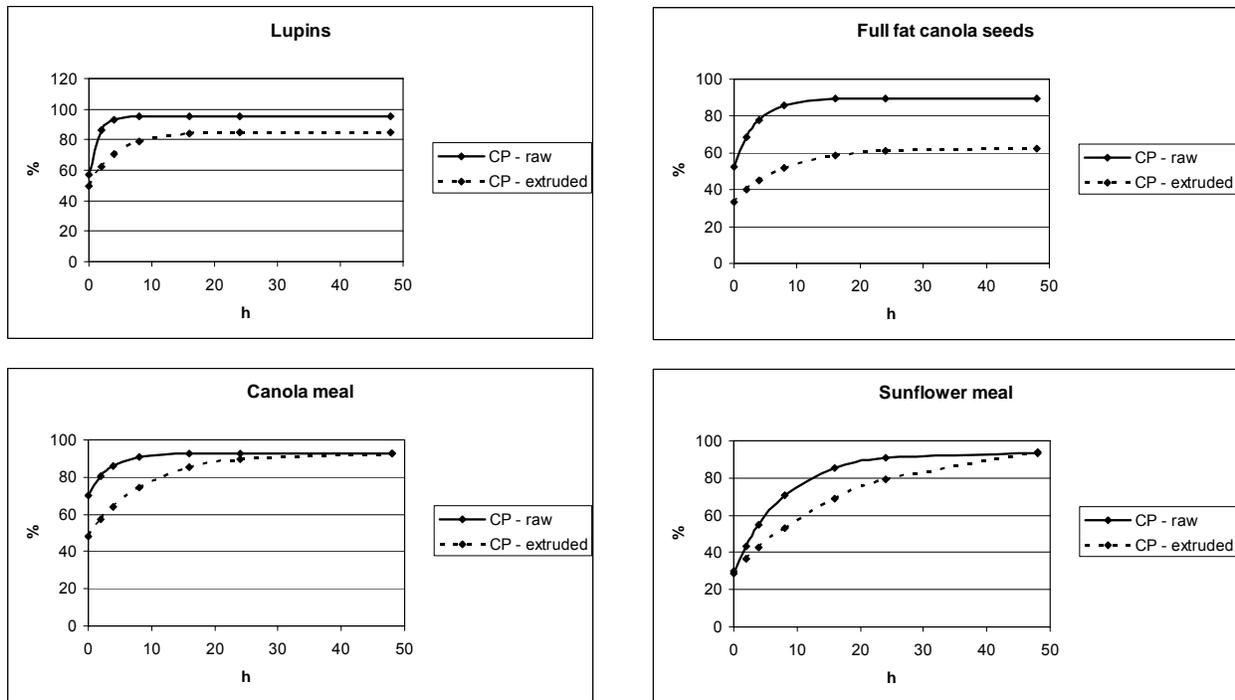


Figure 2: The effect of extrusion on *in sacco* CP disappearance

As a further extension of this trial and due to a lack of comparative data on this behalf, we compared the *in vitro* effective degradability values obtained in this trial with the *in sacco* effective degradability values obtained from the trial discussed in the previous chapter (Table 6).

Actual CP disappearance values at 8h were used to compare the methods in the case of SB and SBM (Table 7).

If it is assumed that the *in sacco* method yields an accurate estimation of protein degradability, then it appears as if the *in vitro* method over-estimates degradation in most cases (Figure 3). Regarding D_{eff} of DM, the values obtained using the *in vitro* method was higher for all the samples. The difference was significant for all protein sources (except FCS). *In vitro* determined values for D_{eff} of CP were significantly higher for raw LUP, FCS, CM and SFM and for extruded SFM. Broderick *et al.* (1988) compared ruminal protein degradation by *in vitro* and *in situ* methods. They also found the rates obtained using the *in situ* method were much slower than that by the inhibitor

in vitro method. Nevertheless, the same ranking of the protein sources were obtained with both methods.

This phenomenon could be due to various possible differences between the 2 methods such as the actual temperature, pH and the composition of the microbial population in the rumen and that inside the vessels in the Daisy^{II} Incubator. Another factor that could have had an effect is that there is constant in and out flow of substrate in the rumen which is not the case in the Daisy^{II} Incubator. These parameters were not investigated, as it was outside the scope of the current study.

Table 6: *In vitro* vs. *in sacco* values for effective degradability ($k_p = 0.08$)

Feed-stuffs ²	Treat-ment ³	D _{eff} of DM ¹				D _{eff} of CP ¹			
		Method ⁴				Method			
		IS	IV	SE _m	P	IS	IV	SE _m	P
LUP	Raw	54.9	73.1	3.87	0.0001	84.4	91.4	1.69	0.0227
	Extr	54.6	64.8	2.25	0.0028	75.3	75.6	0.72	0.8907
SB	Raw	65.5	77.5	2.58	0.0004	-	-	-	-
	Extr	53.2	64.2	2.33	<0.0001	-	-	-	-
FCS	Raw	65.5	70.2	1.27	0.0756	75.5	81.3	1.36	0.0166
	Extr	42.1	45.0	0.73	0.0504	52.7	51.5	0.56	0.3586
SBM	Raw	56.7	69.9	2.87	0.002	-	-	-	-
	Extr	43.3	55.6	2.69	0.0022	-	-	-	-
CM	Raw	63.7	72.3	1.87	0.0015	81.4	88	1.45	0.0022
	Extr	58.0	66.5	1.99	0.0171	67.1	73.7	1.78	0.0684
SFM	Raw	45.6	56.4	2.34	0.0007	53.8	68.4	3.25	0.0043
	Extr	39.5	51.9	2.71	0.0019	45.7	56.8	2.43	0.0022

¹D_{eff}= effective degradability (%), DM = dry matter, CP = crude protein, ²LUP = lupins, SB = full fat soybeans, FCS = full fat canola seeds, SBM = soybean oilcake meal, CM = canola meal, SFM = sunflower oilcake meal, ³Extr = extruded, ⁴IS = *in sacco*, IV = *in vitro*.

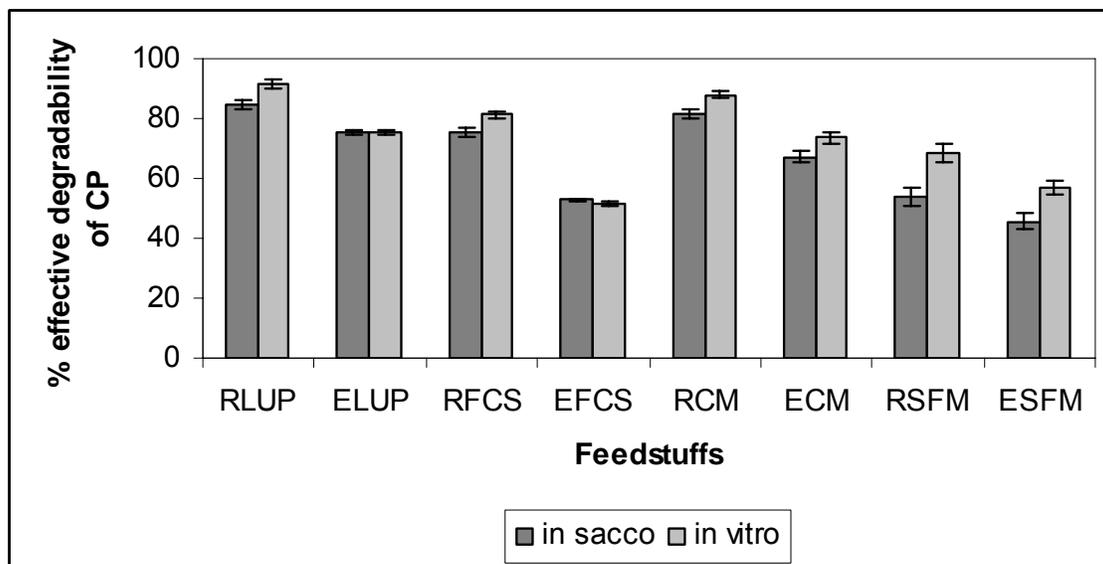


Figure 3: In vitro vs. in sacco methods for determination of effective degradability of protein (RLUP = raw lupins, ELUP = extruded lupins, RFCS = raw full fat canola seeds, EFCS = extruded full fat canola seeds, RCM = raw canola meal, ECM = extruded canola meal, RSFM = raw sunflower meal, ESFM = extruded sunflower meal).

Table 7: Actual crude protein disappearance values at 8 hours for raw and extruded vegetable protein sources

Feedstuffs ²	Treatment ³	D _{eff} of CP ¹			
		Method ⁴		SE _m	P
		IS	IV		
SB	Raw	63.6	76.7	2.94	0.005
	Extr	34.1	46.5	2.68	0.001
SBM	Raw	44.2	67.9	5.21	0.0026
	Extr	23.9	36.4	2.85	0.0082

¹D_{eff} = effective degradability, ⁴IS = *in sacco*, IV = *in vitro*, ³Extr = extruded, ²SB = full fat soybeans, SBM = soybean oilcake meal.

Actual disappearance values after 8h *in vitro* incubation were used to estimate effective degradability values (for $k_p = 0.08$) as determined *in sacco*. Regression analysis yielded the following equations:

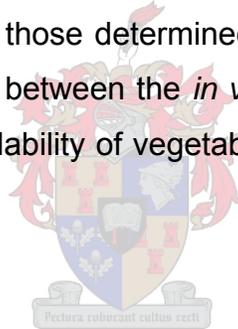
Raw vegetable protein sources: $y = 1.153x - 22.11$ ($R^2 = 0.89$; $P = 0.005$)

Extruded vegetable protein sources: $y = 1.131x - 11.25$ ($R^2 = 0.96$; $P = 0.005$)

It therefore appears as if *in vitro* CP disappearance values at 8h incubation can be used successfully to estimate the effective degradability of vegetable protein sources. This could offer a valuable and cost-effective alternative to *in sacco* trials.

CONCLUSION

Extrusion significantly lowered the effective degradability of DM and CP in vegetable protein sources, as determined *in vitro*. This in agreement with the results of an *in sacco* study (see chapter 2). Upon comparison, it appeared as if *in vitro* determined values consistently over-estimated those determined with the *in sacco* procedure. The results indicated a high correlation between the *in vitro* CP disappearance values at 8h incubation and the effective degradability of vegetable protein sources as determined *in sacco*.



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CHAPTER 4

THE EFFECT OF EXTRUSION ON NITROGEN FRACTIONS OF VEGETABLE PROTEIN SOURCES

ABSTRACT

Six vegetable protein sources were extruded to determine the effect on certain nitrogen fractions. Feedstuffs used were lupins (LUP), full fat soybeans (SB), full fat canola seeds (FCS), soybean oilcake meal (SBM), canola meal (CM) and sunflower oilcake meal (SFM). Buffer insoluble nitrogen (BIN), acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN) fractions were determined to serve as indicators of heat damage to the protein sources. The ADIN-fraction was significantly lower for all extruded feedstuffs, except FCS. Extrusion significantly increased the NDIN content of SB and SBM, CM and SFM, and left that of LUP and FCS unaltered. The BIN values and NDIN : ADIN ratios of the various treatments of the feedstuffs suggest that extrusion would be expected to reduce ruminal protein degradability of most of these feedstuffs without causing extensive heat damage. Feedstuffs that were most favourably affected appeared to be LUP, SB, SBM and SFM.

Key words: extrusion, heat damage, nitrogen solubility, acid detergent insoluble nitrogen, neutral detergent insoluble nitrogen.

INTRODUCTION

Heat damage is detrimental to protein quality and it is therefore not only a significant concern to ruminant nutritionists, but also to the processing industries who aim to increase the rumen undegradable protein (**RUP**) content of commercial protein supplements by heat treatment.

According to the National Research Council (NRC) system, protein is divided into two fractions, being degraded and undegraded intake protein. The Cornell Net Carbohydrate and Protein System (CNCPS), on the other hand, divide protein into five fractions. Fraction A is non-protein nitrogen (**NPN**) and is soluble in trichloroacetic (TCA) acid. The unavailable protein, being the protein bound to the cell wall, is fraction C, and is derived from the acid detergent insoluble nitrogen (**ADIN**) (Sniffen *et al.*, 1992). This fraction contains protein associated with lignin, tannin-protein complexes, and Maillard products that are highly resistant to microbial and mammalian enzymes. It cannot be degraded by ruminal bacteria and does not provide amino acids postruminally (Krishnamoorthy *et al.*, 1982). Fraction B3 is the slowly degraded true protein and is the neutral detergent insoluble nitrogen (**NDIN**) minus fraction C. Fraction B1 represents the rapidly degraded true protein, whilst fraction B2 is the true protein with an intermediate degradation rate. Some of the fraction B2 protein is degraded in the rumen and some of it escapes to the lower gut. This depends on the relative rates of digestion and passage. Fraction B1 is calculated as the TCA-precipitable protein from the buffer-soluble protein minus NPN and fraction B2 is the remaining nitrogen (Sniffen *et al.*, 1992). The article by Licitra *et al.* (1996) provides a good outline of recommended procedures for determination of the various fractions mentioned here.

For a long time already, various authors (Sherrod & Tillman, 1962; Peter *et al.*, 1971; Wohlt *et al.*, 1973; Aitchison *et al.*, 1976) have considered the solubility of dietary nitrogen (**N**) to be a factor that affects protein degradation in the rumen. It does not necessarily relate to susceptibility to enzymatic degradation (Mahadevan *et al.*, 1980). Even though the correlation between protein solubility in mineral buffer and extent of

digestion in the rumen has been high (Hendrickx & Martin, 1963, cited by Krishnamoorthy *et al.*, 1982), there are certain shortcomings to the use of N solubility as an estimate of rumen N degradability. The need for a simple laboratory system to determine the extent of rapidly degradable protein in the rumen makes the use of N solubility as indirect indicator useful.

Another possibility is the detergent system (Goering & Van Soest, 1970) for fibre analysis. Insoluble protein fractions most likely include protein bound to fibre (Nikokyris & Kandyliis, 1997). According to the detergent system, the N insoluble in acid detergent solution is considered as an estimate of indigestible N (Goering *et al.*, 1972). They found the NDIN, which is the N associated with the neutral detergent fibre (**NDF**), to have a positive correlation with the slowly degradable pool of N in the feed. The NDIN includes N associated with the cell wall.

The objective of heat treatment of protein sources is to alter the protein structure chemically, thereby decreasing the rumen degradability. Studies with various feeds showed dietary protein to be degraded at different rates according to their structure and location in plant tissues (Aufrère *et al.*, 2001). The ideal is to decrease the soluble N fraction, increase the NDIN, whilst minimizing the increase in the amount of ADIN, associated with the acid detergent fibre (**ADF**). The increase in the NDIN fraction indicates an increase in the protein fraction that is slowly degraded in the rumen (Van Soest, 1989), while an increase in ADIN would indicate a rise in heat damaged indigestible protein (Goering *et al.*, 1972; Van Soest & Mason, 1991) as the ADIN includes lignified nitrogen and Maillard products and is largely unavailable to the animal.

Whilst moderate heating can increase the RUP fraction with minimal decreasing of protein quality (McKinnon *et al.*, 1991), excessive heating can reduce the intestinal availability of dietary amino acids (**AA**) through the formation of Maillard products (Van Soest 1982, cited by McKinnon *et al.*, 1991). Protein quality is consequently lowered and ultimately this will have a negative effect on animal performance. Van Soest (1989)

suggested that whilst ADIN is a valuable indicator of heat damage, it might not quantitatively represent the Maillard products in a sample.

McKinnon *et al.* (1995) found a negative relationship between crude protein (**CP**) disappearance and treatment of canola meal (**CM**) with heat, and also the duration of heat treatment. Though dry heat treatment was found to be an effective method of decreasing dry matter (**DM**) and CP disappearance of CM from the rumen, heating to 145°C increased the amount of ADIN to nearly 8 times that of the ADIN value after heating to 125°C (McKinnon *et al.*, 1991). Various authors (Goering *et al.*, 1972; Goering & Van Soest, 1970) have used ADIN as measure of heat damage and unavailable protein. Yu & Thomas (1976) found ADIN to be a reliable ($r^2 = 0.86$) predictor of N digestibility.

As research progressed, various authors (Robertson & Van Soest, 1975; Rogers *et al.*, 1986; Van Soest, 1989; Weiss *et al.*, 1989; Britton *et al.*, 1986, cited by Nakamura *et al.* 1994) have started to question the predictive value of ADIN and provided evidence that ADIN is partially digestible. If this should be the case, then using ADIN as indicator would result in over-estimation of heat damage (Britton *et al.*, 1987; Cleale *et al.*, 1987; Weiss *et al.*, 1989).

Nakamura *et al.* (1994) conducted a study to evaluate the digestibility and growth efficiency caused by feeding heat-damaged protein, using dried distillers grains and corn gluten meal. They found a decrease in the N digestibility of heated corn gluten meal. They also found evidence that though some of the protein could possibly be absorbed from the digestive tract, it was not utilized for growth by the ruminants, resulting in a lower daily gain. Mauron (1981, cited by Nakamura *et al.*, 1994) speculated that some of the AA in the escape protein of these damaged protein sources were reduced in availability by the formation of cross-links between peptide chains, thus making the AA absorbable but not metabolizable.

The aim of the experiments reported in this article was to determine the effect of heat treatment on the buffer insoluble N (**BIN**), ADIN and NDIN fractions, as indicators of the protein degradation characteristics of the feedstuffs used in the trials previously reported.

MATERIALS AND METHODS

The following six vegetable protein sources were evaluated: lupins (**LUP**), full fat soybeans (**SB**), full fat canola seeds (**FCS**), soybean oilcake meal (**SBM**), canola meal (**CM**) and sunflower oilcake meal (**SFM**).

Nitrogen solubility in a mineral buffer

The buffer used in this trial is the preferred one by Krishnamoorthy *et al.* (1982), who evaluated six solvents and chose borate-phosphate buffer due to its ability to maintain a stable pH over prolonged storage periods. This buffer consists of 12.2g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and 9.91g $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ per liter of distilled water. The pH of the buffer should be adjusted to 6.8 by bubbling with CO_2 or by adding sodium bicarbonate.

Buffer insoluble nitrogen was determined according to the procedure of Pichard (1977, cited by Krishnamoorthy *et al.* 1982) as modified by Krishnamoorthy *et al.* (1982). The latter evaluated two nitrogen extraction procedures and found the results to be statistically indifferent. The method of Pichard (1977, cited by Krishnamoorthy *et al.* 1982) was consequently chosen due to the simplicity thereof.

According to this method, amounts of 0.5g of the ground samples were weighed into 125ml Erlenmeyer flasks and 5ml of t-butyl alcohol (10%) was added to each flask and swirled lightly. A volume of 25ml of buffer was added to each flask and swirled thoroughly, whereafter another 25 ml of the buffer was added. Samples were left for 1 hour after which the contents of the beakers were filtered through previously weighed sheets of Whatman#54 filter paper, using a mild vacuum suction. The residues were

washed with distilled water and the filter papers and residues dried at 55°C before weighed. The BIN was estimated using a LECO FP-528.

The procedure was repeated four times for every feedstuff.

Nitrogen solubility in detergent solutions

The ADIN and NDIN fractions were determined from the %N in the ADF and NDF residues, respectively. The ADF and NDF determinations were done with the use of an ANKOM Fiber Analyzer (ANKOM Technologies Corp., Fairport, NY) according to the manufacturers specifications (ANKOM Technologies Corp., Fairport, NY). Sodium sulfide was excluded from the neutral detergent solution to prevent nitrogen associated with the NDF from being removed from the residue.

Acid detergent fibre and NDF determinations were done in four-fold for each feedstuff. Due to the small quantity of residue remaining after the detergent treatments, residues were pooled per feedstuff and treatment. Pooled residues were analysed for N in duplicate. The mean N-values were then applied to the respective individual acid detergent and neutral detergent residue values.

Data analysis

The data obtained from the above experiments were submitted to a one-way ANOVA with the aid of SAS PROG ANOVA (SAS, 2000). Significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

Nitrogen solubility in a mineral buffer

The BIN-fractions of the evaluated feedstuffs is summarized in Table 1.

Table 1: The effect of extrusion on nitrogen solubility of various vegetable protein sources

Feedstuffs ²	Buffer insoluble nitrogen (% of total N)			
	Treatment ¹		SE _m	P
	Raw	Extr		
LUP	33.0	58.2	5.73	0.0005
SB	46.3	103.6	12.84	< 0.0001
FCS	66.2	68.9	1.11	0.269
SBM	83.6	91.7	1.98	0.0111
CM	38.4	67.9	6.61	< 0.0001
SFM	73.2	89.6	3.69	< 0.0001

¹Extr = extruded, ²LUP = lupins, SB = full fat soybeans, FCS = full fat canola seeds, SBM = soybean meal, CM = canola meal, SFM = sunflower meal

Extrusion caused a significant increase in the BIN-fraction of all feedstuffs, except FCS. This increase was expected, since there is a high correlation between protein solubility in mineral buffer and extent of digestion in the rumen (Hendrickx & Martin, 1963, cited by Krishnamoorthy *et al.*, 1982). In a previous study (see Chapter 2), we found extrusion to decrease the effective degradability of CP.

The results for lupins are in agreement with those of Murphy & McNiven (1994) who found heat-treatment, by means of roasting at 105°C, to decrease the solubility of N in buffer from 69.8% to 35.8% of the total N in the feedstuff. Extrusion more than doubled the BIN value of SB. This is interesting, because according to the *in vitro* results, SB disappeared from the incubation bags at such a rate that, after 8 h of incubation, there was not enough sample left for a N-analysis. It should be added that the sample size was only 2 g. It is hypothesized that, although extrusion appeared to decrease the soluble protein fraction and therefore also the effective degradability, the degradation rate of the b-fraction was not significantly affected. The BIN value for CM, as determined in this study (38.4%), is much lower than the 74.97% determined by McKinnon *et al.* (1991). Heat treatment of CM to 125°C and 145°C, respectively,

significantly increased the insoluble protein in the feedstuff. McKinnon *et al.* (1995) also found the same type of heat treatment to decrease the proportion of CP soluble in borate-phosphate buffer solution. The increase was much more significant for 145°C than 125°C (618% vs. 35%, respectively).

According to Sniffen *et al.* (1980, cited by Nikokyris & Kandylis, 1997), the soluble protein in SBM is mostly true protein which, furthermore, is very degradable in the rumen.

Zheng *et al.* (1998) found that increasing the temperature of micronization from 115°C to 140° progressively reduced N solubility in 6 legumes tested. They found that micronized samples showed lower N solubilities in water at pH 6.0, 0.5 M NaCl, and 70% ethanol, indicating denaturation of albumins and globulins. Results of extracting insoluble N with 0.5% sodium dodecyl sulfate and 0.6% 2-mercaptoethanol in borate buffer (pH 10) suggested that micronization induced hydrophobic aggregation in legume proteins.

Nitrogen solubility in detergent solutions

Table 2 summarizes the effect of extrusion on nitrogen solubility in detergent solutions.

Crude protein, which is insoluble in acid detergent, is a measure of heat damage that renders carbohydrates and proteins indigestible (Van Soest, 1965, cited by Kohn & Allen, 1992).

Extrusion significantly lowered the ADIN-fraction of all feedstuffs, except FCS. This phenomenon was unexpected as various authors (McKinnon *et al.*, 1991; Sniffen *et al.*, 1992; Aufrère *et al.*, 2001) have found heat treatment to increase ADIN. It therefore appears as if the temperatures reached during extrusion (115°C - 120°C) of the feedstuffs in the current study were not high enough to damage the protein and decrease the quality thereof.

Table 2: The effect of extrusion on the ADIN and NDIN fractions of various vegetable protein sources

Feedstuffs ⁴	ADIN ¹				NDIN ²			
	Treatment ³				Treatment			
	Raw	Extr	SE _m	P	Raw	Extr	SE _m	P
LUP	2.7	0.7	0.38	<0.0001	3.9	3.4	0.14	0.1326
SB	5.6	1.8	0.75	0.0001	15.6	31.3	3.35	0.0033
FCS	7.7	7.1	0.27	0.242	17.1	19.5	0.77	0.1358
SBM	2.5	1.8	0.15	0.013	9.2	21.5	2.34	<0.0001
CM	7.5	5.3	0.45	0.0013	14.3	20.0	1.09	<0.0001
SFM	4.6	3.8	0.17	0.0013	17.7	21.4	0.80	0.0048

¹ADIN = acid detergent insoluble nitrogen as percentage of total nitrogen in the feedstuff, ²NDIN = neutral detergent insoluble nitrogen as percentage of total nitrogen in the feedstuff, ³Extr = extruded, ⁴LUP = lupins, SB = full fat soybeans, FCS = full fat canola seeds, SBM = soybean meal, CM = canola meal, SFM = sunflower meal

McKinnon *et al.* (1991) evaluated heat treatment of CM at 125°C and at 145°C. Heat-treatment was accomplished by treatment in a vacuum tumble dryer. The ADIN content of the control sample was 5.32% of the total N and increased with heat treatment, being approximately 9% at 125°C and 37% at 145°C. McKinnon *et al.* (1995) also found heat treatment of CM at 125°C not to have a detrimental effect on protein quality, but heat treatment at 145°C to cause an unacceptable increase in heat damaged protein.

Murphy & McNiven (1994) reported similar results in their study of the effect of roasting at 105°C on lupins. They found no significant difference between the ADIN content of the raw and roasted lupins (3.31% vs. 3.46%). This could suggest that processing at higher temperatures could be even more beneficial in further reducing N solubility and degradability since practically no heat damage was detected at this temperature.

Sniffen *et al.* (1992) published the values for certain protein fractions of numerous feedstuffs. They found the ADIN-fraction of raw soybeans to be 2.9% of total %N, which is lower than the value (5.6%) obtained in the current study, while that of heated

soybeans was 7.3%, which is much higher than the 1.8% determined in this study. Unlike the results of the current study, Sniffen *et al.* (1992) found an increase in the percentage ADIN with heat treatment. The method of heat-treatment and temperatures reached during this treatment is unknown. It is possible that the heat-treatment of the soybeans used for the study of Sniffen *et al.* (1992) was more severe than the extrusion used in this study. The ADIN-fraction of SFM was 4.6%, which is in agreement with the 4.8% of the total N as determined in the current study.

The NDIN-fraction of SB, SBM, CM and SFM was significantly increased by extrusion. Extrusion left the NDIN-fraction of LUP and FCS unaltered. These results are in agreement with those of McKinnon *et al.* (1995) who found heating to increase the NDIN content of CM relative to that of unheated CM. It is also in agreement with the results of Mustafa *et al.* (2000) who found heat treatment of CM to increase the NDIN content whilst leaving the ADIN content unaltered. In these documented studies, heat treatment was not in the form of extrusion.

Crude protein soluble in neutral buffers or neutral detergents is generally more rapidly converted to ammonia by ruminal microbes than insoluble protein (Nocek *et al.*, 1983; Van Soest & Sniffen, 1984, cited by Kohn & Allen, 1992; Lindberg, 1988, cited by Kohn & Allen, 1992). The increase in NDIN is favourable since it could imply an increase in the protein fraction that would be slowly degraded in the rumen (Van Soest, 1989).

The ratio of NDIN : ADIN was used as an indication of the efficiency of the increase in slowly degradable protein (Table 3) as proposed by McKinnon *et al.* (1995). According to the results, extrusion increased the ratio of NDIN : ADIN for all feedstuffs, except FCS.

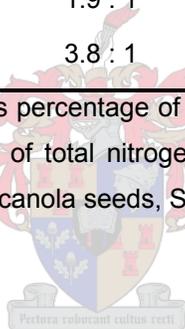
Wider ratios, such as 6 : 1 for CM heated to 125°C (McKinnon *et al.*, 1995) indicate more effective increase in the slowly degradable proteins. The narrower ratios eg. 2 : 1 for CM heated to 145°C (McKinnon *et al.*, 1995) and such as the ratios calculated for

FCS and CM in this study, are presumably indicative of increased amounts of heat damaged protein.

Table 3: The effect of extrusion on the ratio of NDIN : ADIN for various vegetable protein sources

Feedstuffs ³	NDIN : ADIN ¹	
	Treatment ²	
	Raw	Extr
LUP	1.4 : 1	4.9 : 1
SB	2.8 : 1	17.4 : 1
FCS	2.2 : 1	2.7 : 1
SBM	3.7 : 1	11.9 : 1
CM	1.9 : 1	3.8 : 1
SFM	3.8 : 1	5.6 : 1

¹ADIN = acid detergent insoluble nitrogen as percentage of total nitrogen in the feedstuff, NDIN = neutral detergent insoluble nitrogen as percentage of total nitrogen in the feedstuff, ²Extr = extruded, ³LUP = lupins, SB = full fat soybeans, FCS = full fat canola seeds, SBM = soybean meal, CM = canola meal, SFM = sunflower meal



CONCLUSION

Results from the current study suggest that extrusion appears to be an effective way to reduce the rumen degradability of most vegetable protein sources without causing heat damage. Feedstuffs that were most favourably affected appeared to be LUP, SB, SBM and SFM.

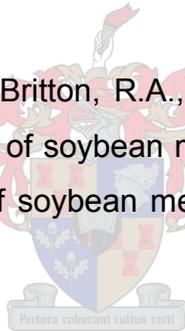
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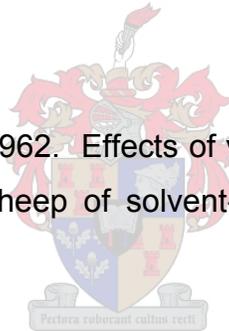
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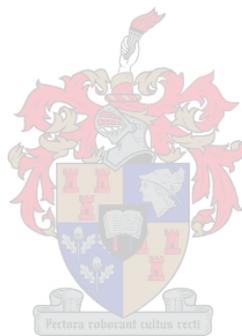
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CHAPTER 5

GENERAL CONCLUSION

The aim of this study was to provide the industry with information on the effect of extrusion on the degradability parameters of vegetable protein sources. The protein sources evaluated, being lupins (**LUP**), full fat soybeans (**SB**), full fat canola seeds (**FCS**), soybean oilcake meal (**SBM**), canola oilcake meal (**CM**) and sunflower oilcake meal (**SFM**), are all commonly used in South African dairy cow diets, but the feed dictionaries of popular dynamic models and programs generally lack data on the effect of heat treatment, especially extrusion, on these vegetable protein sources, except SB and SBM.

Both an *in sacco* and an *in vitro* degradability trial indicated that extrusion lowered the effective degradability of dry matter (**DM**) and crude protein (**CP**) in FCS, CM and SFM. In the case of LUP, extrusion decreased the effective degradability of CP according to the results of both trials. The *in sacco* method indicated no effect on the effective degradability of DM, although the *in vitro* procedure, on the other hand, indicated that extrusion did lower the effective degradability of DM. The *in sacco* and *in vitro* trials indicated that extrusion lowered the effective degradability of the DM of SB and SBM. The effective degradability of CP, as determined by the *in sacco* procedure for these feedstuffs, was lower for the extruded samples. The *in vitro* procedure left too little residue for the N-determination, in which case the actual disappearance after 8 hours incubation was compared. The values for the extruded samples were lower than those of the raw samples, which suggest that effective degradability of CP of SB and SBM would also be lowered by extrusion.

A comparison of the two sets of data obtained from the respective trials, were compared. The *in vitro* determined values appear to be an over-estimation of the *in sacco* results. Further research as to the reason for this observation is validated. This could be especially valuable since it was observed that there is a high correlation

between the 8 hours *in vitro* incubation values and the effective degradability as determined by the *in sacco* procedure. Confirmation of this phenomenon could offer a valuable and cost-effective alternative to *in sacco* trials.

Certain nitrogen fractions were determined to evaluate the extent of heat damage to the proteins and the formation of Maillard products. In contrast to expectation, the acid detergent insoluble nitrogen (**ADIN**) of all feedstuffs, except FCS, were lowered by extrusion. The NDIN fraction of LUP and FCS was lowered, that of SB and SBM was increased, and that of CM and SFM was unaltered by extrusion. Extrusion appears to have the most significant effect on LUP, SB, SBM and SFM.

These results could indicate that the temperature reached during extrusion is not high enough to cause any serious damage to these protein sources. Similar evaluations of these feedstuffs extruded at higher temperatures would be of interest.

To conclude, it seems as if extrusion can be useful as a method of heat treatment, which could lower the rumen degradability of vegetable protein sources, thereby increasing the rumen undegradable protein (**RUP**) content of these feedstuffs. This would mean that these feedstuffs could be included in dairy cow diets at higher rates than normally used.