Effect of Processing Method with Particular Reference to Solvent Extraction and Pelleting on in Vitro Protein Degradation and Pepsin Digestion in Some Pepsin Digestion in Some



THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

(BIHAR)

(FACULTY OF VETERINARY SCIENCE)

PUSA, (SAMASTIPUR)

In partial fulfilment of the requirements
FOR THE DEGREE OF

Master of Veterinary Science

IN

(ANIMAL NUTRITION)

Bharti Singh

Department of Animal Nutrition
BIHAR VETERINARY COLLEGE
PATNA
2000

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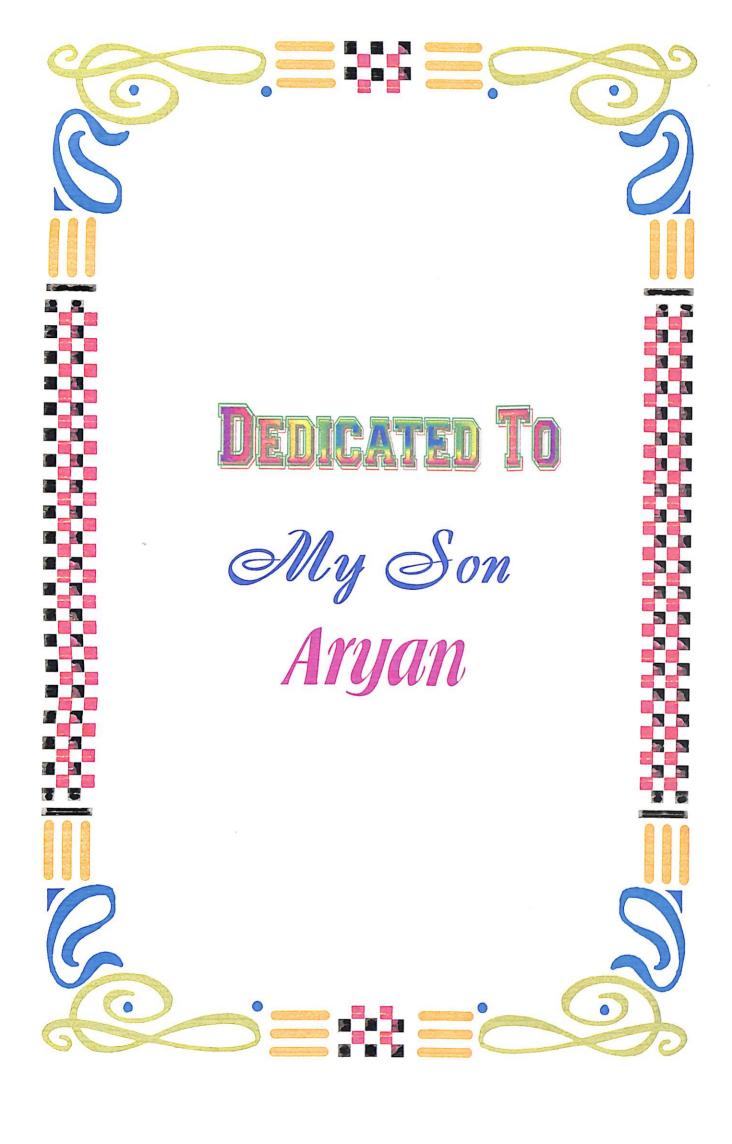
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DEPARTMENT OF ANIMAL NUTRITION

Bihar Veterinary College, Patna – 800014. (Rajendra Agricultural University, Pusa, Samastipur, Bihar)

CERTIFICATE - I

This is to certify that the thesis entitled "EFFECT OF PARTICULAR REFERENCE METHODS WITH PROCESSING SOLVENT EXTRACTION AND PELLETING ON IN-VITRO PROTEIN DEGRADATION AND PEPSIN DIGESTION IN SOME VEGETABLE PROTEINS" submitted in partial fulfillment of the requirements for Master of Veterinary Science (Animal of the Degree Nutrition) of the Faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar is the record of bonafide research carried out by Dr. Bharti Singh under my supervision and guidance. No part of the thesis has been submitted for any other Degree or Diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been duly acknowledged.

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CERTIFICATE - II

We, the undersigned, members of Advisory Committee of Dr. Bharati Singh, a candidate for the Degree of Master of Veterinary Science with Major in Animal Nutrition has gone through the manuscript of the thesis and agree that the thesis "EFFECT OF PROCESSING METHODS WITH PARTICULAR REFERENCE TO SOLVENT EXTRACTION AND PELLETING ON IN-VITRO PROTEIN DEGRADATION AND PEPSIN DIGESTION IN SOME VEGETABLE PROTEINS" may be submitted by Dr. Bharti Singh in partial fulfilment of the requirement for the Degree.

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Bharte Singh

(Bharti Singh)

Contents

Chapter No.	Description	Page No.
1.	Introduction	1-7
2.	Review of Literature	8-58
3.	Material & Method	59-69
4.	Result & Discussion	70-98
5.	Summary & Conclusion	99-108
	Bibliography	i-xxxvi

List of Tables

able No.	`Description	Page No.
1.	Proximate composition of unprocessed and processed vegetable protein sources used in the experiment (on DM basis)	71
2.	Mean value showing the effect of different processing methods of vegetable protein supplement on crude protein content along with their S.E. and CV%.	73
3.	Analysis of variance showing the effect of different processing methods of vegetable protein supplement an crude protein content.	74
4.	Mean value showing the effect of different processing methods of vegetable protein supplement on RDP content along with their S.E. and CV%.	76
5.	Analysis of variance showing the effect of different processing methods of vegetable protein supplement on RDP.	77
6.	Mean value showing the effect of different processing methods of vegetable protein supplement on RDP as % of CP along with their S.E and CV%.	78
7.	Analysis of variance showing the effect of different processing methods of vegetable protein supplement on RDP as % CD.	79

8.	Mean value showing of the effect of different processing methods of vegetable protein supplement on UDP content along with their S.E and CV %	83
9.	Analysis of variance showing the effect of different processing methods of vegetable protein supplements on UDP content.	84
10.	Mean value showing of the effect of different processing methods of vegetable protein supplement on UDP as % of CP along with their S.E and CV %	85
11.	Analysis of variance showing the effect of different processing methods of vegetable protein supplements on UDP as % of CP.	86
12.	Mean value showing the effect of different processing methods of vegetable protein supplement on Pepsin digestibility along with their S.E. and CV%.	90
13.	Analysis of variance showing the effect of different processing method of vegetable protein supplement on pepsin digestibility.	91
14.	Mean Value showing the effect of Pelleting of concentrate mixture on CP, RDP, RDP as % of CP, UDP, UDP as % of CP and pepsin digestibility along with their S.E and CV%.	94
15.	Analysis of Variance showing the effect of Pelleting of concentrate mixture on CP, RDP, RDP as % CP, UDP, UDP as % CP and pepsin digestibility.	95
16.	Physical Parameters of Feeds.	97



INTRODUCTION

The concept of feeding domestic animal is not a recent one. Earlier livestock were kept at a subsistence level only but with the arrival of cash economy system they are now kept for financial gains.

The pace of industrialization and population growth have drastically altered the scenario of agricultural production in last 30 years. There is enormous pressure on land to raise food grains, commercial crops and available raw materials for the biomass based industries. Both qualitatively and quantitatively, still a large gap exists in the demand and availability of feed and fodder resources. It reflects major constrains in the production of feed concentrates and livestock products as well as old system feeding practices itself. There is thus an urgent need to explore new alternative non-conventional feed resources on one hand and to utilize available feed resource more effectively on the other hand.

The ability of ruminants for efficient digestion of cellulosic and allied plant products and utilization of feed residues to produce milk, is unique in nature. However, this is only a partial truth, since high yielders cannot be reared profitably only on roughage diet based on dry fodder and even with high quality green

fodder. They also need optimum level of concentrates to meet the needs of major nutrients as well as minerals and vitamins to express their full genetic potential. Any deficiency in quality or quantity of required nutrients would adversely affect, milk production, weight gain and other vital functions.

Rumen is the first and the largest of the four compartments of stomach of a cow, buffalo and other ruminants. The well grown microbial population in the rumen can very well alter the nutritional content of food of ruminants both quantitatively and qualitatively Dietary protein are also considerably degraded in the rumen in order to synthesize microbial protein. The material which becomes finally available for digestion by the animal, in its abomasum and duodenum, differs considerably from that originally present in the ration. Dietary protein is degraded in the rumen by a set of microbial enzymes into peptides and amino acids and ultimately into ammonia. The degree to which the dietary protein suffers degradation depends largely upon the solubility of vegetable proteins. The end product of degradation i.e. ammonia is utilized by the microorganisms for the synthesis of their body protein (microbial protein). However, the degree to which ammonia could be utilized for the microbial protein synthesis is dependent upon the availability of readily fermentable carbohydrates and the ammonia concentration in

the rumen and other factors. If the ammonia formation is rapid the level of ammonia concentration in the rumen increases sharply and a significant larger portion is absorbed through ruminal wall to blood stream, most of which is ultimately converted to urea and thrown out of body system through urine. Not only that a sizable fraction of dietary protein is lost via this path but also it is an energy wasting pathway. Thus, the utilization of dietary protein, appears to be closely linked to the degree of its degradation, in the rumen and subsequent post ruminal digestion (Satter and Roffler 1975).

Proteins are the fundamental components of all structures in an organism. The amount and turnover of protein at different sites in an animal will therefore, greatly affects its performance and production. Protein requirements, for the ruminants have been expressed traditionally in terms of Crude Protein (CP) or Digestible Crude Protein (DCP). However, the concept of CP/DCP fails to cope up adequately with the complexities of the nitrogen (N) economy of ruminant animal. The new system proposed is an improvement and to overcome the limitations of digestible crude protein system, which requires the description of dietary crude protein in terms of Rumen Degradable Protein (RDP) and Undegradable Protein (UDP), (ARC, 1980). NRC, 1989 (National Research Council) have also recognized this fact and has replaced

digestible crude protein and crude protein by its rumen degradable protein fraction (RDP) and Undegradable Protein fraction (UDP). In India many feed factories have also recognized this system of expressing protein requirement and now they are on a way to adopt this method of evaluation in their feed formulations. However, before switching over to this system, the feeds and fodder should have a value of both RDP and UDP under our conditions. Therefore, considerable interest exists in the routine determination of RDP and UDP fraction of feed. Some works have been conducted in this direction but the list is not complete. Besides other factors, protein degradation in rumen is influenced by its solubility (Jasaitis et al., 1987). Hence availability of protein in feed materials can be estimated from the fraction of the total nitrogen or crude protein in feed material that is soluble in various solvents. More and more quantitative knowledge on the protein requirements of ruminants has been acquired in which the extent of degradation of dietary protein is an extremely important determinant for both feed quality as well as for animal performance.

made estimate protein Attempts have been to (Crooker al..solubility tests et 1978; from degradability Krishnamoorthy et al., 1982; Kumar, 1986; Susmel et al., 1993; Kumar and Walli, 1994; Mehta, 1988). Further in vitro solubility of feed protein is reported to have a positive correlation with their

degradability in the rumen. (Hendericks and Martin, 1963; Lindberg et al., 1982). In vitro method not only have the advantage of being less expensive but also provide the chance to maintain the experimental conditions more precisely, than in vivo trial permit. Nitrogen solubility can be considered as an index for laboratory evaluation of protein quality and is a quick test for screening of feed ingredients used in the formulation of concentrate mixtures for ruminants feeds. Agro-industrial origin of feeds comprising of traditional concentrates like bran and expeller processed oil cake are now being processed by solvent extraction. These solvent extracted oil cakes are comparatively cheaper and protein content is also high. However the degradation of protein through different processing methods are also affected. The protein meals and cereals by-products, available commercially are processed from two different methods, such as solvent extraction through batch & continuos process. As the treatment in these two processes is different and the degree of protein degradability is induced by heat treatment so the variation in protein solubility might be different. Not much is known about the bypassibility and its subsequent digestion, post-ruminally of same protein meal by these two different processing methods.

Other processing method of compounded feed is being carried out mostly in pelleted form and is very popular among farmers in Bihar. Pelleting has certain advantages and has been introduced with the aim of simplifying the feeding system. Blending

of concentrates, mineral & vitamin supplements in a balanced form would minimize labour requirement and at the same time reduce wastage and more importantly facilitates in utilizing industrial byproducts. Pelleted ration, in addition would help in reducing the chewing and rumination time and thus helping increasing the resting time which will have direct effect on production. Adequate information is not available about the influence of pelleting feed ingredients on the level of undegradable protein (UDP) and pepsin digestibility. Thus there is a great need to undertake such studies in order to get more data on degradability of protein in rumen of some cereal by-products and oil cakes either as such or in pelleted form to be utilized in the formulation of ration of ruminants which will form the basis of new system of protein evaluation for ruminants. Moreover, the economics of adopting such system based on RDP and UDP content can be equated with CP content of feeds. It will also give a better concept of different processing methods in nutrient quality of feed ingredients.

Keeping in view the above facts the present experiment was designed to study with the following objectives:

a. To determine the effect of toasting in solvent extraction by batch and/or continuous process, as compared to the respective expeller cake of different protein supplements, on protein degradability and pepsin digestibility.

- b. To have a better appreciation of the superiority or inferiority of a protein supplement in terms of its protein value.
- c. To make a choice and judicious decision in purchase of these feed materials utilized in feed formulations.
- d. To evaluate the different solvent extraction method on overall effect in protein degradability.
- e. To determine the bypassibility and pepsin digestibility of the final product in pelleted form and to asses its superiority or inferiority over unpelleted form.
- f. To asses the expected response on growth and milk production of such processed feeds under field conditions.
- g. To know the protein degradability of protein supplements used in the experiments and its utilization in feed formulation on the basis of RDP and UDP contents.





REVIEW OF LITERATURE

The unique feature of ruminants is that it have two types of nutrient requirements (a) to satisfy the needs of the rumen microbes. (b) to satisfy the needs of the animal tissues. Satisfying the needs of the microbes help to satisfy the needs of the host animal because the microbial protein produced in the rumen makes a large contribution to the protein supplied to organs and tissues. In the qualitative sense, the two requirements differ. In that, the rumen microbes can be satisfied with ammonia as a source of N, while organs and tissues need amino acid. Awareness of these differences has led to recognize that the traditional method of measuring DCP in feeds does not represent the actual protein absorbed from the feed in ruminant nor does it recognize the relationship between the N required and energy intake. Both the U.K (ARC, 1980, 1984) and the U.S. (NRC, 1989) have developed system based on Rumen Degradable Protein (RDP)/undegradable Dietary Protein (U.D.P) values in feeds and their requirements by animals. The newer protein evaluation systems are based on the amount of the UDP and microbial protein available for absorption in the small intestine. In ruminants, the microbial protein synthesized in the rumen is the major protein source utilized for body function. However, high growth rate and milk production levels,

require higher protein energy ratios than usually provided from microbial growth alone. Supplementation of the undegradable dietary protein may enhance growth rate and milk production, especially under condition of higher level of production. If the protein requirement of the host animal is greater than the contributed through rumen microbial protein then the diets needs to be supplemented with slowly degradable protein source (Walli et al., 1993). However, an effective ration formulation using the new ruminant protein feeding system depends upon the accuracy of estimation and microbial nitrogen supply to and digested in the small intestine.

Investigations have been made to know the chemical composition of the vegetable protein supplements, methods of estimating degradability in vegetable protein source as such or in processed form and the effect of processing on degradability on these vegetable protein sources. The subjects will be discussed under the following headings.

- A. Chemical composition with respect to various proximate principles in various vegetable protein supplements used in the experiments.
- B. Methods of estimation of protein degradability with advantages and disadvantages.

- C. Influence of source of protein and processing methods on degradability values of protein and pepsin digestibility.
- **D.** Pelleting and its effect on degradability of protein.
- (A) Chemical composition with respect to various proximate principles in various vegetable protein supplements used in the experiments.

Through the years sunflower cake, mustard cake and groundnut cake are being used by many feed manufacturers and live stock owners in rations meant for different classes of livestock. Chemical composition of feed stuff varies depending upon the method of cultivation, the management practices followed, soil fertility, agroclimatic conditions and the methods of extraction employed.

Mustard Cake

Among edible oil seeds, mustard has received much attention in its production and utility as extracted oil for human consumption and the subsequent residual cake as a protein source in livestock feeding.

Varieties and Production of Mustard and Mustard Oil Cake

Mustard and rape seed belonging to the family cruciferae and genus *Brassica* are cultivated as rabi (Winter) crop in many parts of India viz., Assam, Bihar, Haryana, Orissa, Punjab, Uttar Pradesh

and West Bengal and is the main oil seed crop in Bihar. The commonly found varieties of mustard and rape seed are as follows:

- 1. SARSON: (i) Brassica campestris var sarson (yellow sarson
 - (ii) Brassica campestris var dichotoma (brown sarson)
- 2. RAI: (i) Brassica Juncea (raya or laha)
 - (ii) Brassica alba (safed rai)
 - (iii) Brassica nigra (kali rai)
 - (iv) Brassica tournefortil (Punjabi rai)
- 2. TORIA: (i) Brassica campestris var toria (Lahi)
- 3. *TORI* : (i) *Erucea sativo* (taramira)

In India, the commercially available samples of mustard seed are usually admixtures of sarson and laha (Rao and Reddy, 1981). The oilseed extractors usually refer sarson as mustard and rai as rape (Singh, 1964). The cake derived from Brassica seeds by ghani or expeller or solvent extraction is usually termed as mustard or sarson cake, since little or no species distinction is being made by oil processors (Lodi and Ichhoponani, 1973). The total annual production of mustard/rape-seed in India during 1981-82 was 1.9 million tones which yielded about 1.1 million tones of the residual cake (Anon, 1981).

Nutritive Value Of Mustard Oil Cake (MOC)

The proximate composition of variously processed MOC as reported by different workers is presented below:

Reddy and Vaidya (1973) reported the values for proximate composition of expeller processed Mustard oil cake as crude protein (CP) 35.4, Crude fibre (CF) 8.5, Crude fat 14.2 and total ash (TA) 8.3 per cent respectively.

The value of CP, CF, EE and TA of solvent extracted mustard oil cake as 41.9, 11.6, 1.4 and 9,0 per cent respectively was reported by Banerjee *et al.* (1975).

Lodhi et al. (1976) observed the values as 33.4, 12.2, 11.0 and 8.7 of expeller pressed mustard oil cake for % CP, CF, EE and total ash respectively.

The values of expeller pressed Mustard oil cake for CP, CF, EE and TA as 36.3, 8.4, 13.9 and 8.5 per cent respectively was recorded by Shrivastava *et al.* (1976a).

Prasad (1977) reported the percentage value of expeller mustard oil cake for CP 36'8, CF 7.5, EE 13.0 and TA 8.4 respectively.

The values for expeller processed mustard oil cake as 36.3, 7.8. 13.5 and 7.8 per cent respectively for CP, CF, E.E and T.A was given by Vaidya *et al.* (1979).

Chand et al. (1980) recorded the CP, E.E content of Expeller pressed mustard Oil cake as 36.1 and 10.8 per cent respectively. They further reported CP and E.E content of solvent extracted mustard oil cake as 38.6 and 1.6 cent respectively.

Sadagopan et al. (1982b) reported the crude protein and crude fate content of expeller pressed mustard oil cake in per cent as 36.0 and 8.2 respectively. They further observed the corresponding values for solvent extracted mustard oil cake as 38.8 and 1.0 respectively.

Negi et al. (1989) reported some what higher crude protein (43.05%) value of mustard seed cake.

Ponnappa (1985) reported the CP content of MSC as 31.59 per cent. He further stated that solvent extracted materials in general contain over 12% crude fibre which is the maximum permissible fibre content of animal feeds. The level of crude fibre is mainly influenced by the proportion of outer coat in the seed. Higher proportion of crude fibre in the feed has a depressing effect on the availability of nutrients.

The NRC (1978) recommended a crude fibre level of 17% of dry mater in dairy cattle rations to prevent low butterfat syndrome in milking cows.

Sahoo (1992) gave the value of CP EE, C.F, N.F.E and TA in mustard seed cake (MSC) as 34.50, 1.20, 8.53, 43.11 and 12.66 per cent respectively.

Dutta and Singh (1994) recorded the CP and TA content of MSC as 35.76 and 9.60 per cent respectively.

Vaidya (1974) found that the protein content of expeller pressed mustard oil cake (EMOC) is fairly high (37%) with a range of 33% to 37%.

Chand et al. (1980) recorded very high crude fibre (11.7%) in solvent extracted mustard oil cake when compared to the value (7.5%) in expeller pressed mustard oil cake. They further reported that unlike protein and fibre the fat content of MOC become reduced to 1.6% from 10.8% on solvent extraction of the cake.

Sunflower Cake

During the past decades the quest for newer alternative protein sources has intensified in view of soaring high cost and sometimes due to non-availability of conventional proteinic feeds. Sunflower cake/meal, the proteinaceous residue following oil extraction contain a fair amount of protein (30-45%), mineral, vitamin and essential amino acids particularly rich in methionine content.

Sunflower (*Helianthus annuus L.*) "Suraj-mukhi" in Hindi is well known as ornamental flowering plant far a long time. In recent years its several varieties are being cultivated as Rabi and Kharif crops in many parts of the country. The annual production of sunflower seed meal in our country during 1978-79 has been estimated to 60,000 metric tones (Rao and Rajasekhara, 1981).

The proximate composition of sunflower seed meal reported by many workers vary to a great extent. Such variations may be due to agro-climatic conditions, physical nature and different processing methods employed for oil extraction.

Mitchell *et al.* (1945) reported the proximate composition of expeller sunflower seed meal as crude protein 52.8, total ash 6.5 per cent respectively.

Morrison *et al.* (1953) recorded that the extracted sunflower seed meal contained CP, EE and CF as 37.2, 4.2 and 12.6% respectively.

Morrison (1957) mentioned the CP, EE, CF, NFE, TA of extracted sunflower seed meal as 19.6, 1.1, 35.9, 27.0 and 5.6 per cent respectively.

Merwe (1962) reported a higher figure for crude protein.

He estimated CP, EE, CF of extracted sunflower seed meal as 52.6,

0.9, 5.3 percent respectively.

Ballester et al. (1968) recorded the CP, EE, CF, NFE, TA of expeller sunflower seed meal as 46.6, 4.6, 8.9, 31.7 and 8.5 cent respectively.

NRC (1971) gave the figure in its report for CP, CF of expeller sunflower seed meal as 41.0, 13.0 per cent and that of extracted sunflower seed meal as 46.8 and 11.0 per cent respectively.

Delic et al. (1972) observed CP, EE, CF, NFE and TA of extracted sunflower seed meal as 48.5, 3.1, 8.2, 24.2, 7.0 per cent respectively.

Shrivastava (1977) found CP, EE, CF, NFE and TA of expeller sunflower seed meal as 30.5, 12.2, 16.5, 33.6 and 7 per cent respectively and that of extracted sunflower seed meal for CP, CF, NFE and TA as 31, 20.9, 40.0 and 7.9 percent respectively.

Singh and Prasad (1979) reported CP,EE, CF, NFE and TA of Expeller sunflower seed meal as 49.1, 7.7, 5.1, 27.2 and 10.4 per cent respectively.

Subramanyam (1974) observed a lower crude protein content and reported the crude protein content of sunflower cake as 26.2 per cent.

In the compilation report, Sen et al. (1978) gave the average percentage proximate composition values of expeller

processed sunflower cake as 36.40, 10.70, 11.20, 35.70 and 15.90 for crude protein, crude fibre, ether extract, NFE and total ash respectively.

Ranjhan (1991) in his book described that values of sunflower cake as 26.20, 22.90, 20.50, 23.60 and 6.80 per cent CP, CF, EE, NFE and TA respectively while Sibanda *et al.* (1993) gave the values of sunflower cake as 35.63, 14.80, 95.80 for CP, EE and DM percentage respectively.

Sharma (1990) documented the values of CP, CF, EE and TA in deoiled sunflower cake as 34.50, 19.50, 2.20 and 10.50 per cent respectively.

Bhatt (1993) reported the values of CP, CF and EE, in deoiled sunflower cake as 34.20, 20.00 and 2.10 per cent, respectively.

Kotach (1996) reported the values of sunflower cake as 29.60, 9.00, 8.20, 44.20 and 9.00 per cent for CP, EE, CF, NFE and TA respectively.

Kuldip *et al.* (1995) recorded that values of undecorticated sunflower cake (SFC) as 26.75 per cent CP, 25.32 per cent CF, 6.37 per cent EE, 27.50 per cent NFE and 14.06 per cent TA.

Groundnut Cake

Expeller processed/solvent extracted groundnut cake (GNC) is the most commonly used and well accepted as vegetable protein supplement for livestock feeding in the country. The crude protein contents of either of the processed cake varies due to decorticated or undecorticated nature as well as the methods of extraction employed.

Ponnappa (1985) reported crude protein content of solvent extracted groundnut cake as 40.80 per cent.

Chopra et al. (1971) observed the proximate composition of expeller groundnut cake as crude protein, crude fibre, crude fat, total ash as 32.0, 10.0, 3.8 and 4.5% respectively while the corresponding values for deoiled groundnut cake as 44.3, 12.7, 0.8 and 9.5% respectively.

Raghava Rao (1982) observed crude fibre content of decorticated groundnut cake as 5.93 per cent.

Reddy and Vaidya (1973) found the values of expeller processed groundnut cake for crude protein, crude fibre, crude fat, and total ash as 39.5, 8.7, 8.7, 5.4 respectively while values of deoiled groundnut cake as 48.6, 11.2, 1.3 and 8.4 per cent respectively.

The values of solvent extracted groundnut cake for crude protein, crude fibre, crude fat and total ash as 50.4, 9.6, 0.6 and 9.6 per cent respectively (Banerjee *et al.*, 1975).

Lodhi *et al.* (1976) observed the proximate composition of expeller groundnut cake as crude protein 43.9, crude fibre 11.4, crude fat 6.1 and total ash 6.5 per cent respectively.

Prasad (1977) reported the values of expeller processed groundnut cake for crude protein 38.1, crude fibre 8.7, crude fat 7.0 and total ash 6.7 percent respectively.

Chand *et al.* (1980) reported the values of expeller processed groundnut cake for crude protein and crude fat as 39.6 and 7.7 per cent respectively.

ISI (1982) has specified the composition of expeller processed groundnut cake for crude protein 40.9, crude fibre 8.9 and crude fat 7.9 per cent respectively and corresponding value of deoiled groundnut cake as 48.6, 11.2 and 2.2 per cent respectively.

(B) Methods of Estimation of Protein Degradability With Advantages and Disadvantages

The UDP and RDP content of feeds can be determined by the *in vivo* or the *in vitro* method or by estimating protein degradability in the rumen *in situ*.

In *in vivo* method, the UDP is estimated from the post ruminant sampling of abomasal or duodenal contents. The relatively long time and large amount of feed required restrict the use of the vivo procedures for routine determination of degradability of large number of feed samples. More over the estimation of UDP is an indirect method and is determined by difference which may leads to error (Santosh *et al.*, 1984).

The *in situ* or nylon bag technique involves the incubation of feeds in the rumen in synthetic fibre bags for several time periods. Disappearance versus time of incubation is interpreted to calculate the rate constant for degradation which can be combined with an estimate of fractional outflow rate of protein from the rumen to predict effective protein degradability (Orskov and McDonald 1979, Mather and Miller 1981, Mc Donald, 1981). The limitation in this method is the assumption of constant flow rate, loss of a fraction of protein from the bag due to small particle size and washing. It also requires the use of fistulated animals; moreover, this technique requires correction for microbial contamination of feed residuals left in the bag after incubation in the rumen. The per cent error associated with protein degradability not corrected for microbial contamination may be very high as was reviewed by Noeck (1988).

The *in vitro* method of determining protein degradability involves the use of either rumen inocula or proteolytic enzymes. The varying rate of reutilization of ammonia for microbial synthesis which depends on the rate and extent of fermentation of available substrates

particularly carbohydrates in the incubating medium is the limiting factor in this method (Broderick, 1982). Various workers have improved and modified the technique to estimate protein degradability (Broderick, 1987; Raab et al., 1983). A different approach in in vitro methods is the use of various proteolytic enzymes to estimate ruminal protein degradability and commercially available proteases have been tested (Krishnamoorthy et al., 1983; Mahadevan et al., 1987). The methods involving proteolytic enzymes are very simple and do not require fistulated animals or automated equipments as in other methods.

The extent of degradation determined by *in vitro* methods may differ considerably from that determined by *in vivo*. However the *in vitro* methods are time saving and particularly useful in relative ranking of feeds and in evaluating the effect of processing methods.

More information on these aspects have been reviewed and are presented as follows:-

IN VIVO

Buttery (1977) summarised in vivo method, which measures the proportion of protein reaching duodenum, which is of microbial origin and calculation of undegradable dietary protein by difference. This method involves animal fitted with abomasal or duodenal cannula and require a number of analysis for separation of microbial and dietary protein.

A method involving a single cannulae spot samples and indigestible solid and/or liquid phase marker was described by Orskov *et al.* (1969).

Miller (1973) and Hume (1974) used 35S label to estimate the proportion of dietary protein escaping degradation in the rumen for protein meals and forages by measuring the flow of total non ammonia nitrogen (NAN) and its fraction at duodenum.

Some workers were of the view that it is possible to estimate degradability of feed protein by giving different levels of protein in the diet and measuring the increments in the protein entering the duodenum. When degradability was determined by incremental approach, the problem of determination of microbial nitrogen could be avoided (Orskov et al., 1971; Gonzalez et al., 1979).

Diaminopemetic acid was used as endogenous marker by few workers (Hogan and Weston, 1970; Harrison *et al.*, 1973; Ling and buttery 1978), while nucleic acid was employed as endogenous marker by several others (Coelh. Da Silva, 1972 a; 1972 b; McAllon and Smith, 1974; and Ling and Buttery, 1978). Exogenous markers such as 35 S and 32 P were used by Bliver *et al.* (1972); Hume (1975); Sadanandan and Arora (1975) and Ling and Buttery (1978).

Orskov et al., (1980) reported a change in the outflow rate and consequent change in effective degradability of protein under

varying levels of feeding. The values obtained by *in vivo* studies are thus tenable only to the particular level of feeding.

Stern and Satter (1984) indicated that though *in vivo* method gives a fairly accurate estimate of protein degradability and can be used to test fewer feeds but *in vivo* estimation of protein degradability is always laborious; time consuming, need lot of feed and large number of animals. They suggested that an alternative procedure which provides rapid and reliable estimation of protein degradation for wide variety of feed stuffs should be advocated.

IN SITU METHOD

In situ method involves the measure of protein disappearance using artificial fibre bag suspended in the rumen. The protein remaining in the bag is assumed to be undegraded dietary protein.

Resumer (1752) was first to use this principle to assess the digestibility of leaves placed in perforated brass capsule. Several other workers employed this technique for measuring the digestibility of dry matter in the rumen (Hopson *et al.*, 1963; Neathery, 1969; Figgroid *et al.*, 1972; Uden *et al.*, 1974).

Quin et al., (1938) discussed the feasibility of using cylindrical bags made of very thin natural silk for measuring the digestion of feed in the rumen of cannulated sheep. Since then many workers have used it for various purposes.

With the advent of artificial fibres which are totally resistant to microbial degradation, the bag made of synthetic materials (nylon, dacron etc.) are being used *in situ* studies (Mehrez and Orskov, 1977, Siddons and Paradine, 1981; Kristenean *et al.*, 1982; Ha and Kennely, 1984).

The nylon bag technique has been used to estimate the degradation of dietary nitrogen in the rumen and is the reference to compare different methods of nitrogen degradability estimation (Mehrez and Orskor 1977; Crowford et al., 1978; De Boever et al., 1984; Madsen and Hvelpund, 1985). Several research workers adopted this technique subsequently for estimating the protein degradability values (Minor and Hovel, 1979; Deville et al., 1980; Huges-Jones and Peralta, 1981; Ward et al., 1982; Kung and Huber, 1983; Ha and Kennely, 1984).

Different methods for using dacron bag technique to predict protein degradation were evaluated. Two methods were proposed by Orskov and Mehrez (1977), both involves the dry matter and nitrogen disappearance from dacron bags. In one method bag exposure time was used which gave dry matter disappearance equal to DM digestibility of that diet in the rumen. The amount of N×6.25 that disappeared from the bag at that exposure time was considered as ruminally degraded protein. In other method they suggested that if

true ruminal DM digestion is not known then it could be appropriate to leave the bag in the rumen until the percentage of DM leaving the bag equation to 90% of apparent DM digestibility of that feed stuff in the entire gastrointestinal tract. The corresponding time of rumen exposure could be used on the N- disappearance curve, to estimate ruminal protein degradation.

Several methods have been used to combine *in situ* N-disappearance and rumen dilution rate (Orskov and McDonald 1979; Mathers and Miller; 1981; Mc Donald, 1981; Stern and Satter, 1984).

Madsen and Havelplund (1987) reported that nylon bag method gave a good prediction of the actual protein degradation in the rumen and is recommended for concentrate of unknown origin or when very precise values are required.

Sauer et al., (1987) determined the protein digestibility values by using mobile nylon bag technique.

Lindberg et al., (1987) measured the protein digestibility with 'in sacco method'.

The nylon bag technique is however subject to variables that can influence the estimate of protein degradation. The disappearance of feed stuff from bags suspended in the rumen can be affected by the way in which the sample is prepared, the diet fed to

animals used to incubate the samples, the nature of the fibre bag, ratio of feed to surface area of bag and the incubation period used (Nocek, 1988; Huntington and Givens, 1995).

In situ method has its own limitations such as the feed is confined to a bag and hence not exposed to chewing and rumination etc. and also due to fine grinding some of the particles leave the rumen directly without actually being degraded.

Factors affecting Protein degradability in situ

Incubation Time

It is essential that the degradation of protein is to be described in relation to time, otherwise the measurements are of very limited value.

Rodriguez (1968) reported that large variations between bags and animals were associated with short incubation period and these variations were diminished as the incubation period were prolonged.

Negi et al. (1990) reported that prolongation of incubation period in situ in nylon bags beyond 48 hours may not be necessary.

Sharma and Purohit (1991) found that nitrogen degradability was increased with increases in incubation period.

Position of Bags in Rumen

It was considered possible that some of the bags may be floated on the top of the rumen content and may not be subjected to microbial attack. Miles (1951) reported that there were wide variability in degradability of feeds at different position of bags in the rumen.

Erwin and Elliston (1959) and Rodriguez (1968) observed that position of bags in the rumen has no effect on digestibility of feed. They further noted that weight of anchor attached to the bottom of bag to prevent them floating had no effect on variability but suggested that longer string allows greater movement of bags in the rumen and thus minimized the effect of position.

Steritzer et al. (1990) reported that increased cord length increased DM disappearance from the bag as well as the microbial activity in the nylon bags. The effect of cord length should be taken into account when experiments involving feed evaluation with nylon bags are planned.

Pore Size of The Bags

The importance of pore size of the bag was emphasised by Belasco *et al.* (1958) in regulating the passage of food particle. Generally disappearance of nitrogen tended to increases with increased bag pore size.

Noeck (1985) determined the *in situ* digestion of soyabean meal. He reported that bag porosities of 6 and 20 μ m under estimated, wereas porosities of 40-59, 80 and 102 μ m compared favourably with *in vivo* ruminal available protein.

Size of Bag

The importance of sample weight to bag surface area has been emphasised by many researchers (Trohlin *et al.*, 1967; Uden *et al.*, 1974; Mehrez and Orskov, 1977).

Uden et al. (1974) suggested that a ratio of sample weight to bag surface area of 3.5 mg/cm² which is below the maximum of 10 mg/cm as recommended by Van Hellen and Ellis (1977). However, a bag size of 17×9 cm was recommended for the incubation of 5g DM of test diet by Mehrez and Orskov (1977).

Basal Diet

Basal diet of the animal may or may not influence the disappearance of nitrogen from nylon bag.

Mehra et al. (1982) reported that extent of disappearance of nitrogen after 8 hour of incubation of fish meal and linseed cake was not affected by the protein concentration of diet.

Weakly et al. (1983) observed that high grain diet was associated with lower rate of nitrogen disappearance.

Loerch et al. (1983) determined the effect of dietary protein source and energy level on nitrogen disappearance of various protein sources. The *in situ* nitrogen disappearance varied with variation in dietary protein supplement.

Flow Rate of Rumen Content

Zinn and Owenes (1980) noted that on increase in level of feed intake after increase outflow rate resulting in more by pass protein. On an average for every 10% increase in feed intake above maintenance, 6.5% increase to rumen by pass is obtained.

Lindberg *et al.*, 1982 showed that the solid out-flow rate of various protein source are in range of 4 to 6 per cent per hour and also quite constant at same level of feeding.

Newbold et al., (1987) reported that protein degradability may influence feed intake which in turn influence protein degradability.

Method of Washing

Van Keuren and Heinemann (1962) found that method of washing the nylon bags did not affect the coefficient of variation of DM disappearance.

Mehrez and Orskov (1977) reported that method of washing of bags could only affect 1.2% of DM disappearance.

Homogenity of Feed

Mehrez and Orskov (1977) used rolled barley grains to determine digestibility of artificial fibre bag technique. They found that bags giving low percentage of DM disappearance, contained more grain that the bags giving high percentage of DM disappearance, when whole grain were discarded before in cubation, the variability was reduced. They concluded that homogeneity of test diet is important to minimize the error.

INCUBATION PROCEDURE

Noeck (1985) used soyabean meal to estimate in situ DM and protein digestion. They observed that introducing bags in the rumen at specific time intervals and removing them as a group, resulted in a faster rate constant of soyabean meal DM as nitrogen disappearance than introducing all bags at once and removing them at specific time intervals.

Bacterial Contamination

Researchers (Akin and Amos, 1975) have shown that certain bacteria tenaciously attach to plant particles.

Noeck (1985) did not detect and significant difference between N-disappearance rate constants with or without correction for bacterial N-contamination. Negi et al., (1991) reported that proportion of microbial N-in the nylon bag residue continued to increase with increase in period of fermentation.

Pre Soaking

Presoaking of test feed is done to reduce any lag phase for microbial activity. Noeck (1985) reported that presocking did not result in uniform DM disappearance of feeds. However, pre-soaking could minimize difference between grinding.

Species of Animal

Lohan and Gupta (1991) reported that there were no uniformity among the mean nitrogen disappearance values between cattle and buffalo. Nitrogen of malt sprout was more degradable in cattle than in buffalo at 4 to 6 hr incubation period. At 12 hour incubation period N of mustard cake, maize and de-oiled rice bran was more degradable in buffalo than in cattle and for ingredients like groundnut cake, cotton seed cake, wheat bran and gram chuni, reverse was true.

Structure of Protein

Access to protein by proteolytic enzyme is influenced by the three dimentional structure of protein. The cyclic feature of protein, greatly reduces the proteolysis (Mangan; 1972). Protein with excessive cross linking, such as Di-sulphide bonds are less accurate to proteolytic enzymes and are relatively resistant to degradation (Nugent and Mangan 1978).

In vitro

Use of *in vitro* technique allows the measurement of degradability under standard laboratory condition. Laboratory methods which use buffer, purified enzymes or rumen fluids have been extensively studied (Crooker *et al.*, 1978; Mahadevan *et al.*, 1979, Krishnamoortny *et al.*, 1983; Aufere and cartailler, 1988).

Broderic et al., (1988) compared the estimates of ruminal protein degradation by an inhibitor in vitro (IIV) method with in situ method. In IIV method inhibitor are added to prevent metabolism of protein degraded products.

Roe et al., (1991) used different combinations of Streptomyces griseus, ficin and neutral protease with amylase in in vitro to predict in situ degradation of concentrate feeds. He reported that neutral protease with amylase ranked test feeds, according to the extent of crude protein degradation at 24 h, most similar to that in in situ.

Various workers tried to compare auto claved rumen fluid with in-organic salt solutions and buffer solution for protein solubility (Crawford *et al.*, 1978; Walli *et al.*, 1980; Reddy and Prasad, 1982; Poos-Floyd *et al.*, 1985; Lohan & Gupta, 1990).

Some workers used method consisting of *in vitro* measurement of ammonia on incubation of feed sample with rumen innoculum (Chamberlian and Thomas, (1979); Walli *et al.*, 1980; Daugherty and Cherch, (1982).

Chamberlain and Thomas (1979) explored the possibility of using neutral protease in the determination of rumen degradable nitrogen. They observed that by using enzymes the rate constant can be calculated, but it cannot rank the protein in the same order as degradabilities estimated *in vivo*.

Krishnamoorthy et al. (1982) have suggested an in vitro protease technique to estimate the rates of solubilization, of feeds nitrogen in the rumen and application of these rate constants to estimate undegraded nitrogen.

Rabb et al., (1983) have given an in vitro method which makes use of relationship between fermentation of carbohydrate and microbial protein synthesis to estimate rumen degradable nitrogen.

Lohan and Gupta (1990) and Fulpagare and Krishna (1993) have estimated solubility of protein in distilled water. The method has been used in particular for the evaluation of quality of N-preserved in grain and silages. However, studies are limited on solubility of protein in distilled water.

The N-degradabilities of 10 concentrate feeds by *in vitro* and *in sacco* methods have been compared with significant correlations between the *in sacco* effective degradability and the percentage of N-degrade *in vitro* at each time of incubation (Susmel *el al.*, 1993).

Protein Solubility

The degradability of protein by rumen micro-organisms is directly related to their solubility (Hendericke and Martin 1963). According to Hungate (1966) rumen microbes can rapidly digest such protein as is soluble for instance in saline solution. Wadhwa et al., (1993) conclusively revealed that solubility of protein is an important factor in determining the protein susceptibility or resistance to rumen degradation.

Several workers have indicated a positive relationship between protein solubility and ammonia release in the runes. (Anhison *et al.*, 1954, Chalmer *et al.*, 1954, Syrjala, 1972, Chalupa, 1975, Donaldson and Edwads, 1977).

A relatively simple and rapid method for evaluating the degradation of feed protein in the rumen is the determination of protein solubility. Attempts have been made to estiate protein degradability from solubility tests (Crooker et al., 1978; Krishnamoorthy et al., 1983). The solubility of feed proteins have

been made in various solvents viz. distilled water, NaOH, NaCl, diluted acids auto claved rumen fluid, proteolytic enzymes and also McDougall's artificial saliva and buffers.

Annison et al., (1954); Chalmers et al., (1954) observed that protein of low nitrogen solubility had higher nutritive value for ruminants, because soluble nitrogen sources were rapidly converted to ammonia, most of which is lost through absorption.

As early as 1973, Layman et al. reported correlation between nitrogen solubility and nutritive value of cotton seed cake for chicks. They suggested nitrogen solubility as an index for laboratory evaluation of proteins. Sampath and Sivaraman, 1986b; Crawford et al., 1978; Lindberg et al., 1982; also estimated protein degradability by solubility test by various methods.

Mehrez et al., (1980) showed that solubility and degradability could be very similar in well processed fish meal since the fish meal had very little 'b' value.

Several workers also tried to compare auto claved rumen fluid with inorganic salt solutions and buffer solutions for protein solubility (Crawford *et al.*, (1978); Walli *et al.*, (1980); Reddy and Prasad, 1982; Poos-floyd *et al.*, 1985; Lohan & Gupta, 1990).

Bhirund et al., (1995) compared in vitro DM and CP solubility in 0.15 M Phosphate buffer and by in sacco methods of 11

protein supplements, 6 energy supplements and 4 different combinations and advocated that *in vitro* method; could be used to measure soluble protein from feed stuff.

Solubility in Buffer Solutions

The rumen liquid without its microbial fraction, acts simply as a buffer and shows little enzymatic activity (Susmel et al., 1993). The most commonly used buffers are Mc Dougall's (Mc Dougall, 1984) Burrough's (Burroughs et al., 1950) and Phosphate (Krishnamoorty et al., (1983). Buffer solubility of protein is dependent on incubation period, presence of metal salts, temperature and pH of solvent (White et al., 1968); Wholt et al., 1973). Incubation in buffers are generally performed at a temperature of 39-40°C.

Macrae (1976) proposed that phosphate buffer can be used successfully in estimating the protein solubility as an index of protein degradability in rumen.

Owen et al., (1976) also recommended the use of phosphate buffer to find out the protein degradability in the rumen inspite of the problems when some proteins which are water soluble but show resistance towards rumen fermentation. They reported that apart from some drawback, still this test showed a good 'r' value between the value obtained from 'in vivo' trials.

Lohan and Gupta (1990) used seven solvents consisting of distilled water (DM), Burroughs mineral mixture (BMM), Sodium Chloride (NaCl), auto claved rumen fluid (ARF), Mc Dougall's artificial saliva (MCD), Bicarbonate phosphate buffer (BCP) and borate phosphate buffer to find out the solubility of various fluids. They found that correlation coefficient between nitrogen solubility in various solvent and rumen degradable nitrogen (RDN) at different incubation intervals varied among feed stuff and solvents. The concluded that highest over all significant correlation coefficient with RDN was obtained using BCP buffer as solvent.

Verite et al., (1979) also gave similar information on the solubility of proteins in buffers.

(C) Influence of source of protein and processing methods on Degradability values of protein and pepsin digestibility.

Protein in the diet is partly degraded in the rumen and its degradation is affected by factors like solubility, inhibitory substances like tannins etc and rumen environment. Protein that escape rumen degradation and that passes to lower digestive tract plays an important role in high yielding animals particularly when high biological value protein is fed to cattle.

Feeding of diets of lower protein degradability viz fish meal and formaldehyde or heat treated meals has been found to decrease the DM digestibility in rumen compared to diets of higher RDP value (Zerbin et al., 1988). Growing animals that were fed with protein of lower RDP value showed increased N-retention and a simultaneous decrease in urinary N excretion than when proteins of higher RDP value were fed (Kurilov et al., 1988; Walli et al., 1988). The N-retention is lactating cows was also improved by infusion of casein into the abomasum (Cohick et al., 1986). The flow rates of non ammonia nitrogen and amino N (as % of nitrogen intake) were found to be highest on urea treated straw supplemented with low degradable protein, followed by untreated straw plus highly degradable protein (Kumar and Walli, 1989). The average UDP values of some of the commonly used feed stuffs have been given in a review (Sampath 1990a). These values were calculated for a flow rate of 5% / hour. On that basis he divided feed stuffs into three categories of high, medium and low UDP values. Feed materials of animal origin and heat processed materials usually had high UDP content. The digestibility of UDP of most of the concentrates was about 90% whereas that of roughage varied from 40-85%.

Dimitrova (1980) studied the effect on gain and feed utilization of 12 samples of sunflower seed oil meal produced by the battery extraction and continuous exaction methods in 4 trials with

chickens from hatching to 35 or 56 days old. He found best results with oil meals produced by continuous extraction at 100° C to 110° C and poorest result with oil meals produced by battery extraction at 125° C to 130° C.

Klimov, (1984) observed that availability of crude protein for intestinal digestion varied invariably with dietry crude protein solubility in artificial saliva.

Murphy and Kennelly, (1986) showed that increasing the rumen undegradable dietry protein (UDP) intake of Holstein Friesian cows, either by increasing the dietary crude protein content from about 12.7 % to 16.4 % with canola rape seed meal or to 14 % to 16.1% with maize gluten meal increased the milk yield. They observed similar milk yield response with 16.4 % dietary protein (canola rape seed meal) and the 14.1 % dietary protein (maize gluten meal) and suggested that rumen degradable protein and UDP are better indicator of protein quality than crude protein.

Treat and Stallcup, (1983) conducted a study to estimate the nitrogen metabolism digestible energy and total digestible nutrients of sunflower oil meal extracted by the expeller process or by the solvent extraction process in two series of digestion trial using 3 cattle each in 3 2 design. They concluded that sunflower oil meal is a high quality protein source and that method of processing can affect metabolism of the protein and energy content of the meal.

Mahjeesh et al., (1996) examined the effect of source and degradability of dietry crude protein supplements on amino acid (AA) flow and absorption in the small intestine. They studied the CP supplements of fish meal or meat meal as animal by product supplement and soyabean meal or heated soyabean meal as plant by product supplement. They observed that heat treatment of soyabean meal reduced the availability of most of the essential amino-acids for absorption in the small intestine by 2.3 %, compared with that of soyabean meal and availability of amino acid in the intestine was effected by source and degradability of the CP dietary supplement.

Sinha and Singhal, (1997) estimated effective DM and CP degradabilities of untreated (expeller pressed), CuSo₄ treated and solvent extracted mustard cake using nylon bag technique. Rumen degradable protein content of untreated (expeller pressed), CuSo₄ treated and solvent extracted cakes were 22.82, 23.70 and 26.96 % and corresponding values of undegradable protein content were 14.11, 13.22 and 11.66 %. They stated that processing of mustard cake may effect the crude protein degradability marginally but its utilization was not effected significantly.

Sampath, (1988) discussed the limitations of the digestible crude protein system in calculating the crude protein requirements of ruminants along with the advantages of the system

based on undegradable dietary protein. He urged that the time has come to switch over to the new system of by-pass protein in meeting the protein requirements of ruminants.

Veresegyhazy et al. (1989) noted that sunflower meal had rumen degradation of 69.1 % and heat treated sunflower meal had degradation of 67.1 %. They used 3-phase laboratory procedure to predict degradability of protein in extracted sunflower meal before and after heat treatment. They found digestibility values of rumen undegraded protein as 57.3% for sunflower meal and 57.0% for heat treated sunflower meal with pepsin.

Srinivas and Gupta (1994) found CP degradability of mustard cake as 32.9 % and that of groundnut cake as 45.7 %.

Bani et al. (1995) measured solubility in phosphate borate buffer and in vitro degradation in 2, 4, 8, 18, 24, 48 hr. with proteinase from streptococcus griseus and compared with in situ nitrogen effective degradability in 16 vegetable samples and 11 animal by products. They observed significant correlation between solubility and effective degradability for all 27 samples.

Kuldip et al. (1995) estimated the nitrogen solubility of undecorticated sunflower cake as 54.43 ± 1.98 and 50.24 ± 1.82 percentage in Mc Dougall's buffer solution and autoclaved rumen liquor, respectively. He further reported the solubility of mustard

cake in Mc Dougall's buffer and autoclaved rumen liquor as 33.90 ± 2.33 and 34.88 ± 1.64 percent respectively. Hence they concluded that undecorticated sunflower cake protein had higher solubility than that of mustard cake which agrees with the work of other scientists. (Lohan 1986).

Calsaminglia and Stern (1995) suggested that in vitro procedure provides an alternative to using intestinally connulated animal for estimating intestinal digestion of protein supplements. He also suggested that variations in intestinal digestion of proteins among and within various sources should be considered when determining protein value for ruminants.

Prabhu et al. (1996) reported Rumen degradable nitrogen percentage of groundnut extracted meal, sunflower extracted meal and rape seed extracted meal as 89.90±1.85; 81.83±2.68, and 68.34±2.23 respectively. They further reported Buffer soluble nitrogen percentage of these feeds as groundnut extraction 61.56±0.85, sunflower extraction 43.34±1.44, and rape seed extraction as 37.04±0.89 respectively. They observed that RDN values of same feeds but from different sources of groundnut extraction, sunflower extraction was similar to those obtained from standard samples, however it was lower for rape seed extraction and higher for cotton seed extraction than those obtained from standard

samples. Such differences in nitrogen degradability among the same ingredient from different sources may be due to the change in characteristic of protein associated with processing method. Similar views were also expressed by Waldo et al. (1979).

Kandylis and Nikokyris (1997) showed that protein solubility was lowest (less than 10% of total nitrogen) for oil seed by products, animal and fish by products intermediate (5-30% of total nitrogen) for some cereals and highest (35-45% of total N) for wheat varieties and plant protein source. They also observed that solubility data for various feed proteins demonstrated the variability in solubility of ruminant feed due to protein type or processing.

Reddy and Prasad (1985) found that soluble protein fraction in solvent extracted groundnut cake and sunflower cake was quiet low. They obtained degradability percentage as 30.4% for groundnut cake (solvent extracted) 20.4% for groundnut cake (expeller processed) and 46.1 for sunflower seed cake.

Orskov et al. (1980) reported degradability value to be 70.8% at an outflow rate of 0.05/h for groundnut meal in sheep fed a dried grass diet However same author reported N degradability value of 56.8% at same outflow rate when the sheep were fed a whole barley diet.

The value for expeller processed groundnut cake as reported by Rao and Prasad, (1989) was 70.20. Sampath and Sivaraman (1985) reported RDP and UDP content of groundnut cake to be 30.20 and 14.80% respectively.

Gupta et al. (1987) reported the IVDMD of mustard seed meal as 76.4 % indicating the scope for improving its digestibility by removing the husks.

Miller (1982) reported percent N degradability for sunflower seed meal as 65.5.

Rao and Prasad (1985) reported N solubility values for silk cotton seedcake as 25.2, 23.5 and 16.2% and for tobacco seed cake as 24.6, 25.2 and 21.3% in buffers such as sodium chloride, Mc Dougall's saliva and Wise burrough's solution respectively.

Rao et al. (1989) worked out the effective protein degradability for groundnut cake, niger cake, gingelly cake, coconut cake and cotton seed cake as 72, 83, 57, 71 and 57 percent respectively, at a rumen outflow rate of 0.05/h they observed that differences in the protein degradability values of niger cake, coconut cake and gingelly cake might be due to differences in processing technology adopted during oil extraction.

Rao and Prasad (1989) found N solubility of solvent extracted and expeller processed groundnut cake as 7.96 and 7.53 percent and that of sunflower cake as 6.20%.

For niger seed cake the EPD value of 32.7% was reported by Reddy and Prasad, (1985), whereas value of 76.7% was found by Rao and Prasad, (1989). However Rao *et al.*, (1989) reported a still higher value of 83% for niger seed cake.

Sampath and Sivaraman (1985), Rao and Prasad (1989) and Rao et al. (1989) observed protein degradability values of 85, 67 and 57 percent respectively for gingelly oil cake.

Miller (1982), Vanstraalen and Tamminga (1990) found values of 65.5 and 73.0 percent respectively as EPD of sunflower cake. However Reddy and Prasad (1985) reported a lower value of 46.1%.

Reddy and Prasad, (1985) reported an EPD of 42.90% for tobacco seed cake. Kumar and Walli, (1994) observed percent N-solubility ($in\ vitro$) at 8 hr for groundnut cake in Mc Dougall's buffer, Autoclaved rumen liquor and normal saline as 72.20 ± 2.44 , 43.94 ± 1.19 and 41.10 ± 1.22 respectively.

As compared to the protein degradability value of 19 percent for solvent extracted coconut cake (Sampath and Sivaraman, (1985)) Rao et al., 1989 reported a high value of 71%.

Sampath and Sivaraman, (1985) reported protein solubility of groundnut cake in 0.15 M sodium chloride, Burroughs Mineral solution, Mc Dougall's artificial saliva and strained autoclaved rumen fluid as 31.17, 57.95, 52.24 and 29.46 percent respectively.

Floyd et al. (1985) reported soluble nitrogen as percentage of total Nitrogen in bi-carbonate phosphate buffer for soyabean meal, blood meal, meat meal, corn gluten meal, Dehydrated alfalfa, distillers dried grains, Distillers dried grains plus solubles Breevers dried grain, Bentonite treated soyabean meal as 30.95, 1.29, 13.64, 9.98, 18.78, 5.71, 19.33, 3.63 and 13.29 respectively.

Krishnamoorthy et al. (1980) found soluble nitrogen as percentage of total nitrogen based on solubility in bicarbonate phosphate buffer for solvent extracted rape seed meal as 32.4.

Sampath *et al.* (1997) reported RDP and UDP % for untreated GNC as 37.36 and 9.76 respectively and heat treated GNC at 130° C for 2 hours as 24.82 and 23.20 percent respectively, for heat treated GNC at 130° C for 3 h as 21.13 and 27.56 percent respectively.

Singh *et al.* (1995) observed nitrogen solubility in Mc. Doughal's buffer and bicarbonate phosphate buffer as 60.80 and 65.40 percent respectively for Groundnut cake.

Lohan et al. (1984) observed percentage soluble nitrogen as percentage of total in Distilled water, Sodium chloride, Mc Doughal's buffer, Burrough's mineral mixture, Bicarbonate phosphate buffer and autoclaved rumen fluid for groundnut cake as 51.52, 22.40, 77.28, 38.08 and 67.87 percent respectively, for mustard oil cake 22.40, 54.20, 68.77, 30.08, 53.76 and 52.20, for sunflower cake 20.60, 36.28, 61.15, 39.88, 34.05 and 61.16 percent respectively.

Gupta and Gupta, (1984) noted RDP, UDP and UDP as % of total CP for groundnut cake as 39, 2 and 4 percent respectively, for mustard cake as 33, 4 and 12 percent respectively.

Sampath and Sivaraman (1985) reported RDP, UDP and UDP as % of total CP for G.N.Cake as 30, 14 and 32 respectively.

Reddy and Prasad (1985), found RDP, UDP and UDP as % of total CP for G.N.Cake as 9, 37 and 70 percent respectively, for groundnut extracted as 15, 34 and 69 percent respectively for sunflower seed cake as 16, 18 and 53 percent respectively.

Upadhyay and Gupta (1988), reported for groundnut cake RDP, UDP and UDP as % of total CP as 18, 21 and 53 respectively.

Negi et al. (1989) found RDP, UDP and UDP as % of total CP for G.N.Cake as 25, 7 and 23% respectively and for mustard cake as 36, 7 and 14 percent respectively.

Manget Ram and Gupta (1994) found RDP, UDP and UDP as % of total CP for groundnut extract as 36, 4 and 11 percent respectively.

Kunju *et al.* (1992) calculated RDP and UDP percentage of rape seed meal as 14 and 26 percent respectively.

Mehra et al. (1998) found solubility percentage in autoclaved rumen liquor, Mc Doughall's saliva phosphate buffer and

normal saline for mustard cake as 44.2 ± 0.85 , 47.7 ± 0.84 , 33.6 ± 1.34 41.6 ± 1.32 and 41.8 ± 1.60 percent respectively and for groundnut cake as 41.3 ± 0.45 , 75.4 ± 0.96 , 58.1 ± 2.67 and 22.6 ± 0.22 percent respectively.

Reddy and Prasad (1985) observed effective degradability as 30.4 and 20.4% for solvent extracted and expeller crushed GNC respectively.

Sahoo et al. (1993) found Rumen degradable protein as 24.40 and 33.14 percent in mustard seed cake and groundnut cake respectively and Undegradable protein as 10.10 and 10.15 percent respectively.

Kumar and Walli (1988) reported UDP content of GNC and MSC as 8.78 and 19.36 percent respectively.

Kumar (1996) reported nitrogen solubility in phosphate buffer 18.48% for sunflower cake and 56.28 percent for mustard seed cake. He found N solubility as 64.96% for MSC and 25.20% for SFC in MC Dougall's buffer and 47.13% for MSC and 21.28% for SFC in Sodium Chloride. He further observed percent nitrogen solubility of mustard cake and sunflower cake in Trito X-100 solvent as 18.85 and 13.63 percent respectively.

Lohan et al. (1984) have reported N solubility values of SFC in distilled water, Mc Dougall's buffer and Nacl as 20.60, 61.15 and 36.28 percent respectively.

Walli et al., (1993) reported the percent N solubility in phosphate buffer of sunflower oil cake as 16.04. Sampath, (1985) observed solubility of groundnut cake as 31.17, 57.95, 52.24 and 29.46 percent in sodium chloride (Nacl) Burroughs mineral solution (BMM), Mc Dougall's artificial saliva (MCD) and autoclaved rumen fluid (ARF) respectively.

Sahoo, (1992) found RDP percent for MSC and GNC as 24.40 and 33.14 percent and UDP percent as 10.10 and 10.15 percent respectively.

PEPSIN DIGESTIBILITY OF FEEDS

Very few references are available on the above topic which have been briefly mentioned below.

Kumar, (1996) reported percent nitrogen solubility of Mustard seed cake and Sunflower cake in pepsin as 97.63 and 62.91 percent respectively.

Kim et al., (1998) found pepsin digestibility of feather meal ranged from 51.92% to 74.0%. They further noted that grinding condition of raw feather meal did not affect pepsin digestibility. They worked on effect of physical processing and protein content and pepsin digestibilities of feather meals.

(D) Pelleting

The concept of feeding complete rations to ruminants is quiet recent and is becoming increasingly popular. Feedstuffs after grinding and moistening (called tempering in feed mill) may be forced under pressure through perforated dies. As the strings of materials extruded through the die they are cut off into short lengths. These small, pressed feed particles are called pellets, and the feeds or feed mixtures so prepared are said to have been pelleted. Nutritionally, this method of preparation has some advantages over the mere grinding of the grains. In pelleting small quantities of purified nutrients are added as supplements for maintaining an even distribution through the batch of feed for satisfactory feeding results. It is also claimed by some that when pelleted feeds are offered to livestock there is less wastage, particularly of feed that has been spilled from the feeder, because the animals can recover spilled pellets more easily than spilled ground feed. Pelleting tends to improve the physical nature and hence the acceptability of feed mixtures that contain powdery substances. Pelleting reduces dustiness and animals can be induced to consume large quantities of the feed. One of the problems in Pelleting feeds is to prepare the pellet in a firm enough form to resist breaking and crumbling on handling. Molasses incorporated in the feed to the extent of 5 to 10% will usually keep the

pellets from crumbling. More recently sodium bentonite to the extent of about 2% has been incorporated into such feeds, where it is effective in making a firmer pellet

Mc Donald (1990) reported that fine grinding followed by pelleting has a marked effect on the manner in which roughages are digested hence on their digestibility.

Mehta and Singhal (1983) observed that pelleting did not affect the ammonia N-release, cellulose digestibility, nutrient utilization and microbial protein synthesis significantly. However, they found that nitrogen utilization of pelleted feed was better than un-pelleted feed.

Pena et al. (1986) in their experiment with extruded cotton seed, demonstrated that extrusion markedly reduced ruminal degradation of protein.

Stutts et al. (1988) also worked with extruded cotton seed and found similar results.

Do-reau et al. (1987) in their work with soyabean seeds and Fabar beans, found that extrusion diminished the effective rumen degradability of crude protein.

Walhain et al. (1992) analysed the influence of extrusion on ruminal and intestinal disappearance in sacco of pea (Pisum sativum). They reported that it is unnecessary to increase extrusion

temperature above 140° C. Above this temperature protein protection was not significantly improved. They further reported that the low value of residual N in mobile Nylon bags (0.45 g/kg DM) and high N pepsin solubility (95.8%) of peas extruded at 140° C, demonstrate that extrusion had little or no negative effect on intestinal digestibility.

Mesini (1998) discussed advantages of Hydrotheromic treatment involving heat and steam, in production of pelleted feeds. He found such treatment modifies the physical and chemical characteristics of feeds, improving digestibility and hygiene and consequently animal performance.

Briggs et al., (1998) investigated effect of ingredients, steam pressure and mixing paddle configuration inside the conditioner on pellet quality and observed that increasing the protein content, increased pellet durability while increasing oil content decreased it.

Lindahl and Davis, (1955) concluded that the pelleting usually reduced the moisture content of feed mixtures, even when steam or water was used in the process. Further, they found that there was a trend towards higher digestibility of ether extract and lower digestibility of crude fibre and only slight difference, if any, among TDN values between the pelleted and unpelleted feeds.

Long et al. (1955) fed a ration of long hay and whole grain in three different physical forms viz. natural, ground, ground and pelleted to lambs and studied the digestibility values. They found that average apparent digestion coefficient for organic matter, crude protein, crude fibre and NFE were significantly higher (P<0.01) for pelleted ration than for the ground ration.

Several other workers observed similar result that pelleting of complete mash rations seemed to improve the palatability and thus increased the food intake. Klosterman *et al.*, (1960), Khajuria and Mudgal, (1975) and Hazlett *et al.*, (1960).

Lindahl and Reynolds (1959) found no apparent effect of pelleting on the digestibility of dry matter. Similar results were also reported by Lindhhal and Davis (1955) Long *et al.* (1955), Esplin *et al.* (1957) and Meyer *et al.* (1959).

Long et al. (1955) noticed that grinding of the whole ration lowered its digestibility among sheep. However, pelleting the ground ration restored its digestibility to the same level as observed in the natural state.

Nelson et al. (1968) and Cowsert and Montgomery (1969), recorded a significant increase in apparent crude protein digestibility for pelleted rations.

Reddy and Reddy (1979) also found, an improved crude protein digestibility due to pelleting of the mash feeds.

Reddy (1983) revealed that pelleting of complete feeds, require additional power consumption than those of complete mash feeds and the additional cost of pelleting was due to cost of steam production and the actual pelleting process, He also observed a significantly higher (P<0.05) crude protein digestibility in pelleted ration as compared to corresponding mash ration indicating improved protein digestibility due to pelleting.

Similarly, Meyer *et al.* (1959), Woods and Rhodes (1959) and Reddy and Reddy (1979) reported improved nitrogen retention by pelleting.

Bush et al. (1978) found that pelleted complete rations were advantageous in a fattening lamb programme, both on performance and economic basis.

Murdock and Miller (1951) after working with lambs reported that pelleting finely ground ration increased the digestibility of crude fibre.

Noble et al. (1953) and Bell et al. (1954) reported that pelleting a ground mixed ration increased the efficiency by which it is utilized by fattening lambs.

Tangendjaja et al. (1988) noted that extruding or pelleting the rice bran diet improved feed efficiency. Digestible protein was not affected by pelleting or extruding but Digestible energy and DM were slightly improved by extruding or pelleting.

Greenhalgh and Reid (1973) observed that the general effect of grinding and pelleting rough ages are to increase their acceptability to the animal. He found that feed in take was greater with pelleted feed than with unpelleted feeds. Campling and Freer, (1966) also found similar results.

Reddy et al. (1990) reported higher DMI on pelleted ration as compared to ground form due to increased palatability because of steam pelleting effect. Research was carried out with Twelve adult Nellore brown rams (18-20 m : 28.9 kg) and 12 local male goats (22-21 m; 29.1 kg) each divided into 2 equal groups in a randomized block design and fed sunflower straw either in ground or pelleted farm ad lib along with 200 g/d of concentrate mixture for 20 days.

Morel (1997), studied pelleted or extruded flaked concentrates for rearing calves. He observed that compared with pelleted feed type, extrusion of protein source reduces CP degradability which fell from 75.4 (PLT) to 71.4% (E).

Walczynski *et al.* (1996) reported that high quality pellets were produced using pelleting temperatures above 90° C and lignin as binding material.

Ginste et al. (1998) observed that processing increased the in vitro protein solubility (P<0.05) and reduce the dietry contents of free lysine and Methionine (P<0.05) but had no effect on the available lysine, free threonine and tryptophan contents. They worked on 12 Belgium Landracex Pietrain barrows, weighing 40 kg, giving a grower diet containing barley, wheat and soyabean meal and libitum for 5 weeks. Half the pigs were given the diet unprocessed and half were given were given the diet after it had been expanded at 110xc and than pelleted at 80x.

Reddy et al., 1992 observed increased DMI on pelleted ration due to increased palatability because of steam pelleting effect. Twelve adult Nellore brown ram and 12 local male goats were taken each divided into two equal groups in a randomised block design and fed sunflower straw either in ground or pelleted form ad lib along with 200 g/d of concentrate mixture for 20 days. They further observed that Digestibility % of all the nutrients and nutritive value were comparable on both rations indicating that pelleting of ground sunflower straw had no beneficial effect over grinding.

In general the pelleting of the complete mash ration, improved the palatability and increased feed intake, this may be due to cooking effect and increased density of the pelleted feeds, this was noted by Reddy and Reddy (1983), Hazlett et al. (1960), Klosterman et al. (1960) and Khajuria and Mudgal (1975). It was further observed by Reddy and Reddy (1983) that dustiness of the mash ration may be reason for lower feed intake to that of pelleted feeds.

Keyserlingk et al. (1998) studied two forms pelleted and textured of the same concentrate mix consisting of barley, maiza, rape seed meal and a vitamin mineral premix in a feeding trial. Parameter studied were in situ rumen degradability characteristics of the concentrates, DM in take and yield of milk and milk components. The trial indicated greater degradable fraction of the DM for the textured as compared with the pelleted form. In terms of NE for bactation the diet containing the pelleted concentrate tended to have a higher energy value than the diet containing the textured concentrate. The marked difference in the ratio of milk fat to milk protein for pelleted and textured concentrates, coupled with the impact on rumen degradability characteristics indicated that minor differences in processing can have an impact on product yield from the milk and the efficiency of utilization of the diet.

Ginste et al. (1998) found that feeding of pelleted grower and finisher diets in pigs increased apparent total tract N digestibility. In vitro test showed that pelleting caused an increase in protein solubility which appeared to be paralleled by higher protein digestibility in growing and finishing pigs.

Kumar and Walli, (1994) found percent N-solubility of pelleted GNC in Mc Dougall's buffer, autoclaved rumen liquor and normal saline as 35.09 ± 1.16 , 21.25 ± 0.26 , 17.90 ± 0.50 respectively and that of soyabean meal as 14.48 ± 0.33 , 11.19 ± 0.26 , 9.51 ± 0.35 , respectively.





MATERIAL AND METHODS

The present investigation was designed with a view to study the influence of the processing methods viz. solvent extraction, i.e., batch or continuous process as well as expeller process of some of the commonly used oil cakes and also the effect of machine pelleting of compounded feed with respect to the degree of degradability of protein and to assess the percentage of rumen degradable protein and undegradable protein and its subsequent, post ruminal digestion in the lower gut.

The analytical works were carried out at the laboratory of Department of Animal Nutrition, Bihar Veterinary College, Patna with the collaboration of cattle feed factory, COMFED, Patna. The entire studies were carried out into two parts, namely,

- (i) Proximate analysis and effect of toasting of oil cakes during batch or continuous process of solvent extraction and expeller process on protein degradation and pepsin digestion.
- (ii) Effect of steam treatment and extrusion process in pelleting of compounded feed under study in pellet mill on protein degradation and pepsin digestion.

Part - I

In first phase three vegetable protein supplements viz. mustard oil cake, sunflower cake and groundnut cake processed under different methods were investigated for proximate composition, protein degradability and pepsin digestibility. For comparing various processing methods in different vegetable protein supplement the outline of work was as follows

Name of Feed	Method of Processing	Treatment assigned
Mustard oil cake	Expeller processed	C_1
Mustard oil cake	Solvent extraction (Batch processed)	T_1
Mustard oil cake	Solvent extraction (continuous processed)	T_2
Sunflower cake	Expeller processed	C_2
Sunflower cake	Solvent extraction (Batch processed)	T_3
Sunflower cake	Solvent extraction (continuous processed)	$\mathrm{T_4}$
Groundnut cake	Expeller processed	C_3
Groundnut cake	Solvent extraction (Batch processed)	T_5
Groundnut cake	Solvent extraction (continuos processed)	T_{6}

Part - II

Compounded feed for dairy cattle prepared as per formulae developed in cattle feed factory was determined for its protein degradability and pepsin digestibility which is detailed as below:-

Items	Proportions (%)	
Deoiled Mustard Cake	6.00	
Guar Meal (Korma)	6.00	
Deoiled cotton seed cake	5.00	
Deoiled groundnut cake	10.00	
Soyabean meal	12.00	
Mustard oil cake	3.00	
Maize	3.00	
Deoiled rice bran	28.00	
Rice bran	12.00	
Molasses	10.00	
Calcium Supplement	3.00	
Common salt	2.00	
Trace mineral and Vit AD3	2.50 Kg/MT	

The above compounded feed was analyzed for its proximate composition, protein degradability and pepsin digestibility, both unpelleted and pelleted form for their comparative study.

Name of feed	Method of	Treatment
	Processing	assigned
Concentrate	Unpelleted	С
Mixture	(Mashform)	
Concentrate Mixture	Pelleted	Т

Experimental Design

- (i) Proximate analysis of feed under investigation
- (ii) (a) C_1 Vs. T_1 and T_2 .
 - (b) T₁Vs. T₂
 - (c) $C_2Vs. T_3$ and T_4
 - (d) T_3 Vs. T_4
 - (e) C_3 Vs. T_5 and T_6
 - (f) T₅ Vs. T₆

(iii) C Vs T

Procurement and Preparation of feed samples

The above-mentioned feed ingredients were procured from cattle feed factory (COMFED) Patna. Feed ingredient used for compounded feed in mash form and pelleted form were taken at random from different shifts of cattle feed factory. Material were dried at 70° C in oven and finally grounded and sieved to pass through 850 μ sieve (IS: 78.74, 1975) at the feed processing section of the laboratory of cattle feed factory and packed in separate polythene bags which were labeled for identification. All the samples taken for study were processed in a similar fashion and observations were recorded.

Proximate analysis of feed samples

Proximate analysis of the feed samples under investigation were done as recommended by Bureau of Indian Standards (IS: 7874 Part I, 1975).

Physical Parameters

The following physical parameters of all the feeds were recorded

- (a) Colour
- (b) Odour
- (c) Form
- (d) Solubility in water for 20 minutes based on the usual practice of our livestock owner to make "Sani" of concentrate feeds.

'In vitro' estimation of Rumen Degradable Protein (RDP) and Undegradable Protein (UDP) using phosphate Buffer:

RDP and UDP *in-vitro* estimation of the above mentioned feed material were done as per the methods adopted by Mehta (1988).

Detailed procedures of RDP and UDP estimation by *invitro* method was as follows:

Samples of feed stuffs, dried at 70° C in oven and finally grounded were weighed accurately (1 g) was taken in 150 ml. Conical flask and 50 ml of phosphate buffer was added. Flasks were closed

with rubber cork. Water bath with device was started to bring the temperature to 39-40 degree centigrade. Flasks were placed in shaker holders of the water bath. Shaker was started maintaining 120 RPM by RPM regulator of the shaker.

The samples were run exactly for two hours. After completion of two hours samples were taken out from the water bath and kept for sometime to bring it to normal room temperature.

The samples were filtered using Whatman's filter paper no. 41. After completion of filtration, filtrate was mixed thoroughly and 10 ml was taken out and placed inside the Kjeldhal flask. Thereafter 2 grams of digestion mixture and 10 ml of H₂SO₄ (Conc.) was added in the Kjeldhal flask and digested till the colour becomes greenish. The samples were distilled after adding 50 ml. of 50% NaOH solution till the colour changed to deep blue.

The distillate was received in $2\%~H_3BO_3$ solution. After collecting distillate upto 100~ml., the material was titrated with 0.1~(N)~Hcl and burette reading was taken.

Preparation of Buffer Solution

- (a) 0.15 Molar NaH₂PO₄ (Sodium dihydrogen orthophosphate).
- (b) 0.15 Molar Na₂HPO₄ (Disodium hydrogen orthophosphate) 26.6988 gm./litre.

195 ml. of solution (a) and 305 ml. of solution (b) was taken and made upto the volume of one litre with distilled water working buffer solution had pH. 7.

Calculation

$$RDP = \frac{Burette \ reading \times Acid \ factor}{W \ eight \ of \ sample}$$

Multiplication with 5 was done since only 10 ml. of aliquote was used out of 50 ml.

In-vitro Pepsin Digestibility

Digestibility of protein in the intestine can be determined from the fraction of total nitrogen in the feed material that is potentially digestible by the action of proteolytic enzymes. The enzyme is active in the abomasum of ruminants at pH 2-3. It is believed that if the if the protein nitrogen is not released insoluble form after incubation with pepsin, it is not likely to be digested by the acid digestion process in the animal.

In vitro pepsin digestibility of the feeds under study was estimated as per the methods adopted by Mehta (1988). The details of the procedure of estimation is was follows:

Samples of feed stuff dried at 70°C in oven and finally ground was weighed accurately to 1 g., taken in 150 ml. conical flask and 50 ml. of pepsin – Hcl solution was added. Flask was closed with

rubber cork. Water bath shaker was started to bring the temperature to $39-40^{\circ}$ C. Flasks were placed in shaker holder. Shaker was started, maintaining 120 RPM by RPM regulator of the shaker.

The samples were run exactly for 2 hours. After completion of 2 hours samples were taken out from the water bath and kept for sometime to bring it to normal room temperature.

The material was filtered using Whatman's filter paper no. 41. After completion of filtration, filtrate was mixed thoroughly and 10 ml. was taken out of it and placed inside the Kjeldhal flask and then 2 gm. of digestion mixture and 10 ml. H_2SO_4 (Conc.) was added in the Kjeldhal flask and digested till the colour become greenish. The distillate was received in 2 per cent H_3BO_3 solution. After collecting the distillate upto 100 ml., the material was titrated with 0.1 (N) H_2SO_4 and burette reading was taken.

Preparation of Pepsin - HCl Solution

2 gm of pepsin (1: 10,000) was dissolved in 100 ml. of 0.1 Hcl and the volume was made to 1 litre with distilled water. The pH of solution was within 2-3.

Calculation

Pepsin solubility = $\frac{B urettereading X A cid factor X 5}{W eight of Samle}$

Multiplication with 5 was done since only 10 ml. of aliquate was used out of 50 ml.

% of Pepsin Digestibility =
$$\frac{\text{Pepsin solubility }\%}{\text{Total protein of feed stuffs}}$$

Statistical Analysis

Data obtained with respect to different parameters were subjected to statistical analysis, as per methods of Snedecor and Cochran (1967) using ANOVA for CRD and significant differences of obtained mean were determined using Duncan's multiple range test.

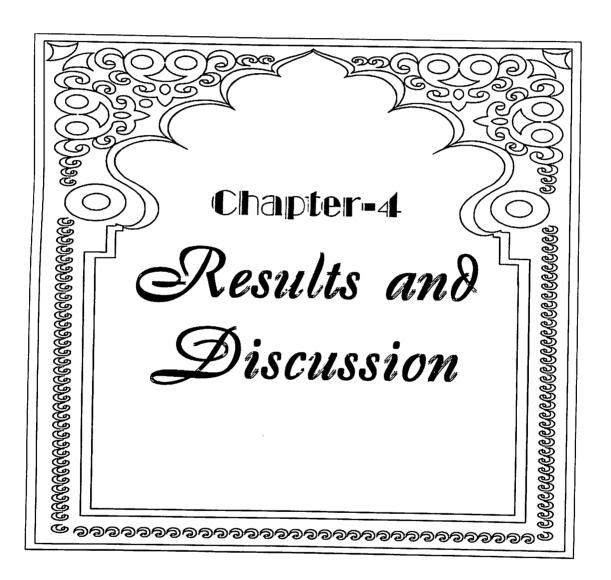
A brief account of the processing methods

In general there are three oil milling methods in the process of fat extraction of oil bearing seeds. The old ghani method popularly known as rural method of extraction which was used earlier to extract oil from oil seeds contained more amounts of oil content in residual cakes. In expeller process the seed after cracking and drying is heated in a steam jacketed apparatus and subsequently the mass is subjected to pressure in a screw press. A considerable amount of heat is generated from friction in this process. The residue is again ground into a meal. The method is an improvement over ghani method and more fat is extracted by this process but the residual cake still contain 7-8% of fat. Solvent extraction of oils is carried out in certain organic solvents. The comparative advantage of solvent extraction over the

mechanical process is that the recovery of oil is high and the cake produced is richer in nitrogen content. In this process the powdered material either in seed or cake form is brought in contact with solvent like chloroforms, hexane, CTC which dissolves the oil in it. The oil solvent mixture is separated from the cake either by gravity or by filtration and then heated to distill off the solvent. The recovered solvent is again used for dissolving further quantity of oil from fresh oil seed or cake. Solvent extraction can be classified into three distinct groups according to their mode of operation such as batch type, semicontinuous type and continuous type. The batch process is one in both oil extraction and solvent extraction is done intermittently in batches. In semi-continuous type the extraction of oil and circulation of solvent is continuous and automatic but recovery of solvent is done separately. The continuous method consists of both extraction and solvent recovery being carried out continuously and automatically. Taking into consideration the relative advantages of the three methods with regard to cost of working, solvent losses, time of operation etc., the continuous process is considered the most economical and efficient. However there are various objection raised against solvent extraction of oil seeds and oil cakes. Firstly it is said that the process extracts the oil so effectively that it renders the cake practically useless for cattle feeding. Some are of the view that the solvent extracted cake is better than the expeller pressed cake as it is

richer in protein. Investigations carried out at IVRI, Izathagar showed that the digestibility of ether extract and CP of the ration of bullock is increased in solvent extracted cake. The second objection is that it is very difficult to eliminate the last traces of solvent which makes both the oil and cake unfit for edible purposes. However, some oil plant manufactures claim that the oil and cake produced by their plant is absolutely solvent free and can be used for edible purposes. The third objection is to find a proper solvent possessing requisite specifications. At present there are nine solvent extraction plant working in the country carrying a total capacity of 390 tonnes of cake per day. Out of these four are of the continuous type one semicontinuous and rest of the batch type. Besides licenses have been granted for setting up of 22 more plants which will add to the present capacity by about 825 tonnes of cake a day.





RESULTS AND DISCUSSION

Proximate Composition of vegetable protein sources and processing effects on chemical composition

The proximate composition of three vegetable protein sources viz. mustard cake, sunflower cake and Groundnut cake and the effect of processing (Expeller and solvent extraction consisting of batch process and continuous process) on chemical composition on DM basis are presented in Table -1.

From the results of proximate composition it indicated that an increase in crude protein, crude fibre and ash percentage were observed when these vegetable protein sources were extracted by solvent extraction methods than expeller method of processing. A reduction in ether extract percentage was noted in solvent extraction with more reduction in continuous than batch process of solvent extraction. Such type of reduction in ether extract percentage and increment with a positive correlation between crude protein and crude fibre could be regarded as standard pattern, however there was no marked differences in CP % with respect to groundnut cake extracted by solvent extraction process either through batch processes or continuous processes. The figure obtained for different proximate composition for vegetable protein sources used in this study were within the normal range.

Table 1.: Proximate composition of unprocessed and processed vegetable protein sources used in the experiment (on DM basis)

Feed	Crude	Ether	Crude	Ash	Acid in-	Moisture
	protein	Extract	Fibre	%	soluble	
	- %	<u></u> %	%		Ash %	%
Mustard oil cake		<u> </u>	<u> </u>			
Expeller process	31.51	8.43	8.61	7.83	1.56	9.02
Solvent Extraction						
Mustard oil cake	34.44	1.49	10.90	7.94	3.01	8.41
Batch process						
Solvent Extraction						
Mustard oil cake	33.46	1.16	11.65	7.98	3.51	7.22
continuous process						
Sunflower cake						
Expeller process	24.45	12.42	18.86	6.70	1.66	9.97
Solvent extraction						
Sunflower cake	27.09	1.80	21.68	7.71	2.52	8.21
Batch process						
Solvent extraction			İ	İ		
Sunflower cake	27.58	1.72	23.71	7.92	2.82	7.85
continuous process						
Groundnut cake				İ		
Expeller process	38.34	8.56	9.24	6.97	2.51	9.56
Solvent extraction						
Groundnut cake	43.93	0.94	14.44	7.53	3.02	8.37
Batch process						
Solvent extraction						
Groundnut cake	43.93	0.73	15.52	8.36	3.25	8.04
continuous process						
Unpelleted						
concentrate	24.32	2.51	7.86	8.57	3.01	9.51
mixture						
Pelleted						
concentrate	24.54	2.81	7.02	8.10	2.75	10.47
mixture						

The effect of different processing methods on crude protein content of mustard oil cake, sunflower cake and GN cake and their analysis of variance (mean value converted to arc sin percent) are presented in Table -2 and Table -3.

Results indicated a significant (P<0.05) effect of processing methods used in the extraction of different vegetable protein sources. There was a significant (P<0.05) increase in crude protein content in solvent extraction process as compared to expeller process. Between batch and continuous processes in solvent extraction a significant increase in crude protein content was observed in continuous process with respect to Mustard oil cake and sunflower cake but there was no significant (P<0.05) differences in crude protein content of groundnut cake, extracted either through batch or continuous processes. The mean value of CP content in expeller, solvent extraction in batch and in continuous process corresponding to arc sin percentage were 31.51, 34.44 and 33.47 for mustard cake; 24.45, 27.09 and 27.58 for sunflower cake and 38.34, 43.93 and 43.93 for groundnut cake, respectively. Among vegetable protein sources groundnut cake showed highest protein content followed by mustard oil cake and sunflower cake. The variability in the crude protein content for all vegetable protein sources and the pattern of result obtained in this study were also reported by several

Mean value showing the effect of different processing methods of vegetable protein supplement on crude protein content along with their S.E. and CV%. Table 2.

	Mustard oil cake	ake	Sunflower cake	ake	Ground	Groundnut cake	$\overline{}$
	Mean ± S.E.	CV %	Mean ± S.E.	CV %	Mean + S.E.	% Ai)	
Expeller process	$34.15^a \pm 0.03$	0.25	29.63 ª ± 0.02	0.15	38 31 8 + 0 04	5 . 6	
	(31.51)		(24.45)		(38.34)	0.40	
			(01				
Solvent Extraction	$35.94^{\text{ b}} \pm 0.02$	0.14	$31.37^{\mathrm{b}} \pm 0.03$	0.26	$41.52^{b} \pm 0.05$	0.28	т —
"Batch process	(34.44)		(27.09)		(43.93))	
Solvent Extraction	$35.34^{\circ} \pm 0.01$	0.08	31.68° ± 0.02	0.15	41.51 ^b + 0.09	0.14	_,_
"Continuous, process	(33.47)		(27.58)		(43.93)	# 1.0	
							_

Means with different superscript differ significantly (P<0.05) in a column; Figure in parenthesis indicate average percentage corresponding to Arc Sin V percentage given by C.I. BLISS.

Fig. 1.: Histogram showing mean value showing the effect of different processing methods of vegetable protein supplement on crude protein

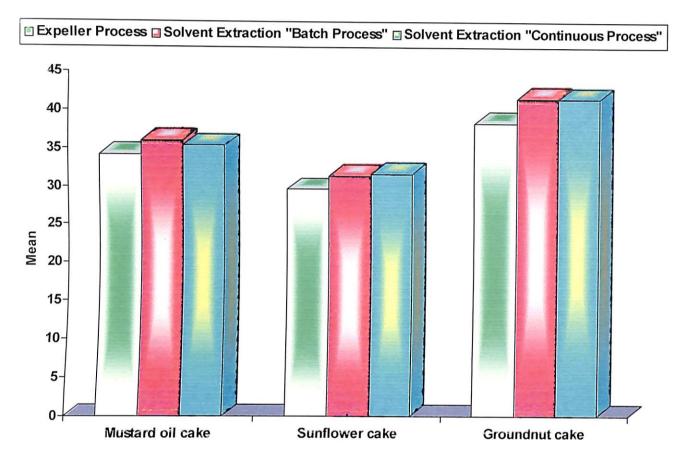


Table 3.: Analysis of variance showing the effect of different processing methods of vegetable protein supplement an crude protein content.

Source of Variation	d.f.	Mustard oil cake	Sunflower cake	Groundnut cake
Between processes	2	4.17**	6.08**	17.204**
Error	12	0.003	0.004	0.016

** P < 0.05

workers (Reddy and Vaidya 1973, Sadagopan et al., 1982b; Banerjee et al., 1975; Dutta and Singh, 1994; Kuldip et al., 1995; Katoch 1996). All these workers recorded a higher crude protein content in solvent extraction than expeller method of processing with some variability. The variability were attributed to different factors such as fertilizer application and agroclimate conditions which directly or indirectly affect the nutrient content of vegetable protein sources but there was no difference in opinion that a reduction in oil content through solvent extraction would significantly (P<0.05) increase the crude protein content. The observed values of solvent extracted mustard oil cake as reported by Banerjee et al. (1975), Chand et al. (1980), Sahoo (1992) and Dutta and Singh (1994) and for solvent extraction groundnut cake as reported by Chopra et al. (1971), Reddy and Vaidya (1973) also corroborated these findings.

Effect of processing on degradability of crude protein content of vegetable protein sources

The mean value of RDP in % and the value of RDP as % of CP of vegetable protein sources extracted under different processing methods along with their analysis of variance are presented in Table -4, Table -5 and Table -6, Table -7.

The mean RDP value of mustard oil cake in expeller, solvent extraction in batch and continuous processes were

Table 4.: Mean value showing the effect of different processing methods of vegetable protein supplement on RDP content along with their S.E. and CV %

	Mustard oil cake	il cake	Sunflower cake	cake	Groundnut cake	ut cake
	Mean ± S.E	% AO	Mean ± S.E	% AO	Mean ± S.E	% AO
Expeller process	$24.27^{8} \pm 0.07$	0.70	$20.17^{a} \pm 0.15$	1.68	$29.24^{a} \pm 0.03$	0.24
	(16.90)		(11.87)		(23.87)	
Solvent Extraction	$23.25^{b} \pm 0.13$	1.31	18.83 b ± 0.08	0.99	30.93 a ± 0.11	0.82
" Batch Process"	(15.59)		(10.38)		(26.42)	
Solvent Extraction	$17.61^{c} \pm 0.17$	2.16	14.28 ° ± 0.24	3.76	26.92 ^b ± 0.12	1.03
"Continuous Process"	(9.16)		(5.93)		(20.48)	

Means with different superscript differ significantly (P<0.05) in a column Figure in parenthesis indicate average percentage corresponding to Arc Sin / percentage given by C.I. BLISS.

Fig. 2.: Mean value showing the effect of different processing methods of vegetable protein supplement on RDP

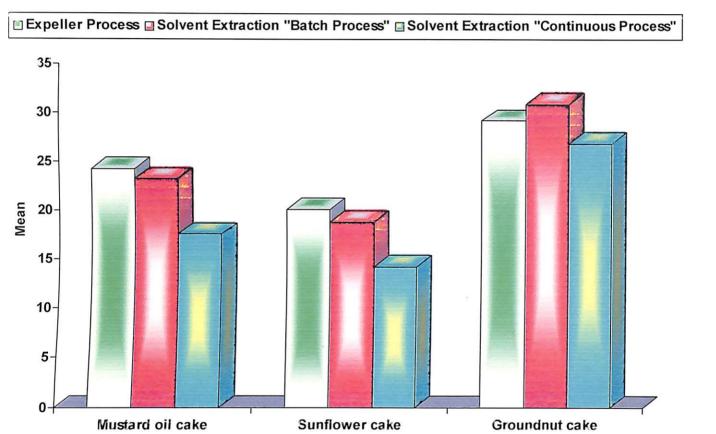


Table 5.: Analysis of variance showing the effect of different processing methods of vegetable protein supplement on RDP.

Source of Variation	d.f.	Mustard oil cake	Sunflower cake	Groundnut cake
Between Processes	2	64.30**	47.53**	20.31**
Error	12	0.08	0.14	0.04

** P < 0.05

Table 6.: Mean value showing the effect of different processing methods of vegetable protein supplement on RDP as % of CP along with their S.E and CV%.

Groundnut cake	% AO	0.16		0.71		1.19	
Ground	Mean ± S.E	52.09 a ± 0.03	(62.26)	50.32 " ± 0.26	(60.14)	43.06 ^b ± 0.22	(46.62)
Sunflower cake	% AO	2.01		0.68		3.43	
Sunflow	Mean ± S.E	44.03 a ± 0.39	(48.56)	38.23 ^b ± 0.11	(38.30)	27.64 ° ± 0.42	(21.51)
cake	% AO	0.72		1.47		2.25	
Mustard oil cake	Mean ± S.E	$47.08^{8} \pm 0.15$	(53.62)	$42.28^{\text{b}} \pm 0.27$	(45.26)	$31.53^{\circ} \pm 0.37$	(27.34)
		Expeller process		Solvent Extraction "	Batch Process"	Solvent Extraction	"Continuous Process"

Means with different superscript differ significantly (P<0.05) in a column Figure in parenthesis indicate average percentage corresponding to Arc Sin / percentage given by C.I. BLISS.

Fig. 3.: Mean value showing the effect of different processing methods of vegetable protein supplement on RDP as % of CP

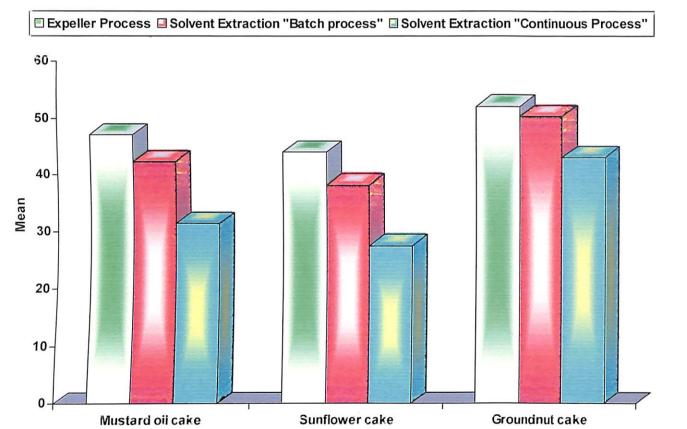


Table 7.: Analysis of variance showing the effect of different processing methods of vegetable protein supplement on RDP as % Cp.

	 _	-г
Groundnut cake	120.59**	0.13
Sunflower cake	351.04**	0.58
Mustard oil cake	316.94**	0.33
d.f.	2	12
Source of Variation	Between Processes	Error

 $^{**}P < 0.05$

16.90, 15.59 and 9.60 percent respectively. A significant effect (P<0.05) in the reduction of RDP value was seen in solvent extraction method and the reduction in RDP value was found to be almost half in continuous process of solvent extraction than from expeller process. The reduction of RDP value in continuous process was significantly (P < 0.05) less than batch process of solvent extraction processing. The mean value of RDP in sunflower cake were 11.87, 10.38 and 5.93 for expeller process, solvent extraction in batch process and solvent extraction continuous process respectively. Processing significantly (P<0.05) affected the RDP value and a significant (P<0.05) reduction in RDP value was noted in respective extraction methods. The value was significantly (P<0.05) lowest in continuous process than batch and expeller processes. A similar reduction in RDP percentage was also observed in groundnut cake processed by solvent extraction continuous method than expeller method, but the RDP value (26.42) in batch process was found to be somewhat higher than expeller and continuous process. The RDP value for GNC were 23.87, 26.42 and 20.48 processed under expeller, solvent extraction in batch and continuous process respectively.

Results indicated a lower RDP value in sunflower cake than mustard oil cake and groundnut cake, irrespective of the methods of processing used. The RDP value expressed as % of CP showed a similar trend for processing method for each vegetable protein sources as was observed earlier. Sunflower cake showed the lowest RDP value as % of CP followed by MOC and GNC. The highest RDP value (26.42) in GNC extracted in batch process was compensated when RDP value expressed as % of CP and the value was found to be lower than expeller process. Groundnut cake showed highest RDP values which might be due to solubility effect of protein source. The percent of RDP value as obtained in this study was in agreement with the values reported by Kunju et al. (1992) and the observed value was in accordance with the values used by NDDB for feed formulations. However, Krishnamoorthy et al. (1980), Negi et al. (1989) Mehra et al. (1998), and Kumar (1996) obtained a lower value than the present value which might be due to difference in methods of estimation adopted.

The RDP values obtained for SFC agreed well with the values reported by Rao and Prasad (1989) for solvent extracted- SFC.

A slight different values were given by Veresegyhazy et al. (1989), Kuldip et al. (1995) and Prabhu et al. (1996). The values obtained for GNC were within the range of values as obtained by Sampath and Sivaraman (1985) and Lohan et al. (1984), however Manget Ram and Gupta (1994) reported a higher value for GNC

extractions. The higher value in batch process than continuous process for GNC could not be substantiated for want of evidence.

The mean value of UDP in percentage and the value of UDP as % of CP of vegetable protein sources extracted under different processing methods along with their analysis of variance are presented in Table -8, Table -9 and Table - 10, Table -11.

The mean UDP value for mustard oil cake in expeller, solvent extraction in batch and continuous processes were 14.60, 18.77 and 24.26 per cent respectively. A significant effect (p<0.05) in increase of UDP was seen in solvent extraction method and the increase in UDP value was found to be almost to half in expeller method than continuous process of solvent extraction. The lowest UDP value was obtained in expeller and highest in continuous followed by batch process. The increase in continuous process was significantly (p<0.05) more than batch process of solvent extraction processing. The mean value of UDP in sunflower cake were 12.57, 16.72 and 21.64 for expeller process, solvent extraction in batch process and solvent extraction in continuous process, respectively. Processing significantly (p<0.05) affected the UDP value and a significant increase (p<0.05) in UDP value was noted in solvent extraction methods. The value was significantly highest (p<0.05) in continuous process than batch process and expeller process and the

Table 8.: Mean value showing of the effect of different processing methods of vegetable protein supplement on UDP content along with their S.E and CV %

	Mustarc	Mustard oil cake	Sunflower cake	ır cake	Ground	Groundnut cake
	Mean ± S.E	% AO	Mean ± S.E	% AO	Mean ± S.E	CV %
Expeller process	22.46° ± 0.05	0.57	20.73 a ± 0.15	1.62	22.34 ª ± 0.04	0.44
	(14.60)		(12.57)		(14.46)	
Solvent Extraction	25.69 ^b ± 0.10	0.90	$24.12^{b} \pm 0.02$	0.23	24.73 b ± 0.06	0.57
" Batch Process"	(18.77)		(16.72)		(17.51)	
Solvent Extraction	29.50° ± 0.32	0.77	27.72° ± 0.09	0.79	28.94° ± 0.12	96:0
"Continuous Process"	(24.26)		(21.64)		(23.42)	

Means with different superscript differ significantly (P<0.05) in a column Figure in parenthesis indicate average percentage corresponding to Arc Sin / percentage given by C.I. BLISS

Fig. 4.: Mean value showing of the effect of different processing methods of vegetable protein supplement on UDP

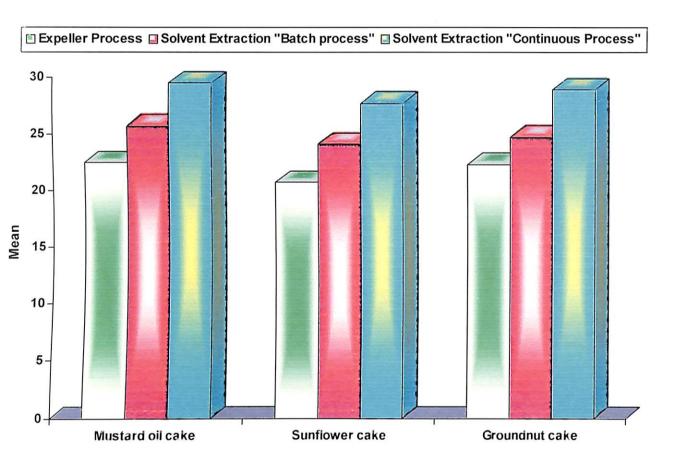


Table 9.: Analysis of variance showing the effect of different processing methods of vegetable protein supplements on UDP content.

Groundnut cake	55.71**	0.03
Sunflower cake	61.04**	0.05
Mustard oil cake	62.12**	0.04
d.f.	2	12
Source of Variation	Between Processes	Error

** P <0.05

Table 10.: Mean value showing of the effect of different processing methods of vegetable protein supplement on UDP as % of CP along with their S.E and CV %

	Mustard oil cake		Sunflower cake		Groundnut cake	
	Mean ± S.E	CV %	Mean ± S.E	CV %	Mean ± S.E	CV %
Expeller process	$42.89^a \pm 0.14$	0.75	$45.83^{a} \pm 0.39$	1.93	$37.89^{a} \pm 0.03$	0.23
	(46.34)		(51.43)		(37.70)	
Solvent Extraction	$47.57^{\rm b} \pm 0.27$	1.27	51.71 ^b ± 0.16	0.69	39.14 ^b ± 0.16	0.92
" Batch Process"	(54.49)		(61.68)		(39.85)	
Solvent Extraction	$58.37^{c} \pm 0.31$	1.19	$62.36^{\text{ c}} \pm 0.41$	1.48	$46.88^{\text{ c}} \pm 0.43$	2.09
"Continuous Process"	(72.48)		(78.47)		(53.29)	

Means with different superscript differ significantly (P < 0.05) in a column Figure in parenthesis indicate average percentage corresponding to Arc Sin $\sqrt{\text{percentage}}$ given by C.I. BLISS.

Fig. 5.: Mean value showing the effect of different processing methods of vegetable protein supplement on UDP as % of CP.

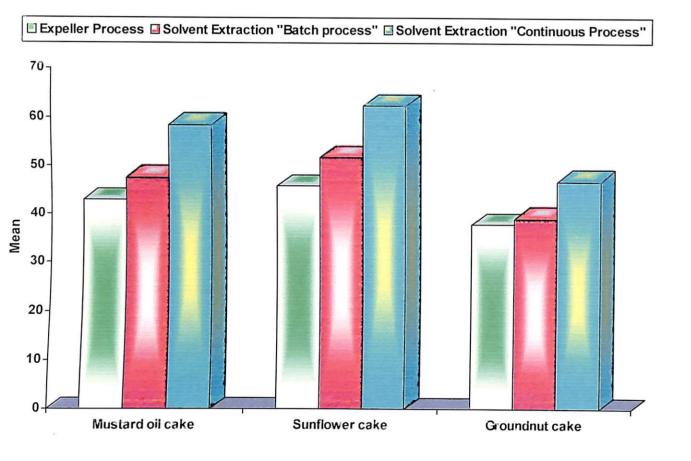


Table 11.: Analysis of variance showing the effect of different processing methods of vegetable protein

supplements on UDP as % of CP.

_			
	Groundnut cake	118.56**	0.36
	Sunflower cake	351.19**	0.59
	Mustard oil cake	315.17**	0.32
0 -	d.I.	2	12
Some of Vonice	Source of Variation	Between Processes	Error

 $^* P < 0.05$

latter was significantly different from the former. A similar increase in UDP percentage was also observed in groundnut cake processed by solvent extraction continuous method than solvent extraction batch method and expeller method. The UDP value for GNC were 14.46, 17.51 and 23.42 processed under expeller, solvent extraction in batch and continuous process respectively.

Results indicated higher UDP value in mustard oil cake than sunflower cake and groundnut cake irrespective of the method of processing used. When UDP value expressed as % of CP it showed a significantly (p<0.05) higher value for sunflower cake irrespective of the method of processing used followed by MOC and GNC.

The observed value for RDP and UDP showed that in MOC among the methods used for extraction, continuous process had the lowest RDP % followed by batch process of solvent extraction while expeller process had highest RDP value. A reverse trend was observed in case of UDP in which continuous process of solvent extraction had the highest UDP value. The same trend of results were obtained in SFC and GNC. The variability in results of processing methods could be attributed to the difference in processing methodology as pointed out by some workers that the degree of protein degradability could be induced by toasting in the processes involved. The ideal temperature for improving rumen bypassibility is

to be around 120 $^{\rm o}$ C, however the solvent extraction industry employs toasting of solvent extracted material at around 95-105 ° C for removing the solvent from the extracted residue. The superior effect of continuous process as compared to batch process of solvent extraction might be due to maintaining uniform conditions of heat. In batch method the material usually in the form of cake is taken in a steam jacketed vat and is submerged in the solvent (usually Hexane). Even after draining out the solvent along with the fat the residue remains still soaked with solvent and in order to remove excess solvent the residue is heated by applying surface heat exchange technique. On the other hand in continuous process the material in fragmented form passes continuously through the solvent and then it enters the toaster in which the material is exposed to uniform heat being of a cost effective method it besides also destrovs antinutritional factors present in material making feed more useful.

Many workers suggested that a reduction in protein degradability of solvent extraction cake might be attributed to the application of heat during solvent extraction (Waldo et al., 1979; Prabhu et al., 1996). Mercer and Annison (1976) also indicated that processing techniques involved during oil extraction influenced the protein degradability. Nature of protein in different protein sources also affect the degradability value of protein. The observed differences

between the value of present study and values reported by some workers could also be due to feed protein structure and feed solvent interaction (Singh et al., 1995), processing effect or length of extraction (White et al., 1968; Wohit et al., 1973; Crooker et al.; 1978; Kumar and Walli, 1994 and Singh et al., 1995). It has also been reported that different fraction of feed have different solubility in different solvent (Blethen et al., 1990) and the differences in solubility ultimately affected the nitrogen solubility of cakes (Sood and Walli, 1986).

Results indicated that sources of protein had an influence on the degradability of protein in the rumen. Among the three vegetable protein sources studied the crude protein of sunflower cake showed least degradability and a higher percentage of by-pass protein. Protein from GNC was found to be more degradable than MOC. Results obtained in the study can be exploited in the formulation of rations of high yielding dairy cows with respect to method of extraction. Solvent extraction by continuous process seemed to be a better method in reducing the degradability of protein in the rumen.

Pepsin Digestibility

The mean value of pepsin digestibility in percentage of vegetable protein sources as affected by processing methods and their analysis of variance are presented in Table -12 and table -13.

Table 12. : Mean value showing the effect of different processing methods of vegetable protein supplement on Pepsin digestibility along with their S.E. and CV%.

ıt cake	CV %	0.16	•	0.16		0.35	
Groundnut cake	Mean ± S.E	$69.81^{a} \pm 0.04$	(88.09)	70.39 b ± 0.05	(88.73)	71.20° ± 0.11	(89.56)
cake	% AO	0.22		0.05		60.0	
Sunflower cake	Mean ± S.E	$56.40^{\mathrm{a}} \pm 0.11$	(69.38)	$58.30^{\mathrm{b}} \pm 0.01$	(72.39)	59.02° ± 0.02	(73.50)
l cake	% AO	0.23		0.71		0.09	
Mustard oil cake	Mean ± S.E	$64.80^a \pm 0.06$	(81.87)	$65.44^{b} \pm 0.20$	(82.73)	$66.31^{\circ} \pm 0.02$	(83.84)
		Expeller process		Solvent Extraction	" Batch Process"	Solvent Extraction	"Continuous Process"

Means with different superscript differ significantly (p<0.05) in a column Figure in parenthesis indicate average percentage corresponding to Arc Sin / percentage given by C.I. BLISS.

Fig. 6.: Mean value showing the effect of different processing methods of vegetable protein supplement on Pepsin digestibility.

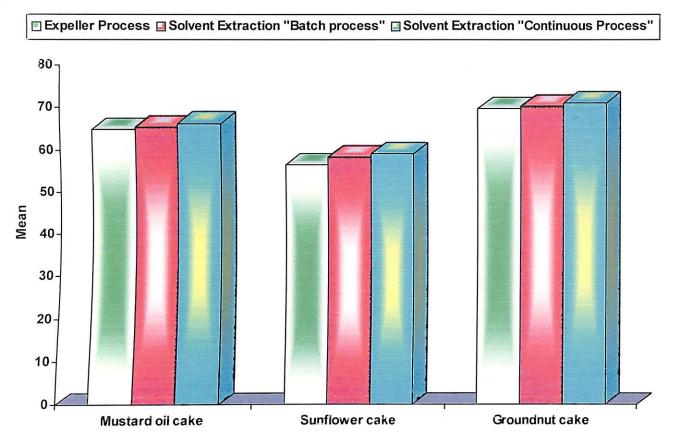


Table 13.: Analysis of variance showing the effect of different processing method of vegetable protein supplement on pepsin digestibility.

	GNC		1.31**		0.02	
	SFC		9.20^{**}		0.006	
	MOC		2.88**		0.08	
1	d.f.		72		12	
Source of Variation		D.±	Detween Frocesses	F	Error	

** P < 0.05

In MOC the % pepsin digestibility ranging from 81.9 to 83.8 was significantly (P<0.05) affected by processing methods. The highest digestibility was obtained in continuous process of solvent extraction and then in batch process and lowest in expeller process. The same trend in pepsin digestibility % was also obtained in sunflower and GNC. However in SFC there was marked improvement in digestibility in solvent extraction processed cake and there were not much differences between batch and continuous process, though these values were significantly (P<0.05) different. The values of pepsin digestibility of sunflower cake for expeller, solvent extraction in batch and continuous process were 69.38, 72.39 and 73.50 respectively. Processing methods though produced significant (P<0.05) effect in pepsin digestibility of GNC but the values were not much different in different processing methods. It ranged from 88.09 to 89.56 %. The values obtained for pepsin digestibility could be considered the crude protein digestibility of the protein source and has been shown a positive correlation between these two. Solubility of the protein might be another factor for increasing the pepsin digestibility of protein source. In the present study GNC showed highest % of pepsin digestibility followed by MOC and SFC and this finding testified the fact that protein of GNC is more soluble than MOC and SFC.

Some workers found that pepsin digestibility of untreated or heat treated sunflower meal was same (Veresegyhazy et al., 1989).

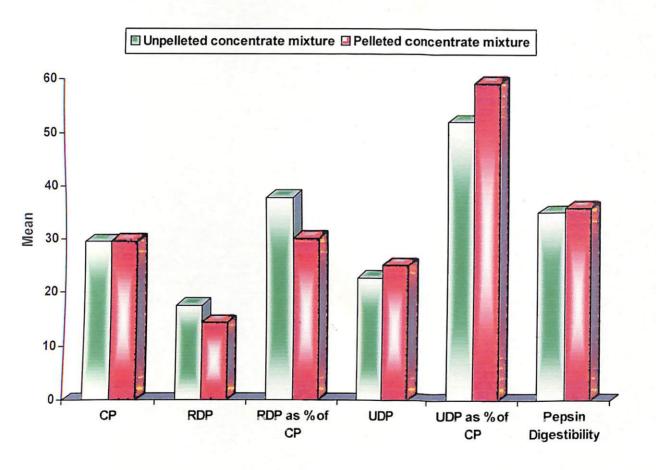
Pelleting

The mean value of unpelleted and pelleted concentrate mixture for CP, RDP and UDP and both as % of CP along with pepsin digestibility and their analysis of variance are presented in **Table -14** and **Table -15**.

The values for different parameters was significantly affected by pelleting of the concentrate mixture. An increase in crude protein, UDP and pepsin digestibility was observed by pelleting of concentrate mixture. The degradability of protein in rumen was significantly (P<0.05) reduced by pelleting and the values were reduced to 6.26 from 9.21 in unpelleted concentrate mixture. The mean values for undegradable protein was 15.19 and 18.19 and as % of crude protein the values were 62.47 and 74.13 for unpelleted and pelleted concentrate mixture respectively. Pelleting of concentrate mixture significantly (p<0.05) increased pepsin digestibility and value was increased to 34.68 from 33.23 %.

Results indicated that bypass protein could be increased through pelleting of the concentrate mixture as obtained in this study. A simultaneous increase in pepsin digestibility by pelleting of concentrate mixture also showed beneficial effect. The higher UDP value and pepsin digestibility value might be attributed to the steam pelleting effect in pellet making process of the concentrate mixture.

Fig. 7.: Mean value showing the effect of Pelleting of concentrate mixture on CP, RDP, RDP as % of CP, UDP, UDP as % of CP and pepsin digestibility



CP, UDP, UDP as % of CP and pepsin digestibility along with their S.E and CV%.

	Unpelleted concentrate mixture	ate mixture	Pelleted concentrate mixture	trate mixture
	Mean ± S.E	% AO	Mean ± S.E	% AO
CP	$29.55^a \pm 0.03$	0.37	$29.70^{\mathrm{b}} \pm 0.02$	0.25
	(24.32)		(24.54)	
RDP	$17.67^{a} \pm 0.11$	1.90	$14.44^{b} \pm 0.12$	2.52
	(9.21)		(6.26)	
RDP as % of CP	$38.00^{\text{ a}} \pm 0.27$	2.16	$30.32^{\mathrm{b}} \pm 0.22$	2.25
	(37.89)		(25.50)	
UDP	$22.85^{a} \pm 0.13$	1.72	$25.25^{\mathrm{b}} \pm 0.07$	0.87
	(15.19)		(18.19)	
UDP as % of CP	$52.23^{a} \pm 0.23$	1.35	$59.45^{b} \pm 0.25$	1.27
	(62.47)			
Pepsin	$35.20^{\text{ a}} \pm 0.02$	0.22	$36.08^{\mathrm{b}} \pm 0.02$	0.23
Digestibility	(33.23)		(34.68)	

Means with different superscript differ significantly (p<0.05) in a column Figure in parenthesis indicate average percentage corresponding to Arc Sin percentage given by C.I. BLISS.

Table 15.: Analysis of Variance showing the effect of Pelleting of concentrate mixture on CP, RDP, RDP as % CP, UDP, UDP as % CP and pepsin digestibility.

	Pepsin	Digestibility	3.46**	0.007
	UDP as % of CP		234.05	0.54
Mean Square	UDP		25.97**	0.09
Mea	RDP as % CP		264.89**	0.57
	RDP		46.96	0.12
	CP		0.094**	0.009
d.f.			-	16
Source of Variation			Between process	Error

** P<0.05.

Such beneficial effects of pelleting have also been recorded by Reddy and Reddy (1983), Hazlett *et al.* (1960), Klosterman *et al.* (1960) and Khajuria and Mudgal (1975), Morel (1997), Nelson *et al.* (1968) cow sert and Reddy and Reddy (1979).

Physical Parameters

The physical parameters of vegetable protein sources processed under different methods are presented in **Table -16**.

No appreciable effect of processing was seen in colour of vegetable protein sources processed under different processes except a slight changes of colour in continuous process. SFC under expeller process had sweet smell and become odourless in solvent extraction processes. No marked changes was noticed in mustard and groundnut cake. Results of solubility in plain water with maximum limit of time allotted for 20 minutes showed that SFC processed under expeller method took minimum time of 1 minute which was increased to 2 minutes to 3 minutes in batch and continuous process of solvent extraction to become soft. A comparatively higher time was noticed in MOC processed under expeller process and took 9 minutes time to become soft as compared to GNC and SFC. However, adoption of same method of processing for both MOC and GNC processed under solvent extraction method took comparatively more time than expeller or any of the methods used for SFC. GNC when processed by solvent extraction took 5 to 6 times more time to become soft as compared to expeller method.

Table 16.: Physical Parameters of Feeds.

Feed	Colour	Odour	Consis- tency	Form	Solubility in plain water in 20 minutes
Mustard oil cake Expeller process	Charcoal Black	Pungent	Solid	Cake	Become soft in 9 minutes
Mustard oil cake solvent Extraction Batch process	off white	Pungent	Solid	Cake	Become soft in 10 minutes
Mustard oil cake solvent extraction continuous process	Deep Brown	Pungent	Solid	Chips	Become soft in 18 minutes
Sunflower cake Expeller process	Grey Black	Sweet Smell	Solid	Cake	Becomes soft in 1 minutes
Sunflower cake solvent extraction Batch process	Grey Black	Odour less	Solid (softer side)	Cake	Become soft in 2 minutes
Sunflower cake Solvent extraction Coatinuous process	Grey Black	Odour- less	Solid	Form Chips	Become Saft in 3 minutes
Groundnut cake Expeller process	Brown	Sweet Smell	Solid	Cake	Become soft in 2 minutes
Groundnut cake solvent extraction Batch process	Brown	Smell of nut	Solid	Cake	Becomes soft in 10 minutes
Groundnut cake Solvent extraction continuous process	Deep Brown	Smell of nut	Solid	Chips	Becomes soft in 19 minutes
Unpelleted concentrate mixture	Light Brown	Sweet smell	Solid	Mash	Becomes soft in 3 minutes
pelleted concentrate mixture	Deep Brown	Sweet Smell	Solid	Pellet	Become soft in 8 minutes

Pelleting of concentrate mixture took more time (8 minutes) to become soft as compared to unpelleted concentrate mixture which become soft in 3 minutes. In general processing of cake under solvent extraction processing method and pelleting of concentrate mixtures increased the solubility time in water and will provide an idea for including these items in the ration of farm animals.





SUMMARY AND CONCLUSION

About two third of the crude protein supply to the ruminants is derived from the basal forages and energy feed stuffs (cereals, cereal milling by products etc) and the remaining from vegetable protein Supplements. Protein reaching to the rumen suffers microbial intervention, of which some are degraded and other escape from the rumen, intact. The bacterial conversions in the rumen of protein results on the one hand, in a considerable degradation of dietary protein and on the other hand, in synthesis of bacterial protein. As a result, the animal receives two sources of protein in the intestine to meet its requirement for aminoacids that is the undegraded dietary protein and the single cell protein synthesised in the rumen. In order to evaluate the protein supply the amount of bacterial protein synthesis and the degradability of the dietary protein will have to be known. Published work indicate that bacterial protein can supply only 55-65% of the requirement in high yielding dairy cows when given sufficient feed to meet energy needs. This problem can be overcome by feeding proteins of low degradability values to dairy cows. The use of such low degradable protein improves the amino acid supply to the animal and simultaneously decreases the surplus ammonia, reduces stress on liver metabolism and hence

improves conditions of good fertility. Similarly the use of protein with low degradability can be expected to improve performance in growing animals especially during the starting phase when there is relatively a high rate of protein deposition and protein requirements are high.

At present in India the protein requirements and evaluation of protein in feeds is worked out on the basis of apparent digestibility (DCP). This system is found inadequate due to

- (a) extensive degradation of dietary protein in rumen.
- (b) The faecal nitrogen used for the estimation of DCP consisting largely of undigested microbial protein from rumen.
- (c) Insufficient appreciation of the fact that a balanced rumen fermentation is dependent on the relative quantities of degraded N and that readily fermentable carbohydrates in the rumen is essential for maximum voluntary intake of food and efficiency of feed utilization.

ARC (1980, 1984) proposed a new system of expressing the protein requirement of ruminants in terms of rumen degradable protein (RDP) and rumen undegradable protein (UDP). The extent to which a protein source is degraded in rumen has a marked influence on the manner in which it is utilized by the ruminants. The RDP fraction is converted largely to ammonia, fatty acids and CO₂ in the rumen and depending upon the concentration of NH₃, a part of

ammonia is being used to cater the nutrient need of rumen microbes through microbial protein synthesis. The protein which escapes from rumen undegraded becomes available for intestinal digestion and absorption in a similar way as in ruminant. Various factors influences the degradability of protein in the rumen, among them solubility of protein is one of the major factor. Therefore, the availability of protein in feed materials requires to be estimated from the fraction of total nitrogen that is soluble in various solvents. Usually the oilseeds are extracted by expeller process but with the adoption of solvent extraction method the residual oil seed cake contains as little as 0.5% of oil only as compared to the oil seed cake obtained from expeller method which had about 7 - 8% oil. This solvent extraction method has an added advantage that it increase CP content of the residual cake. This process comprises of two different methods i.e. solvent extraction through batch and/or continuous process. Not much is known about the rumen degradable fraction (RDP) and undegradable fraction (UDP) and its subsequent digestion, post ruminally of protein meal by these two different processing methods. Now a days, different feed factories are providing compounded feed in pelleted form for the livestock as balanced concentrate mixture. Whether pelleting of such compounded concentrate mixture has any beneficial effect in the degradability of protein needs to be explored. At present no adequate information is available about the influence of pelleting feed

ingredients on the level of undegradable protein (UDP) and pepsin digestibility.

Thus, the present investigation was undertaken to determine the effect of toasting in solvent extraction by batch and/or continuous process as compared to the expeller processed cake of different protein supplements on protein degradability and pepsin digestibility and also to determine the bypassibility and pepsin digestibility of the compounded concentrate mixture in pelleted form and to asses its nutritional status over unpelleted form. For this, three vegetable protein supplements viz. Mustard oil cake, Sunflower cake and Groundnut cake, processed under different methods were taken to study about their proximate composition, protein degradability and pepsin digestibility. Compounded feed for dairy cattle prepared as per formula developed in cattle feed factory was also studied for its protein degradability and pepsin digestibility by in vitro estimations. Physical parameters related to colour, odour, form and solubility in water for 20 minutes for all the feeds were recorded. Results indicated a significant (P<0.05) effect of processing method used in the extraction of different vegetable protein source. There was a significant (P<0.05) increase in crude protein content in solvent extraction process as compared to expeller process. Between batch and continuous process in solvent extraction a significant

(P<0.05) increase in crude protein content was observed in continuous process with respect to mustard oil cake and sunflower cake while there was no significant (P<0.05) differences in crude protein content of groundnut cake, extracted either through batch or continuous processes. The mean value of crude protein content in expeller, solvent extraction in batch and in continuous process were 34.15, 35.94 and 35.34 for mustard cake, 29.63, 31.37 and 31.68 for sunflower cake and 38.31, 41.52 and 41.51 for groundnut cake, respectively. Analytical results indicated that among vegetable protein sources, groundnut cake showed highest protein content followed by mustard oil cake and sunflower cake.

The mean RDP value of mustard oil cake in expeller, solvent extraction in batch and continuous processes were 16.90, 15.59 and 9.60 percent, respectively. Statistical analysis of data revealed a significant effect (P<0.05) in the reduction of RDP value in solvent extraction method and the reduction in RDP value was found to be almost half in continuous process of solvent extraction than expeller process cake. Result of RDP value in continuous process was found to be significantly (P<0.05) less than batch processed solvent extraction. The mean value of RDP in sunflower cake were 11.87, 10.38 and 5.93 for expeller, solvent extraction in batch and solvent extraction in continuous process, respectively. Statistical analysis

revealed that processing significantly (P<0.05) affected the RDP value and a significant (P<0.05) reduction in RDP value were seen in respective extraction methods. However, in G.N. cake the value was significantly (P<0.05) lowest in continuous process than batch and expeller processes. The RDP value for GNC were 23.87, 26.42 and 20.48, processed under expeller, solvent extraction in batch and continuous process, respectively. Though RDP percentage in G.N. cake was noted to be lowest, processed by solvent extraction continuous method, but the RDP value (26.42) in batch process was found to be somewhat higher than expeller and continuous process.

Results further indicated a lower RDP value in SFC than MOC and GNC, irrespective of the methods of processing used. The RDP value expressed as % of CP showed a similar trend for processing method for each vegetable protein sources. Sunflower cake showed the lowest RDP values as % of CP followed by mustard oil cake and GNC. A reverse trend in UDP values were obtained, the mean UDP value for mustard oil cake in expeller, solvent extraction in batch and continuous processes were 14.60, 18.77 and 24.26 per cent, respectively. Statistical analysis showed a significant effect (P < 0.05) in increase of UDP in solvent extraction method and the increase in UDP value was found to be almost to half in expeller method than continuous process of solvent extraction. The lowest

UDP value was obtained in expeller and highest in continuous followed by batch process. Data further indicated that the increase of UDP value in continuous process was significantly (P<0.05) more than batch process of solvent extraction processing. A similar trend in UDP value in SFC was observed and value of UDP in sunflower cake were 12.57, 16.72 and 21.64 far expeller process solvent extraction in batch process and solvent extraction in continuous process respectively. The value was significantly highest (P<0.05) in continuous process than batch process and expeller process.

The UDP values for GNC were 14.46, 17.51 and 23.42 processed under expeller, solvent extraction in batch and continuous process respectively. Contrary to higher % of RDP in batch process than expeller, the UDP percentage was found to be higher in batch than expeller processed groundnut cake. Results indicated a higher apparent UDP value in mustard oil cake than sunflower cake and groundnut cake irrespective of the method of processing used but when UDP value expressed as % of CP it showed a significantly (P<0.05) higher value for sunflower cake irrespective of the method of processing used followed by MOC and GNC.

The observed value for RDP and UDP showed that in MOC among the methods used for extraction, continuous process had lowest RDP % followed by batch process of solvent extraction while

expeller process had highest RDP value. A reverse trend was observed in case of UDP in which continuous process of solvent extraction had the highest UDP value. The same trend of results were obtained in SFC and GNC.

The % pepsin digestibility in MOC was 81.9, 82.7 and 83.8 for expeller, solvent extraction in batch and continuous process respectively and was significantly affected by processing methods. The highest digestibility was obtained in continuous process of solvent extraction followed by batch process and lowest in expeller process. In SFC the % pepsin digestibility was 69.38, 72.39 and 73.50 in expeller, solvent extraction in batch and continuous process, respectively and the values were significantly (P < 0.05) different. A marked improvement in pepsin digestibility was noted in solvent extraction processed cake with no method difference between batch and continuous process. In GNC percent pepsin digestibility was 88.09, 88.73 and 89.56 for expeller, solvent extraction in batch and continuous process respectively. None of the processing methods showed beneficial effects on pepsin digestibility.

The mean value of unpelleted and pelleted concentrate mixture for CP was 24.32 and 24.54, respectively, for RDP was 9.21 and 6.26, for RDP as % of CP was 37.89 and 25.50, for UDP was 15.19 and 18.19, for UDP as % of CP was 62.47 and 74.13 and pepsin

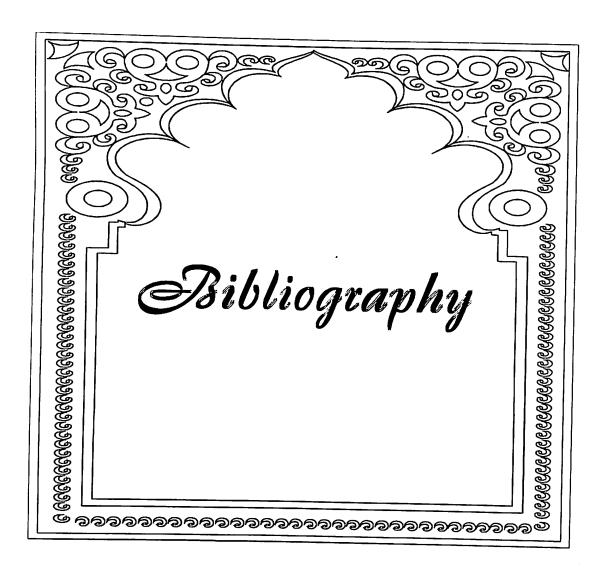
digestibility was 32.23 and 34.68. Pelleting of concentrate mixture showed beneficial effects in a positive way on different parameters studied. An increase in CP, UDP and pepsin digestibility value was seen, and the degradability of protein in rumen was significantly (P < 0.05) reduced by pelleting of concentrate mixture.

Results of physical parametry indicated that there was no effect of processing in colour of vegetable protein sources processed under different processes with slight change of colour in continuous process. SFC under expeller process had sweet smell and become odourless in solvent extraction processes. No marked change in colour, smell and odour was observed in mustard and groundnut cake. Time taken to become soft in water showed that SFC processed by expeller method took minimum time of 1 minute which increased to 2 minutes to 3 minutes in batch and continuous process of solvent extraction. MOC processed under expeller process took 9 minutes to become soft while under solvent extraction batch and continuous process took 19 and 18 minute respectively. GNC processed under expeller process took 2 minutes but under solvent extraction batch and continuous process took 10 and 19 minutes respectively. A comparatively higher time was noticed in MOC processed under expeller process as compared to GNC and SFC. It was further observed that adoption of same method of processing for both MOC

and GNC processed under solvent extraction method took comparatively more time than expeller or any of the methods used for SFC. Concentrate mixture in pelleted form took 8 minutes to become soft as compared to unpelleted concentrate mixture which became soft in 3 minutes.

Hence it can be concluded from the results that sources of protein had an influence on the degradability of protein in the rumen. Among the three vegetable protein sources studied, the crude protein of sunflower cake showed least degradability and a higher percentage of by-pass protein. Protein from GNC was found to be more degradable than MOC. Results obtained in the study can be exploited in the formulation of rations of high yielding dairy cows. With respect to method of extraction, solvent extraction by continuous process seemed to be a better method in reducing the degradability of protein in the rumen.





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ABBREVIATIONS

AIA : Acid Insoluble Ash

ANOVA : Analysis of Variance

AV./av. : Average

CF : Crude Fibre

CP : Crude Protein

DM : Dry Matter

EE : Ether Extract

N : Nitrogen

TA : Total Ash

MOC : Mustard Oil Cake

EMOC : Expeller Pressed Mustard Oil Cake

SMOC : Solvent Extracted Mustard Oil Cake

GNC : Groundnut Cake

EGNC : Expeller Pressed Groundnut Cake

SGNC : Solvent Extracted Groundnut Cake

SFC : Sunflower Cake

ESFC : Expeller Pressed Sunflower Cake

SSFC : Solvent Extracted Sunflower Cake

C.F. : Crude Fat

RDP : Rumen Degradable Protein

UDP : Rumen Undegradable Protein

MSC : Mustard Seed Cake.

CTC : Carbon Tetra Chloride

IVRI : Indian Veterinary Research Institute

DM : Dry Matter

* Standard abbreviations are not included in this list.