"IN VITRO" STUDIES ON

THE EFFECT OF TANNINS OF SOME FEEDS & ADDED TANNIC ACID ON PROTEIN DEGRADATION BY GOAT'S RUMEN MICRO-ORGANISMS

THESIS

Submitted to the

RAJENDRA AGRICULTURAL UNIVERSITY

in Partial Fulfilment of the Requirements for the Degree of

MASTER OF SCIENCE (Animal Husbandry)

AWADHESH KUMAR TRIPATHI

B. V. Sc. & A. H.

I. C. A. R. Research Scholar

POST-GRADUATE DEPARTMENT OF NUTRITION

BIHAR VETERINARY COLLEGE, PATNA

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PATNA,

The 11th February, 1972.

I certify that this Thesis has been prepared under my supervision by Shri A. K. Tripathi, a candidate for the degree of M. Sc. (A.H.) with Animal Nutrition as major subject and that it incorporates the results of his independent study.

D. B. Mukherjee

(D. B. Mukherjee).

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The author is extremely grateful to the "Indian Council of Agricultural Research" for the award of Junior Fellowship in Animal Nutrition in shape of financial assistance for undertaking this project.

(A.K. Tripathi)

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C H A P T E R - I

INTRODUCTION.

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INTRODUCTION

With the start of habitation on the earth, animals have been found to be closely associated with the human society. Animals serve as the sole source of supply of animal protein essentially needed to meet the requirements of human population. With the growth of both human and livestock population reaching to a critical point, the demand of food has increased enormously and the available resources of various feeds have failed to cope with the demand. The position of various feeds and fodder available in India as compared with its requirement for the livestock population presents a gloomy picture which has been reviewed from time to time in post-independence period (Kehar, 1953 ; Gullickson, 1958; Whyte & Mathur, 1965; and Bhattacharya, 1965). A Joint Committee of I.C.A.R. & I.C.M.R. (1959) observed overall shortage of roughages and concentrates in the country to the tune of 58% and 80% respectively. In terms of digestible nutrients the shortage has been estimated to the order of 77% in D.C.P. and 62% in S.E. (Kehar, 1953). Majumdar (1967) estimated that the present resources are able to meet only 1/3rd of the maintenance requirement of the whole livestock population. In addition, various parts of the country is every year hit either by famine or flood making the situation further worse.

Under the present circumstances it becomes the challenging task to the Animal Nutritionists to adopt various ways and means to fight with the situation. Until this situation is not

improved, it is impossible to expect any improvement in the livestock production which is essentially connected with the human needs.

To meet with this situation the possibility of utilisation of various subsidiary feeds and agro-industrial wastes has been investigated by various workers (Curasson, 1951; Bose et al, 1952; Patel & Patel, 1957; Hossain, 1959; Mathur, 1960; Mia et al, 1960a, b; Majumdar & Momin, 1960; Hossain, 1961; Jayal & Kehar, 1962; Patel et al, 1962; Patel et al, 1967; Nayak et al, 1967; Panda & Pradhan, 1967; Saxena, 1967; Patnaik, 1967; Johri etmal, 1967; Sharma et al, 1968; Panda et al, 1969; Pal, 1969; and many others) for livestock feeding.

It is equally important on the part of Nutrition research workers to investigate for the maximum possible utilisation of the nutrients pumped in to the digestive system of livestock so that the wastage could be cut to the minimum.

It has been reported that various naturally occuring substances distributed widely in plant kingdom are either deterimental to the animal health or interfere with palatability and utilisation of nutrients. Some of the subsidiary feeds investigated are not able to be utilised fully due to these reasons, otherwise they could have served as potential source of energy and protein. So, with the aim of meeting the situation from all corners it is essential that such feeds should be processed in a way that may render them suitable for livestock consumption without any deterimental effect on animal health or production.

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One of such substances are "Tannins" occuring widely in the plant kingdom.

"Tannins" are naturally occuring complex chemical compounds. Their chemical constituants are rarely known with certainity. They impart an astringent taste and are fairly soluble in water. "Tannic acid" is a name reserved for the tannin extracted from oak galls. The aquous solution of tannic acid forms precipitate with albumin and alkaloid (Bentley & Driver, 1945). Tannins have been found in appreciably high concentration in salseed (Shorea robusta), Oak Kernels (Quercus incana), carob beans (Ceratonia siliqua) and Lespedeza sericea. Tannins are also present in comparatively lower concentration in some species of sorghum grains, banana leaves and various other tree leaves. The chemical composition of these feed materials shows that they can serve as a good source of energy and protein for the livestock.

Various workers have reported the detrimental effect of tannins upon the digestibility of protein, growth rate and palatability by feeding trial experiments in cattle (Herman et al, 1953; Hawkins, 1955; Hawkins & Autrey, 1957; Pal, 1969), sheep (Wilkins et al, 1955; Bissel & Weir, 1957; Oslage & Becker, 1958), pigs (Husby & Presthegge, 1952; Becker et al, 1955; Neto, 1964) and poultry (Chang & Fuller, 1964; Vohra & Kratzer, 1964; Vohra et al, 1966; Fuller et al, 1966; Potter, 1968; Clandinin & Heard, 1968; Conner et al, 1969). Tagari et al (1965) have studied the effect of carob pod tannin in "In vitro" incubation

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on proteolysis, deamination, protein bio-synthesis and cellulolysis in sheep.

In the present work the effect of salseed tannins on its protein degradation has been studied and compared with that of tannin extracted sample, with a view to investigate the prospect of its better utilisation as a livestock feed.

Forests of sal tree are spread in a vast tract in the sub-himalyan region from Punjab to Assam and in other forests of Bihar, West Bengal, Orissa and Madhya Pradesh. It has been calculated that it may be possible to collect 48,000 mounds of sal fruits per square mile of the forest (cf. Associateship thesis of R.N. Pal, I.V.R.I., 1969). Salseed has also been found as a new source of fat for soap industry and the by-product salseed cake can be made available for livestock and poultry feeding.

Banana leaves and stems are also abundantly available in various parts of the country. After the banana fruit is taken out its, plant may be used for livestock feeding. Such possibility has been explored by Johri et al (1967). Its chemical composition has shown that it can serve as a good source of protein. But it is also important to investigate the effect of tannins present in them on protein degradation which will ultimately indicate the extent of its utilisation by the ruminants. This aspect has also been investigated in the present work.

It has been reported that the process of protein dea-

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degradation in rumen proves to be disadvantageous due to extensive break down of soluble proteins like casein and G.N. Cake protein resulting in influx of ammonia in the rumen, a major part of which goes waste and excreted ultimately as urea through urine. Under this situation the economy of nitrogen utilization is severly affected. Therefore, it appears to be of practical advantage to limit the degradation of dietary protein of soluble nature at a slower rate so that liberated ammonia may be utilized for microbial protein synthesis or the undegraded dietary protein may pass to the lower parts of alimentary canal for enzymetic digestion. But simultaneously, it is also important to take into consideration that such measures may not in any way affect V.F.A. production or water soluble vitamins synthesis in the rumen. Tannins have been tried for such an attempt by many workers (Leroy et al, 1964; Delort et al, 1968; Murty & Khan, 1969 and Zelter et al, 1970). To throw further light on this aspect of the problem effect of tannic acid on degradation of G.N.Cake protein has been undertaken for study.

As the present work undertaken for study is connected with the Goat's rumen it will be proper to discuss the important events of nitrogen metabolism in ruminants.

In ruminant's digestion rumen plays the most important part which may be evident owing to its large size to store large amount of coarse fibres for formentation. The extent of digestion in the rumen exceeds other parts of the alimentary canal. It has been estimated that 61.7 to 85.4% of digestible D.M. is

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utilised in the rumen of goat (Kameoka & Morimoto, 1959) and 70 to 85% of the same is the rumen of sheep (Gray, 1947).

With the advance of the present century new tools and methods have been devised to unravel the mystries of the rumen which was regarded as the "darkest place on the earth" by Hoard (1906). Our recent knowledge on digestive processes in the rumen are based on techniques of rumen fistula, duodenal fistula, use of cathetar in the portal vein, washed suspension of rumen microbes,use of radioactive elements and 'In vitro' techniques of study of digestion. Since last thirty years, the field of rumenology has remained an active area of research. Recent studies o=n nitrogen metabolism by various workers, as reviewed by Hungate (1966), Waldo (1968) and Smith (1969), have well established the broad outlines of digestive processes of protein metabolism in the rumen.

Rumen is regarded as a large fermentation vat which provides suitable environment for the growth and multiplication of the mixed type of microbial population. It is an anerobic, highly reducing system,at slightly acids but buffered pH,at temperature of about 39°C and under a gas phase composed mainly of CO_2, methane and nitrogen. The host animal provides shelter and desired nutrients to the micro-organisms who in turn help in the digestion of cellulose, synthesis of good quality protein and water soluble vitamins. In this way the host and micro-organisms remain in symbiosis with each other. The difficulty in isolation of so many species of micro-organisms is also indicative of

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the fact that they remain in a remarkable symbiosis among themselves as well. The micro-organisms include bacteria and protozoa of a number of species, the number of which vary according to the diet and other conditions. The mean values on average diet are estimated to be 10^9 to 10^{10} of bacteria and 10^6 of protozoa per gram of rumen content.

Nitrogen Metabolism in ruminants:

Rumen micro-organisms play major role in the metabolism of nitrogenous compounds, the protein as well as non-protein nitrogenous substances.

The dietary protein serves as the source of nitrogen in the rumen. The main nitrogenous compounds entering the rumen comprises chiefly of proteins and varying amounts of NPN compounds like peptides, amino acids, amides, purine and pyrimidine bases, pyrolles, simple bases like ammonia, nitrites and nitrates and traces of other substances. Enough experimental evidences have been accumulated to demonstrate that the dietary proteins suffer extensive degradation by micro-organisms in the rumen. Although, Sym (1938) observed strong proteolytic activity of the rumen content and Pearson & Smith (1943c) demonstrated by 'In vitro' technique the protein degradation by rumen contents, its significance was fully appreciated by the pioneer works of Mc Donald (1948a, b) who showed that the large quantities of ammonia were produced in the rumen under normal feeding conditions and the amount formed was dependent upon the type of protein and carbohydrate materials present. It was also observed

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by him that ammonia was absorbed directly from therumen into the ruminal veins and a considerable amount of nitrogen as urea was returned to the rumen through saliva.

The dietary protein is degraded to amino acids and amino acids are deaminated to ammonia. In addition, NPN compounds including salivary urea are readily degraded to ammonia. Protein is degraded by the action of proteolytic enzymes of the micro-organisms to peptides and amino acids. There is no evidence of extra cellular proteolytic activity of rumen contents. The amino acids and peptides are attacked by deaminases, enzymes of microbial origin, to form ammonia. Thus, ammonia formation is an important event in the nitrogen metabolism of ruminants. Liberated ammonia, in turn, is utilised by the micro-organisms for the synthesis of their cell protein which is of higher biological value. Not all of ammonia liberated is utilised in synthesis of microbial protein but a considerable part of it, depending upon the situation, is absorbed through the rumen wall to the portal blood vessels and is carried to the liver where some of the ammonia is utilised in the synthesis of non essential amino acids and remaining is converted to urea. A part of the urea formed reenters the rumen through saliva and major part of it is excreted through the urine. Some of the blood urea may also enter in to the rumen directly through ruminal wall by diffusion (Houpt, 1959). The rate of ammonia absorption is positively correlated to the ammonia and VFA concentration is the rumen (Hogan, 1961). Amino acids are not absorbed through the rumen wall and are passed downwards in the alimen-

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-tary canal (Lewis, 1955). It must be realised that there is always a simultaneous breakdown and synthesis in the rumen. Amino acids are also utilised by the micro-organisms but many bacteria have been found to prefer ammonia-N than amino-N for the synthesis of their cell protein. The synthesis of microbial protein from ammonia in the rumen increases the value of dietary nitrogen for the host because the protein formed in the body of microbes, is acted upon by the host's enzyme when they pass through the abomasum and small intestine where they are hydrolysed to amino acids and then absorbed in the small intestine (Axelsson, 1942; Pounden et al, 1950). Thus, the nitrogenous substances presented to the abomasum and intestine of the ruminant consist to a considerable extent of those present in micro-organisms rather than those in the diet which is not the case with monogastric animals.

Ammonia was shown to be a major end product of protein degradation of several different proteins. The amount of ammonia formed was dependent both on the nature of the dietary protein and proportion of the carbohydrates in the diet (Mc Donald, 1948a, b, 1952). It has been demonstrated that casein, a soluble protein is degraded more rapidly than zein, an insoluble protein (Mc Donald, 1952; Chalmers et al, 1954; Mc Donald & Hall, 1957; and Blackburn & Hobson, 1960). Extensive ammonia production in the rumen of cattle has been shown on feeding Groundnut meal as compared with feeding soyabean meal or cotton seed meal (Butz et al, 1958). The main factors governing the extent of protein breakdown in the rumen are the solubility and

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physical form of the dietary protein.

It is important to note that the changes occuring in t=he rumen are advantageous to the animal when its diet contains mainly poor quality protein or a NPN material such as urea and are disadvantageous when larger amount of dietary nitrogen consists of good quality protein owing to its extensive degradation resulting in wastage of nitrogen ultimately as urea. The merits and demerits of such variations, as they affect the nitrogen economy of the animal as a whole has been extensively studied and attempts have been made to modify the process for the benefit of the animal. Most research works have been directed at one of the two main subjects - (i) to make it possible to use effectively and safely, a high proportion of inexpensive NPN substance in the diet or (ii) to avoid as far as possible, the degradation of good quality food protein in the rumen. Thus, the nitrogen of several different proteins was retained more efficiently by sheep when the proteins were added directly to the abomasum through the fistula than they were given orally or added to the rumen (Chalmers et al, 1954; Blaxter & Martin,1962; Little & Mitchell, 1967). It appears, therefore, that a practical advantage might be gained by limiting the degradation of dietary protein, although it should be born in the mind that this would probably lead to a reduced microbial population and the possibility of secondary effects on cellulolytic activity and VFA production. It has been shown that dietary protein in liquid solution or suspension can be induced to bypass the rumen in adult sheep by encouraging closure of oesophageal groove

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(Ørskov & Benzie, 1969), but this is unlikely to be of practical value. The most promising approach appears to be chemical or physical modifications of good quality protein in such a way as to render it resistant to microbial attack without greatly reducing its nutritive value in the small intestine. Chalmer's et al (1954) and Chalmers, Jaysinghe & Marshall (1964) respectively found that heat treated casein in diets of sheep and heat treated G.N. Cake in diets of goats gave better overall nitrogen utilisation than did the untreated ones. Lower ammonia concentration in the rumen with the heated protein indicate that this was due to decreased degradation in the rumen. Similar improvements in nitrogen retention by feeding different heated proteins have been reported by Tagari et al (1962), Whitelaw et al (1961) and Broaster (1966). Other protein modications have been attempted by treatment with formal dehyde (Ferguson et al, 1967) or tannins (Leroy et al, 1964). Both the treatments have shown to reduce the solubility of the protein and its succeptibility to microbial attack.

Microbiology of the rumen in relation to proteolysis and synthesis of protein :

Reis et al (1959) suggested that protozoa contribute significantly to deamination in the rumen. Weller et al (1962) opined that they play limited role in nitrogen metabolism in the rumen. Applby (1955) found bacillus lichiformis to be the main proteolytic bacteria isolated from three sheeps. Proteolytic activity of bacteria in the rumen is not confined to one kind, but a variety of bacteria possess proteolytic activity in

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addition to their contribution in the breakdown of the other feed constituents (Blackburn & Hobson, 1962). Abou Akkada & Blackburn (1963) suggested that not more than 10% of the rumen bacteria of sheep, maintained on different diets, are proteolytic. The exo- and endo-peptidase activities are possessed by Bacteriodes amylophilus, B. ruminatium, most of the gram positive rods and coci. Deaminase activity appears to be confined to B. ruminocola and selenomonas ruminatium groups. Many strains possess amidase activity and most of the strains used ammonia-N in preference to amino-N.

Bacteria engaged in synthesis of protein from NPN were isolated by Smith & Baker (1944) and they were called iodophilic as they took stain with iodine. Some of the organisms isolated by Phillipson et al (1959) were similar to bacilus bifidus. Some cellulose fermenting organisms also assimilate ammonia-N. Bacteriodes succinogenes has been adapted so much to ammonia that growth does not take place in absence of ammonia-N (Bryant et al, 1959). Much informations have accumulated on the factors affecting the nitrogen metabolism of the rumen bacteria. One such field is the effect of naturally occuring substances like tannin present in certain feeds.

The present work has been undertaken with a view to study the effect of "Tannins" at high and low concentrations on the protein degradation in goat by 'In vitro' incubation technique. Salseed has been selected for study owing to its high level of tannin and banana leaves for low tannin level. At the

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same time the present investigation will also ascertain possible advantages of tannin extracted samples of salseed and banana leaves over the unextracted ones, if any, on the extent of protein breakdown. In addition, studies on the effect of added tannic acid on the breakdown of Groundnut cake protein has also been undertaken with a view to ascertain the possible utility of tannic acid or tannin containing feeds in limiting the extensive breakdown of G.N. Cake protein in the rumen and thus to check the wastage of dietary nitrogen and effect better economy in the utilisation of groundnut cake and other soluble proteins by the ruminants.

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C H A P T E R - II

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

Various aspects of ruminant metabolism have received attention in past by research workers in this field and as a result, considerable knowledge has been gained to broadly outline the events of protein metabolism in ruminants. But not much attention has been paid on the particular aspect of the problem under study i.e. effect of "Tannins" on protein degradation in ruminants. Economic aspects of nutrition has forced the research workers to attempt for the better utilisation of nutrients in the digestive tract of ruminants.

Out of the vast field of protein nutrition, attention has been restricted to the following items directly related to the pro=blem under study :-

(i) Factors affecting protein utilisation in the rumen.
(ii) Effect of tannins on the nutrition of livestock.
(iii) "Artificial rumen" techniques.

(I). FACTORS AFFECTING PROTEIN UTILISATION IN THE RUMEN:

The change in the amino acid composition of dietary protein in ruminants, which results from the conversion to the microbial protein in the rumen, can be an advantage or a disadvantage depending upon the composition of food protein. Ammonia formation is an important event in the process of conversion of dietary protein to microbial protein. Quantitatively, the rate of ammonia liberation (deamination) and the rate of conversion

of liberated ammonia to microbial protein (protein biosynthesis) is of great importance in the economics of nitrogen metabolism in ruminants, the two events are controlled by a number of factors. A number of research works have been projected towards these aspects of nitrogen metabolism. Attempts have been made to prevent influx of ammonia due to rapid degradation of soluble dietary proteins to avoid possible wastage of ammonia and to stimulate the rate of protein synthesis by rumen micro-organisms. Some attempts have also been made to bypass the good quality protein of soluble nature to the abomasum and avoid microbial breakdown in the rumen.

Pearson & Smith (1943a) studied the 'In vitro' attack upon proteins by incubating casein, gelatin and blood meal with rumen contents. They reported that there was a difinite evidence of protein hydrolysis with casein and gelatin. Blood meal was attacked very little. This was attributed to the different solubilities of the proteins, since blood meal unlike casein and gelatin was found to be largely insoluble.

Pearson & Smith (1943b) further studied the effect of various added carbohydrates on protein utilisation on incubation with rumen liquor. They observed that starch was more effective in promoting protein synthesis than glucose or maltose. Sucrose was partially effective in promoting synthesis and dextrin or lactic acid were found to be in effective.

Mc Donald (1948) reported that in the rumen casein was extensively degraded to ammonia which was absorbed through

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rumen wall and largely lost to the animal.

Cuthbertson & Chalmers (1950) observed that casein supplements fed to ewes on a low plane of nutrition were surprisingly ineffective in improving the nitrogen retention of these animals. These findings were explained on the basis of observations made by Mc Donald (1948).

Mc Donald (1952) further investigated the role of ammonia in ruminal digestion of protein. He observed that the chief form of soluble nitrogen in the rumen was ammonia. Casein, gelatin and zein were administered by rumen fistula in the rumen of sheep fed on a meadow hay ration and it was observed that the micro-organisms were capable of rapidly deaminating both casein and geletin, with consequent increase in ammonia concentration of rumen liquor. Zein did not yield ammonia although the protein was digested. He further investigated the interrelationship of protein and carbohydrates. He demonstrated a fall in ruminal ammonia concentration in sheep fed on a ration containing much soluble carbohydrate and he suggested that this happened probably due to the uptake of ammonia by rumen micro-organisms in the presence of readily available sources of energy.

El-Shazly (1952) in his 'In vitro' studies has provided informations on the fermentation of mixtures of amino acids. He found that amino acids of an acid hydrolysate of casein were attacked under anaerobic conditions by washed suspension of rumen micro-organisms. The fermentation products were ammonia, CO_2 and VFA in roughly equi-molecular amounts. In contrast to

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the results reported with proteolytic enzymes the deaminating ability was increased in parallel to the protein intake of the host. However, it was not investigated whether the increase in deaminating activity was due to increased number of micro-organisms with deaminating activity or increase in the amount of enzyme in each cell.

Investigations carried out by Sirotnak et al (1953) indicated that only small number of amino acids were individually attacked by washed suspensions of rumen micro-organisms. The main degradation products of amino acids were ammonia, CO_2 and VFA. The optimum pH for deamination was found to be 6.9 which is close to the usual pH of therumen contents in general.

Kameoka et al (1953) studied the change in nitrogen distribution and pH in the rumen content of goats fed on rations containing different levels of protein. Two goats with rumen fistula were fed on high, medium and low protein rations and on a low protein diet supplemented with urea. It was observed that nitrogen concentration of rumen content decrease with time, most rapidly after feeding high protein diet. The pH of the rumen was between 5.4 to 6.6 except the samples which were taken after 24 hours of feeding which showed a high pH. The lowest pH was observed 4 hours after feeding.

With a view to avoid the extensive degradation of good quality soluble protein and their improved utilisation by the animal, Chalmers et al (1954) made a comparative study of the fate of protein supplements administered into the rumen and

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duodenum. In their experiment, pregnant ewes 5 or 6 weeks before lambing or non-pregnant ewes with rumen and duodenal fistula and all on low plane of nutrition, received 50 g. casein by drip into the rumen or duodenum andthe effect on their nitrogen balance was measured. Overall apparant retention of nitrogen was found to be greater with duodenal thanwith ruminal administration. The extensive breakdown of casein to ammonia in the rumen with absorption of NH_3 into blood stream was also confirmed. The course of dissolution in the rumen of casein in the form of tough lumps was followed by use of stains. A comparision of the nitrogen retention of casein given as commercial casein, tough lumps or ground lumps showed the highest value with ground material, the concentration of ammonia was greater with the commercial casein than when ground material was the supplement.

Chalmers & Synge (1954) made comparative study of two different types of proteins supplements - namely two varieties of herring meal and two varieties of casein with cheviot ewes. They observed that the ammonia concentration in the rumen liquor was higher and retention of supplemental nitrogen was less with casein than with herring meal. In growth experiments they observed that in the high plane animals, there was no significant difference in weight or carcass grading between the groups during 91 days but in the low plane animals herring meals gave much greater live weight gain and the animals had more bloom than casein receiving animals.

Annison et al (1954) measured the ruminal ammonia

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formation with various diets in sheep. In their experiment cheviot sheep with rumen fistulae, fed on basal diet of hay or hay and meals, received different protein supplements viz. groundnut meal, casein, herring meal, maize product and the concentration of ammonia was measured in the rumen liquor. Ammonia production was observed highest from groundnut meal and lowest from maize products. The extent of ammonia production from a given protein supplement decreased as the amount of starch or cereal meal was increased.

Lewis (1955) studied the deamination of individual amino acids. It was observed that there was a slow deamination of individual amino acids when incubated with washed suspensions of rumen microbes. When the condition in the rumen was modified to increase the rate of reaction, there was no significant increase in the rate of deamination of individual amino acids suggesting that, the deamination occured almost exclusively by a stickland reaction. However, it was found that only alanine and proline were attacked. There was not significant deamination of any other pair of amino acids, whether a recognised stickland pair or not. Ammonia production could only be augumented by increasing the numbers of amino acids with suspensions. A mixture approximating to a casein hydrolysate rapidly gave rise to ammonia.

'In vitro' studies carried out by Annison (1956) have revealed, that the protein constituents in the diet of the animals from which the samples of rumen contents, were withdrawn,

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did not influence the rate of fermentation of incubated protein supplements. It was observed that considerable amounts of amino a-cids, peptides and ammonia were always produced from casein, soyaprotein, groundnut protein but not from bovine albumin, zein or wheat gluten. Bovine albumin a soluble protein was an exception as it degraded very slowly.

Rys et al (1956) studied the influence of different diets on the production of ammonia in the rumen of fistulated sheep. They observed that, the concentration of ammonia and urea in the blood was increased only with the diets containing urea and green grass or clover, which gave a high concentration of ammonia in the rumen. The high concentration of ammonia and urea in the blood of animals given green diets indicated serious lossess of nitrogen from this type of fodder.

Warner (1956b) investigated enzymatic breakdown of protein by rumen micro-organisms. The study was made with washed cell suspensions under toulene, an artificial rumen and cell free enzyme preparations. Substrates used were casein, herring meal, paisley meals, aradein and groundnut meal with and without heat treatment. It was shown that both rumen protozoa and bacteria are concerned in protein breakdown, with deamination of amino acids, and that proteases do not depend on high protein intake. Starch and cellulose reduced ammonia production in presence of living organisms, and effect was due to increased utilisation of protein degradation products more than to inhibition of breakdown. It was concluded that protein breakdown in

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the rumen occurs by the normal steps, about half the product being utilised for microbial growth.

Mc Donald & Hall (1957) studied the extent of conversion of casein in to microbial protein. A sheep with fistula in rumen and duodenum was fed on a ration of alkali treated straw, casein, rice starch, sucrose, molasses and salt mixture; a control sheep received casein digest instead of casein. The average maximum concentration of casein in abomasal protein was 47%, 2 hours after feeding and it was calculated that atleast 90% of the casein was degraded in the rumen as compared to 40% for zein.

Lewis and Mc Donald (1958) in their investigation confirmed that the active fermentation in the rumen was encouraged in the presence of both protein and adequate amount carbohydrates and that, the growth and synthetic reactions were increased by a more balanced system. It was concluded that the protein supplements added to the diets of the sheep were best utilised when a carbohydrate material fermented at a comparable rate was also fed. Ammonia concentration in the rumen was reduced by all carbohydrate supplements, the effect of levan and starch was greatest and that of cellulose was slight.

El-shazly (1958) carried out investigations on nitrogen retention and ruminal ammonia production in ewes fed common Egyptian feeding stuffs. Wheat straw was used as roughage and concentrates were given to supply 10 g. of nitrogen daily. The concentrates used were meat meal, fishmeal or casein or common

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Egyptian feeding stuffs as sources of N. positive nitrogen balance was maintained with decorticated cotton seed cake, linseed meal and berseem. Proportion of ammonia nitrogen in the rumen was found to be proportional to soluble nitrogen in the feed.

Reis & Reid (1959) observed in their 'In vitro' experiments with sheep that optimum pH for ammonia accumulation in artificial rumen after 4 hours of incubation at 39°C varied between 6.0 to 6.7, on different diets. They also observed that glucose reduced the accumulation of ammonia with increase in protein-N level suggesting a protein synthesis.

Blackburn and Hobson (1960) carried out experiments to study protein degradation in the rumen of sheep and redistribution of protein nitrogen after feeding. Two cheviot sheeps were maintained on diets similar in all respects except that casein was added in one of the three forms : soluble, insoluble and partly soluble. Changes in the rumen ammonia, protein nitrogen, residual nitrogen and protozoal and bacterial nitrogen were studied for a period of 7 hours after each sheep received each diet. It was observed that the soluble protein (casein) was rapidly degraded and except for a short time after feeding, there was no measurable amount of it in the rumen contents. Compounds of non-ammonia-non protein nitrogen were likewise broken down but at a slower rate. The insoluble casein provided a more constant supply of NANPN than the other casein. Rumen ammonia concentration after three hours of feeding was highest in soluble casein and lowest in insoluble (hardened) casein

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among the three diets. It was concluded that the rumen ammonia concentration depended on the physical state of the casein in the diet and the rate of utilisation of nitrogenous material is governed by its rate of liberation from the insoluble food stuff particles.

Whitelaw et al (1961) made a comparative study on the efficiency of commercial groundnut meal, heat treated groundnut meal and fish meal as a major protein source in the diet of calves. These supplements were each included in a concentrate mixture so that they provided 45% of the total N and the resulting diets had 16.5% of crude protein. The diets were given to 11 weeks old calves. The respective values of the three diets for nitrogen retention were 17.2, 18.9 and 21.6 g. per day. Daily live weight gains were 363, 442 and 636 g. respectively. It was concluded that heated groundnut meal was superior to commercial groundnut meal.

Jayasinghe (1961) studied the effect addition of fat on the breakdown of protein and subsequent production of ammonia in the rumen of sheep. He observed that when 10 to 15% of fat was added as groundnut oil to the protein supplement, less ammonia was produced in the rumen.

Jasiorowski (1961) conducted growth trials and nitrogen balance studies with lambs to evaluate the utilisation of nitrogenous compound in meadow hay, leucerne hay and dried leucerne for ruminants. It was concluded that the nitrogenous material of leucerne hay was not well utilised as that of meadow

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hay. In balance trials dried leucerne gave a better nitrogen balance than leucerne hay but in long term feeding it was little better than the hay. Starch to replace 15% of the leucerne hay gave growth as good as meadow hay but did not affect nitrogen balance. On leucerne hay there was more ammonia production in the rumen than on meadow hay and starch had little effect. Dried leucerne gave intermediate value. He suggested that the poor utilisation of nitrogen in leucerne was caused probably by its greater solubility and more deamination in the rumen.

While studying the inter-relationship of protein and carbohydrate, Lewis (1962) observed that in the presence of starch, groundnut protein (arachin), soyaprotein, gelatin and casein were utilised with an increased rate, though in the begining ammonia production was stimulated.

Tagari et al (1962) studied the influence of heating on the nutritive value of soyabean meal for ruminants. Soyabean meals subjected to different heat treatments were fed to rams. Heating of soyabean caused a considerable decrease in the solubility of its protein. 'In vitro' and 'In vivo' experiments showed a striking decrease in the amount of ammonia liberated by the rumen micro-organisms from heated soyabean meal in comparison with the untreated one. The apparent digestibility coefficients of protein in heated soyabean meal were found to be some what higher than untreated soyabean meal. Nitrogen retention by adult sheep from diet containing heated soyabean meal was also considerably higher than that with untreated meal.

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Commercial untreated meal, which is heated during removal of the solvent, showed an intermediate behavior between untreated and properly treated meals with regard to all properties examined. They concluded that the ammonia is liberated from untreated soyabean meal to such an extent that the micro-organisms cannot use it profitably for synthetic purposes, a greater part of the ammonia diffusing the rumen wall to the blood and therefore being wasted. On the other hand, the superiority of heat treatment was due to its reduced solubility and gradual degradation to supply ammonia at a constant rate for synthetic purposes.

Blaxter and Martin (1962) while studying the utilisation of protein as source of energy in fattening sheep observed increased nitrogen retention when casein was infused directly to abomasum than when it was infused in to the rumen. The mean daily amount retained was 10.1% of the nitrogen of the casein for the sheep receiving casein by rumen and 16.2% for those given it by infusion in to the abomasum.

Henderickx & Martin (1963) carried out extensive 'In vitro' experiments to study nitrogen metabolism in rumen of cattle. Rumen fluid obtained at slaughter from four cattle starved for atleast 48 hours and the pooled sample of fluid was incubated in artificial rumen. Ammonium carbonate, chloride, phosphate and bicarbonate, ammonium salts of fatty and organic acids, urea, thiourea, biuret and amides all provided nitrogen of which 40 to 50% was incorporated in to microbial protein.

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For ammonium sulphate and succinate conversion was about 80%. The presence of ammonium nitrate, sodium nitrite, or potasium nitrate or aspargine was inhibitory to protein formation. When source of nitrogen was ammonium bicarbonate, addition of sulphate or phosphate increased protein synthesis. When ammonium sulphate was the source of nitrogen, addition of cellulose or lignin inhibited protein synthesis; simpler polysaccharides favoured, but monosaccharides other than arabinose and xylose did not. Sugar acids and sugar alcohols did not support protein formation. Natural starches from rice, wheat, maize and potato had the most favourable effect and much more than manufactured products such as soluble starch. Organic acids, fatty acids and sodium salts of glyceal, ethanol were uniformly inhibiting when added alone, but in the presence of glucose and starch the effect was reversed. Protein synthesis occured all over pH range of 6 to 8 and was greatest at pH 7.

A series of 39 proteins in purified form were tested as source of nitrogen. All led to the net breakdown of microbial protein and in addition, 2/3rds were themselves broken down to some extent, in direct relation to their solubility in a mineral buffer, with correlation r = +0.99. Added carbohydrates generally inhibited the breakdown and stimulated synthesis of microbial protein. Interactions of carbohydrates and protein depended on the solubility of each and the extent to which the carbohydrates were fermentable.

Hogan (1964) analysed rumen samples taken from different part of the reticulo-rumen of grazing sheep to study the

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production of ammonia in the rumen content. It was observed that ammonia concentration rose rapidly when a casein supplement was given. Concentrations were higher with a diet of oat grass leaves than with stems and leaves even the latter provided more N. The ammonia concentration of grazing sheep varied enourmously with season and time of day, accompanied by parallel variations in pH and the concentrations of VFA.

Sherrod & Tillman (1964) conducted digestibility, nitrogen retention and growth trials to study the effect of various heat treatments upon utilisation of cotton seed protein by sheep. Cotton seed meal used were - no heat, autoclaved under 15 lb. pressure per square inch at 250°F for 20 minutes, 60 minutes, 120 minutes, 180 minutes and 240 minutes. Autoclaving the raw cotton seed meal for 60 minutes improved sheep gains and feed efficiency significantly, when compared with unheated meal and the meals autoclaved for 120 and 240 minutes.

Brochers (1965) studied 'In vitro' the proteolytic activity of rumen fluid. Rumen content was obtained from cattle fed on leucerne hay and grain were filtered through 4 layers of cheese cloth. The filterate was incubated at 40°C at pH 6.5 with an equal volume of 1% casein or a suspension of lucerne, maize or soyabean previously ground and soaked in water at a concentration of 1% protein. Thymol was added at the rate of 1 mg. per ml. to prevent deamination of amino acids liberated by proteolysis. There was rapid increase in amino acids after incubation with casein. The protein in leucerne, maize or soyabean meal resulted in no significant increase in amino acids.

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Microbial protein sisolated from rumen fluid under went proteolysis more rapidly than plant proteins but less readily than casein.

While studying the digestion of protein in ruminants Hoshino (1965) observed the effect of carbohydrates and antibiotics on the ammonia concentration of the rumen content. He concluded that the production of ammonia on incubation of casein with rumen fluid from sheep was reduced by addition of sugars, carbohydrates, and in some cases, by streptomycin and tetracyclin. However, no effect was marked when cellulose and penicillin were added.

Czerkawski et al (1966) studied the effect of linseed oil and of linseed oil fatty acids incorporated in the diet on the nitrogen metabolism in sheep. They observed that the urinary nitrogen excretion was lower in six determination in diet containing slightly less than 3% N and added glycerides and in five out of six determinations in added fatty acids than the control diet and was consistent with the observation of depressed rumen ammonia. They concluded that depression of fiber digestibility, when feeding lipids, may limit the use of this method of improving nitrogen utilisation.

Ely et al (1967) conducted experiments to estimate the conversion of dietary zein to microbial protein in the rumen of lambs. Six lambs were fed two purified diets containing 12.8% of zein as the only source of nitrogen and two different cellulose to starch ratios. There was no difference

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between the two treatments in the apparent digestibility of nitrogen, N-balance and proportions of zein and non-zein protein in the abomasal fluid. With both treatments nitrogen balance was negative and the apparent digestibility was low. Microbial protein in the abomasal fluid was 26.3 and 30.5% respectively for the high cellulose and high starch diets. The diet and abomasal fluid differed in concentration of most of the essential amino acids, but the difference was great in case of lysine.

Glimp et al (1967) studied the effect of reducing soyabean protein solubility by dry heat on the protein utilisation of young lambs. In growth trial with 48 lambs and digestibility and nitrogen balance with 24 lambs, the diets had 12.1 or 17.2% crude protein, contained heated or unheated soyabean meal and were given without or with neomycin. Growth and efficiency was better with 17% protein. With 12% protein from heated soyabean meal gain was improved to a point comparable with that of lambs given 17% unheated protein, and there was increased retention of nitrogen. Iso-valeric and valeric acids were decreased and digestion of cellulose was increased by the reduction in solubility of protein due to heating. Neomycin had no significant effect.

Little & Mitchell (1967) made comparative study on abomasal versus oral administration of proteins to wethers. Four sources of purified protein were given as a supplement to a basal diet of straw and maize to supply 60% of total intake

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of N for wethers. The protein was given in the diet or by infusion as a slurry in water through an abomasal canula. Soyabean protein was digested as well after infusion as when given by mouth but retention of nitrogen was greater with infusion. When zein was infused digestibility and retention of N were both less. The method of giving casein or gelatin did not affect digestibilities of protein, dry matter or cellulose but infusion increased N retention, which was similar when either protein was given in the feed but favoured casein when given by infusion.

Ferguson et al (1967) have studied the effect of formaldehyde treatment of casein upon protein utilisation in sheep. When formaldehyde treated casein was fed to the sheep it was well utilised as compared with the untreated casein. It was shown that treatment with formaldehyde reduced the solubility of the casein and its succeptibility to microbial attack. Marked improvement was shown in wool growth, suggesting improved utilisation of the sulphur containing amino acids.

Miron et al (1968) investigated the relative usefulness of protein and NPN with and without ready source of energy in growing calves. A starting feed was given to Holstein calves in four groups of 8 each with 15% soyabean meal or 1.9% urea, each with or without 0.6% of a mixture of iso-butyric, valeric and iso-valeric acids. The calves were given whole milk upto 6 weeks of age and the starting feed and good leucerne hay to appetite for 10 weeks from 2 weeks of age. They

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observed that calves given soyabean meal gained faster than those given urea, VFA had no effect on gain.

Susova (1968), while studying the nitrogen metabolism in the rumen of bullocks given different diets observed that the presence of large amounts of quickly hydrolysable proteins in grass resulted in a high concentration of ammonia, 79 to 369 mg. % N, in the rumen of grass fed cattle, the high level was maintained for 6 to 10 hours after feeding. In cattle fed on roots, ammonia concentration in rumen was low, 8.2 to 60.7 mg.% N due to presence of readily fermentable carbohydrate in the diet which stimulated protein bio-synthesis by micro-organisms. On all the diets the ammonia concentration was highest at 2 to 4 hours after feeding.

Devuyst et al (1968) studied the effect of glucose and starch on ammonia content, VFA, changes in pH in the rumen fluid of sheep. With glucose they observed decreased pH, ammonia and acetic acid concentrations and increased concentrations of propionic and butyric acids. The starch had slower but more lasting effect than glucose and its presence in the diet decreased pH, NH_3 and propionic acid concentration and increased acetic and butyric acids.

Ørskov & Benzie (1969) have shown in their study that the dietary protein in liquid solution or suspension can be induced to bypass rumen with a view to avoid extensive degradation, by encouraging closure of the oesophageal groove in sheep.

Broster et al (1969) studied the effect of energy

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intake on the utilisation of protein in growth and lactation. They observed that the part of the nitrogen from a protein supplement was retained in the body despite the excretion of most of it in urine and feces. Nitrogen retention was increased by a supplement of readily available source of energy through a decreased lo ss of N in urine. However, nitrogen balance was not increased by a supplement of a fibrous source of energy because that increased loss of nitrogen in the feces. In another feeding trial milk yield was found to be affected in similar manner to growth.

Kumar & Murty (1970) conducted the experiment to study the effect of molasses on ammonia concentration in the rumen. It was shown on a rumen fistulated Hariana bullock that, at higher level of nitrogen intake the addition of molasses to the concentrate part of the ration reduced the rumen ammonia level.

Tannins have also been found to have inhibitary effect nitrogen utilisation by various workers which have been reviewed seperately in the following pages.

(II). EFFECT OF TANNINS ON THE NUTRITION OF LIVESTOCK:

Various workers have reported the deletarious effect of tannins in different species based on palatability, nitrogen balance, digestibility, growth, production and 'In vitro' experiments. Efforts have also been made recently by some workers to utilise tannins for better nitrogen utilisation in ruminants under certain circumstances. A detailed review on this aspect

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of the problem by various workers is stated below specieswise.

CATTLE :

Herman et al (1953) estimated tannin percentage of a variety of feeds and reported that korean lespedeza contains 8.94 to 2.89%, Alfalfa hay 10%, silage 22.9%, dry beet pulp 15.0% and hedge apple 26% tannins on D.M. basis.

They observed no effect on growth, feed consumption or milk production of cows and no digestive disturbance on a ration containing about 12% tannins with a further addition 5% tannic acid to it.

Hawkins (1955) conducted palatability and digestibility trials on 6 months old dairy calves with sericea lespedeza and alfalfa hay with added gallotannin. Alfalfa hay alone (without added gallotannin) was kept as control ration. On chemical analysis lespedeza hay was found to have less crude protein and more lignin than alfalfa hay. The experimental rations were equal in tannin but the lespedeza hay tannin was of the catechol type and alfalfa tannin of gallotype.

Less dry matter and water was consumed on lespedeza hay in palatability trial and the calves lost weight. Digestibility of dry matter and crude protein of lespedeza hay was found to be less than either control or experimental alfalfa hay.

He concluded that the palatability of lespedeza was affected by its astringent taste and digestibility by precipi-

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-tation of protein as insoluble tannates, properties possessed by both forms of tannin found in the analysis and the low values for lespedeza hay were not, therefore, thought to be due to its high tannin content as it was same in the experimental alfalfa ration. It might be that some of the tannin in to the lespedeza was already combined with some of protein in the plant as insoluble tannates or that tannic acid added to alfalfa was inactivated in the upper part of the gut before it could affect the protein. The more likely reason for the low values of sericea was thought to be its high lignin content. The loss of weight by the calves in lespedeza group could be ascribed to the low feed intake.

Bejovic (1958) reported detrimental effects of oak leaves in cattle and also the same changes were observed when calves were given by mouth solutions of 2 to 10% of tannic acid and 5 litres of strongest solution caused death in 22 hours and this co=rresponded to the amount which an animal might obtain from oak leaves.

Oslage & Becker (1958) conducted digestibility trials in lactating cows using carob pods and observed low digestibility of protein. They also reported that natural tannins and tannin containing substances have long been known to inhibit decomposition processes of organic matter.

Volcani & Levi (1962) and Drori & Volcani (1963) have studied the effects of carob pods on the growth of calves and concluded that the deterimental effects of carob pods in calf

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nutrition were due to lowered digestibility of all ration components.

Volcani et al (1963) reported that carob pod meal and its various extracts inhibited cellulolysis and deamination 'In vitro' and it has been also observed 'In vivo' after prolonged feeding of carob meal by Y. Rabi (cited by Tagari et al, 1965).

Henis et al (1964) reported that carob pod extract and its tannin, as well as gallotannic acid at very low concentrations, have been shown to inhibit saprophytic cellulolytic bacteria and modify their colonies, especially cell vibrio.

Mc Cosker & Hunt (1966) studied the poisoning of cattle with Acacia salicina ground bark of black wattle, Acacia decurrens was found to contain 35% tannins. Two calves of about 360 lbs. body weight each were given 8 kg. of ground bark wattle through rumen fistula. One died within 48 hours and the other was killed after 10 days showing severe signs of poisoning. Signs were similar to those seen in cattle which had eaten from cut branch of trees including A. salicina, leaves of which had 14% tannins.

Delort-Laval & Helter (1968) reported that treatment with tannins strongly inhibited the deamination of many proteins without adversely affecting digestibility of the diet, rumen microflora cellulolytic activity and volatile fatty acid production. They concluded that tannins reduce the degradation of protein in the rumen.

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Donnelly & Anthony (1969) studied dry matter digestibility with low and high tannin variety of sericea leaves and stems by Lusk's method in which ground material in nylon bags was placed in the rumen of bullocks. Average crude protein percent of the low and high tannin varieties was 15.9 and 16.0 respectively. Significantly different dry matter digestibility was observed between two varieties. The digestible D.M. for high tannin variety was 63.4% and that of low tannin variety was 71.8%. No correlation was found between digestable dry matter and protein.

Mc Ginty (1969) conducted digestibility trials in cattle to know the factors affecting digestibility of varieties of sorghum grain and found that the factors causing poorer digestion was in the pericarp. When pericarp from the poorly digested varieties was added to fermentation tubes with endosperm of any variety gas production was less, and the soluble material of the pericarp, extracted with organic solvents and water had the same effect. Tannic acid also reduced gas production. The decrease caused by poorly digested pericarps could be lessened by polythene glycol. When soluble material from the pericarp was added to centrifuged rumen fluid there was a significant increase in the amount of nitrogen precipitated.

Murty & Khan (1969) used 'In vivo' and 'In vitro' digestion techniques to study the concentration of ruminal ammonia in buffaloes using G.N.Cake with and without the addition of salseed cake. The addition of salseed cake to G.N.Cake was

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reported to reduce significantly the ruminal ammonia production. The reduction in ammonia level in the rumen concided with the lower excretion of urinary nitrogen and high nitrogen balance from G.N.Cake protein.

Pal (1969) reported on the basis of observations made during a feeding trial with Hariyana bullocks that salseed cake was unpalatable to the cattle. It was, however, consumed in appreciable quantities when offered in admixture with groundnut cake. It was further observed, that when salseed was fed as the sole source of concentrate to the bullocks, the digestibility of the protein was nil, that of other nutrients was also less in comparison to the animals to which G.N.Cake was fed as sole source of concentrate. A comparision of the average D.M. intake in group I (with salseed cake as sole ration) and group III (with G.N.Cake as sole ration) showed that supplementation of the wheat bhusa ration with G.N.Cake instead of salseed cake improved intake and that the same was bettered when salseed cake was offered simultaneously with G.N.Cake. During the experimental feeding period extending over a month, the animals supplemented with sal seed cake alone lost 9% of their live weight on average, while in the groups supplemented with G.N.Cake alone and in combination with salseed the animals gained 3 and 4% live weight respectively. The inclusion of salseed cake with G.N.Cake increased the total D.M. intake in the ration but it had adverse effect of bringing about depression in average D.M. digestibility from 51 to 48%, and that in wheat bhusa supplemented with salseed cake alone averaging about 45% only. The

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digestibilities of organic nutrients followed the trend of D.M. digestibility.

Zelter et al (1970) studied 'In vitro' the behavier of some proteins tanned with tannin from chestnut wood or some aldehydes (formaldehyde, glutaral-dehyde, glyoxal). Proteins of groundnut, soyabean, linseed, rapeseed and sunflower seed oil meals, skimmed milk powder or dried leucerne meal or casein were complexed with different tanning agents, chestnut wood extracts, formaldehyde, glutaraldehyde or glyoxal,were incubated in an artificial rumen. The minimum amount of each tanning agent which would prevent protein degradation depended on the original physico-chemical properties and heat treatment applied to the protein. The original values for enzymic solubility of the protein were not affected by formaldehyde or glyoxal but were reduced by 5% by chestnut extract or glutaraldehyde. The minimum dose of each aldehyde which would completely protect the protein decreased cellulolytic activity of an inoculum of rumen contents on a wheat straw substrate by 13 to 20%, while doses which gave 90% protection reduced cellulolytic activity by 3%. Chestnut extract did not have this negative effect on cellulolytic activity of rumen inoculum.

SHEEP :

Wilkins et al (1955) studied the influence of tannins present in Lespedeza sericea on the palatability in sheep. The preliminary results showed a definite effect of tannin on palatability. As the tannin content in the herbage increased, the

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amount consumed by the two sheep declined very appreciably. The results were preliminary in the sense that the quantity of herbage and the number of sheep fed were inadequate for more conclusive result.

Bissel & Weir (1957) carried digestibility trial of the new growth of chamise (Adenostoma fasciculatum) and interior live oak (Quercus Wislizenii) on deer and sheep. They reported that the digestibilities of crude protein and crude fibre in both the feeds were poor in deer and sheep. The digestibility of crude protein in live oak was only 10%.

Oslage & Becker (1958) studied the nutritive value of carob beans for ruminants with special reference to the adverse effect of tannic acid on digestibility of protein. In digestibility trial pairs of sheep received legume hay 300 g. and carob meal 500 gm. or leucerne hay 700 g. and carob meal 100 g. daily. They observed that the digestibility of carob meal protein was nil and that of leucerne hay protein 71%; the larger amounts of carob meal significantly reduced the digestibility of the leucerne hay, that of its protein to 50%. With the smaller proportion of carob meal the reduction of digestibility was slight.

Leroy et al (1964) studied the effect of tannic acid on deamination of protein in artificial rumen with sheep inoculam.

Water or an aquous preparation of tannin was added at 13% or 23% to oil cakes, 5 of groundnut and 6 of soya, which

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were then dried. Amounts with equal nitrogen and cellulose content were tested in artificial rumen. Ammonia-N and other soluble N first rose, then were fairly constant for 12 to 14 hours. They were not significantly different between oil cake untreated or with water, or between 13% or 23% tannin solution, but were significantly higher without than with tannin. In earlier tests 6% tannin was not enough.

With pepsin and trypsin 'In vitro'; 13% tannin solution did not affect cellulose digestion of leucerne hay. Enzyme solubility of nitrogen from either G.N.Cake or soya cake was significantly less, about 6%, with water than with untreated cakes, and not significantly less with 13 or 23% solution than with water. Later work on sheep with rumen fistulae seemed to confirm that tannin prevent bacterial deamination of feed without upsetting digestive processes.

Tagari et al (1965) on 'In vitro' study compared carob pod extract and its tannin and sugar fractions with gallotannic acid and sucrose for their effect on the cellulolytic, proteolytic, protein biosynthesis and deaminative activities of rumen micro-organisms. The source of micro-organisms was the rumen fluid of sheep fed on leucerne hay, concentrates, and cotton seed hulls, used in incubation for 48 hours in containers to which cellulose and minerals were used. Proteolytic activity was studied in a similar manner with casein as substrate. Protein biosynthesis and ammonia formation were tested in a similar manner with amino acids added. Carob pod extract was a hot

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water extract and tannins and sugar were prepared from the extract.

Cellulose decomposition was completely arrested by the addition of carob extract to a final tannin concentration of 1.5 mg./ml. Gallotannic acid imparted a similar effect, the tannin fraction of the carob extract alone did not inhibit cellulolysis significantly.

In contrast to cellololysis, proteolysis was very slightly inhibited by gallotannic acid. On the other hand, the tannin fraction of carob extract inhibited proteolysis completely at a concentration of 1 mg./ml.. A most significant inhibition of proteolysis by carob extract, on the basis of its tannin or sugar content. A 100% inhibition was observed at 0.5 mg./ml. of tannins and 6 mg. sugars per ml. The significant inhibitary effect of carob extract on proteolysis apparantely resulted from the additive effect of tannins and sugar. The tannin-sugar ratio in the carob extract was 20. This sugar concentration (10 mg./ml.) inhibited proteolysis by more than 75%. In contrast, to the inhibitory effect on proteolysis at relatively high concentration of sugars, low concentrations enhanced proteolysis.

As compared with sugar containing fractions, tannins alone exerted a greater repressive effect on protein biosynthesis by rumen micro-organisms. Carob tannins were more deterimental to protein biosynthesis than gallotannic acid. The sugar fraction of carob pod extract enhanced protein biosynthesis to the same extent as did the sucrose. The addition of carob pod

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extract (tannin and sugars) increased protein biosynthesis as compared with tannin alone. Deaminating activity, as measured by the amounts of free NH_3 found in the reaction mixture, was significantly reduced by the addition of any of the sugar fractions, this meant a greater uptake of NH_3 for protein biosynthesis. The deaminating activity was not affected by the tannin to the same extent as was protein biosynthesis. The increasing amounts of free NH_3 at high tannin concentrations resulted from the inhibition of protein biosynthesis.

It was concluded that carob pod extract inhibited proteolysis and protein biosynthesis and inhibition appeared principally due to the tannin but the inhibition of cellulolysis and deamination was mainly caused by sugar fractions.

PIG :

Husby & Presthegge (1952) in their experiment with carob meal reported the digestibility of the protein to be 15% only. The digestibility was carried out with wethers.

In growth experiment four groups, each of 4 young pigs were given a basal meal to cover half of their requirement and for the rest barley, millet, carob or potato, with a little fishmeal and/or manioc to balance protein and feed units. The carob pigs lost appetite and condition after 2 weeks and had to be rested till they recovered. They were then continued with carob meal as only 20% of the ration. In that case, the pigs grew, but did not look, as well as the others. There was little

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difference in growth and condition between the pigs given barley, millets and potato.

Becker et al (1955) studied the effect of tannic acid on the nutritive value of carob bean for pigs. They found the digestibility of carob bean protein with pepsin and HCl 'In vitro' was nil, compared with that of fishmeal protein, 87 to 88%. In mixtures of the substances the digestibility of the fishmeal protein decreased with increasing proportions of carob bean. In digestibility trials pairs of pigs received rations containing 2/3, 1/2 and 1/10th of the total as carob meal. Larger proportions of carob meal was found to greatly reduce the digestibility of the rest of the ration, especially that of protein. With 10% carob meal reduction of the digestibility was slight.

Neto (1964) investigated the possibility of utilising carob beans as pig feed. Pig 20 to 25 kg. live weight at the start were fed for 140 days on diets without carob meal (control) or with carob bean from 20 to 60% included at the expense of maize. Estimated digestible protein was some what less in the diets with carob. Average daily gain were 577 g. without carob, between 450 g. and 500 g. with 20 to 40%, 436 g. with 50% and 386 g. with 60% and per kg. gain estimated feed units eaten were 3.8 by cereals, 5.1 with 20 or 30% carob, 5.4 with 40 or 50% and 6.1 with 60%. The diets cost progressively less per kg. with increasing amounts of carob, but cost per unit live weight gained remained the same, always more than for the diet without carob, except that with 60% included cost was highest.

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For the second trial experimental diets had 10 or 20% carob. Daily gains of pigs from 30 kg. live weight for 84 days were 685 g. for controls and 597 and 603 g. for groups given 10 or 20%. Per kg. gained intakes were 3.73, 4.01, and 4.08 F.U. respectively. Cost per unit gain was 3 to 4% more with carob.

In 3 pigs diets with 10, 20, 70, and 88% carob digestibility of all constituents except fibre, decreased as carob increased. The digestibility of carob meal in 2 diets with most, calculated by difference was negative for protein and ether extract.

In a digestibility trial experiment at I.V.R.I., Izatnagar (1969) six adult yorkshire pigs were divided into two groups of 2 males and 1 female each. To start with 3% each of salseed meal and cane molasses replaced maize and by gradually decreasing by the end of 25 days, 30% maize was replaced by 20% salseed meals and 10% molasses. The digestibility coefficients of organic nutrients were significantly depressed in the salseed meal group.

POULTRY :

Kratzer & Williams (1951) observed that growth of chicks was retarded significantly when they were fed ration containing as little as 5% ground carobs.

Uri et al (1954), have shown that at higher levels of addition of ground carobs to chick ration, growth depression increased and mortality occured.

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Bornstein & Lipstein (1959) also showed that higher levels of ground carob in chick rations caused growth depression and mortality.

Bornstein et al (1963) treated pods and seeds of 3 varieties of carob (Ceratonia siliqua) in different ways in an attempt to improve their nutrient value for chicks. The 3 varieties were given in 9 trials to leg horn chicks at the expense of grain portion of the diets. Feed consumption tended to increase with increasing amounts of carob meal in the diet, but not significantly. None of the ways of teating carob meal substantially reduced its growth inhibiting effect. Extraction with hot water increased feed consumption and counteracted inhibition of growth to some extent. Diets including pods soaked in hot solution of ferric chloride or calcium chloride were slightly better than other diets containing carob. Unavailability of the protein of carobs did not seem to be responsible for inhibition of growth. A substance extractable with hot water and which depressed appetite, possibly a plant phenol was suggested as preventing chicken from fully compensating by increased consumption, for deficiency of digestible nutrients in carob meal.

Chang & Fuller (1964) made extensive studies on the effect of tannin content of varieties of grain sorghums on their feeding value for growing chicks. In a series of four feeding trials from one day to four, seven or eight weeks of age, triplicate groups of white rock cockrels were given a diet based

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on maize, soyabean meal with different amounts of tannic acid or diets with 50% sorghum grain in place of maize. There were six varieties of sorghum with tannin contents from 0.2 to 2.0%. Tannic acid as 0.1% of the diet did not appear to be toxic but with 0.5 to 2.0% growth was depressed in proportion to the amount of tannic acid added. Growth of chicken given diets with similar energy and protein and containing different varieties of sorghum grain varied inversely with the tannin content of the sorghum. Livers had more total lipids in chickens given the varieties of sorghum with most tannin. Supplements of choline and methionine hydroxy analogue prevented the depression of growth caused by 1% tannic acid. When twice as much choline and methionine hydroxy analogue as usual were added to the diet, chickens given sorghum with 2.0% tannin grew as well as those given sorghum with 0.2%, but when choline and methionine were omitted from the diet growth of chickens was retarded by sorghum with 2.0% tannin.

Vohra & Kratzer (1964) concluded with their experiments with day old chicks that it was possible to replace part of the cereals in the diet by 20% ground carob pods, a tannin containing feed, when the diet had 5 to 10% of soyabean oil. With 49% ground carobs growth retardation could not be checked even when the diet contained 13% soyabean oil.

Alumot et al (1964) conducted trials to elucidate the cause of the growth inhibiting effect of carob meal in chick rations, with special emphasis on estimating its productive

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energy value and found that the presence of tannin like compounds affected appetite and resulted in reduced feed intake. But the low calorie value of carobs was the major factor responsible for growth depression in chicks.

Zorita & Sanz (1965) investigated the possibility of utilising acorn meal, a tannin containing feed, for feeding table poultry. In experiments with unsexed chickens in batteries, maize in the diet was partly or completely replaced by acorn meal as 5, 10, 15, 20.5, 30 or 40.5% of the feed. Content of the soyabean meal was changed to keep the protein content of the diet constant, assuming the protein from acorns was not digested. Weight and efficiency were worse with 30 or 40% acorn meal but were not significantly affected by smaller amounts. This suggests that the deleterious effect of the acorn meal at 30 to 40% level may be due to the presence of larger amounts of tannins in the ration.

Fuller et al (1966) reported the growth depressing action of sorghum gmain tannin on the basis of feeding trials with chickens. Twenty one varieties of grain sorghum as 50% of the diet replaced a mixture of maize, maize oil and soyabean meal in the basal diet in trials with chickens from day old to 8 weeks of age. All diets were equal in M. E. and protein content and were adequately fortified with minerals and vitamins. Some varieties of sorghum were unsatisfactory. Those varieties had characteristically dark seed coat and much tannin. Amounts of 1.6% or more tannin in grain sorghum depressed growth when

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given as 50% of the diet.

Vohra et al (1966) studied the growth depressing and toxic effects of tannins in chicks. They found that different plant tannins, tannic acid, D-catechin, gallic acid or pyrocatechol at 1% level in the diet based on maize, soyabean meal and fishmeal significantly reduced body weight of chickens 3 weeks old. With 5% tannic acid in the diet 70% of the chickens died between 7 and 11 days of the test, 0.5% significantly reduced body weight. Addition 0.1% methionine or ornithine or 0.5% choline or betaine did not prevent the growth depressing effect caused by 1% tannic acid. Body weight, efficiency of food conversion, metabolisable energy of the diet and nitrogen retention were significantly less with 2% tannic acid in the diet. Cholesterol in the blood was found to be increased but the absorption of fat remained unchanged.

Fuller et al (1967) conducted series of trials with chicks to adopt the means to detoxify the effects of dietary tannic acid. Experiments were with cocks from day old to ages from 18 to 28 days. In the first experiment the diet was supplemented with additional 0.09 or 0.18% methionine hydroxy analogue (MHA), 0.045, 0.09, or 0.135% choline and no tannic acid or 0.5 or 1% tannic acid according to a factorial design. Growth was depressed significantly by 1% tannic acid and slightly by 0.5%. Toxicity of 1% tannic acid was reduced by 0.18% MHA. Increasing amounts of choline with each amount of MHA had no effect on growth rate with either amount of tannic acid. In the next

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experiment diets were given alone or with betaine, choline or MHA each 0.1 or 0.2% and 1% tannic acids according to a factorial design. Depression of growth caused by tannic acid was reduced slightly by 0.2% MHA without extra choline or betaine, choline or betaine reduced toxicity of tannic acid only when MHA was given. In the next 2 experiments diets were supplemented with 0.5, 0.10, or 0.20% MHA or chline, 1% arginine, 0.75% ornithine, or 0.5 or 1% tannic acid. Depression of growth caused by tannic acid was reduced by arginine especially when MHA and choline were given. The depressing effect of 0.5% tannic acid was prevented by MHA, choline, and either arginine or ornithine. In 2 other experiments MHA and arginine had an additive effect in reducing growth depressing effect of tannic acid. The following were the feed requirement per gram of gain by different groups : no supplements, 1.48 g.; with 1% tannic acid and no MHA or extra arginine, 1.71 g. and with 1% tannic acid and MHA and arginine, 1.51 g.

Potter et al (1967) studied the effect of tannic acid on egg production and egg yolk mottling in hen. Three groups of 24 laying hen in individual cages were given for 5 weeks a diet based on maize and soyabean meal alone or with 1 or 2% tannic acid. All groups were given the basal diet for 3 weeks. Eggs were collected for 2 days before tannic acid was given and thereafter on 2 days each week. Egg production was significantly reduced by 2% but not by 1% tannic acid. By the end of the recovery period egg production in all the three groups was similar to that at the start of the trial. Egg yolk mottling was

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significantly increased by either 1% or 2% tannic acid and the effect disappeared in the first week of the recovery period. Yolks of eggs from hens given tannic acid was coloured olive green. The white of the egg was not affected.

Potter (1968) studied the toxicity of tannic acid and the mechanism(s) for its detoxification in chickens. Tannic acid was fed to young chicks at levels of 0, 0.5 and 1.0% of their diet. Choline, methionine, arginine, ornithine were added to the diets singly and in various combinations at each level of tannic acid fed. The growth rate of chicks was significantly depressed when either level of tannic acid was fed, the depression being more severe at the highest level. Supplemental choline and methionine were partially effective in alleviating tannic acid induced growth depression. The combination of methionine, choline and arginine (or ornithine) further reduced the toxicity of tannic acid at 1.0% level and completely alleviated the effects of 0.5% tannic acid in a manner suggesting additive effect of these nutrients.

✓Clandinin & Heard (1968) estimated the tannin content of 8 samples of prepress solvent and solvent extracted rapeseed meal ranging from 2.71 to 3.35%. On the basis of the work of Vohra et al (1966) they have opined that the tannin content of the rapeseed meal may affect adversely the growth of chickens and M. E. value of the diet when a high proportion of it is incorporated in the mash mixture.

Conner et al (1969) reported the growth depressing

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effect of tannins of sorghum grain to growing chickens. Grains of three varieties of sorghum with different tannin contents were given to growing chickens as 70% of their diet in three experiments. Tannic acid was given also as 0.1% or 1.0% of the grain component in one of the experiments. Growth was retarded as tannin content of the grain increased. The retardation produced by an increase in plant tannin or addition of tannic acid could be only partly alleviated by supplementation with large amounts of methionine and choline. Increase of tannic acid or plant tannin seemed to depress feed intake and had a toxic effect. Addition of tannic acid significantly depressed fat content of liver and weight of testis. Additional methionine plus choline prevented the depression of liver fat but did not prevent the retardation in testis weight.

Kaushal et al (1971) investigated the possibility of utilisation of oak (Quercus incana) kernels in poultry mashes. Oak kernels were incorporated in chick starter mashes at 0, 2, 5 and 10% levels replacing an equal quantity of maize. There was no significant difference in growth rate of chicks during the age of 10 to 59 days on 0, 2, and 5% level of incorporation though chicken on 10% level showed a significant growth depression. There was also a gradual decline in feed efficiency and N-retention per gram of mash intake with the increasing level of oak kernels in the mash. The high tannin content in oak kernels was suspected to play an incriminating role. On boiling and washing with water, the tannin content in oak kernels was reduced slightly from 5.2 to 4.2% on D.M. basis. The effect of incorpo-

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-rating treated kernels in chick mash at 0, 5, 10 and 15% levels at the expense of maize was studied. During the age of 10 to 50 days, there was an increasing growth depression with increasing levels of treated kernels in mash. The feed efficiency also declined with increase in proportion of treated kernels in the mash.

RATS AND MICE :

Joslyn & Glick (1969) studied the comparative effects of gallotannic acid and related phenolics on the growth of rats. Weanling male rats were given free water and diets containing gallotannic acid, 3 types of condensed tannins, ellagic acid, gallic acid or catechin at 5% and from 2 to 10% for periods upto 30 days. Food intake and growth were reduced by catechin and gallic acid but less than by tannic acid. Tannic acid at 5, 8, and 10% caused a higher mortality in a shorter time than did other compounds. Mortality was not necessarily correlated with growth depression. Ellagic acid had no adverse effect.

Glick & Joslyn (1970a) further investigated the adverse effect of tannic acid in the rat. Food intake, weight, haemoglobin and fat in liver were estimated for weaning male rats weighing 50 g. and older rats weighing 100, 130 and 200 g. Rats in control group had free access to the basel diet while those in the test groups had free access to the diets containing 4, 5 or 8% tannic acid or were pair fed on the same amount of basal diet. Tannic acid depressed food intake and growth and at 8% caused 90% mortality in weanling rats. Rats weighing 130

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and 200 g. tolerated large amounts of tannic acid better than weanling rats and partly adopted to the tannic acid diet after a few days. Growth depressions thereafter was caused by factors other than reduced food intake. There was no anaemia or fatty liver with 5% tannic acid. When 40% casein was given with 5% tannic acid, food intake and weight gain were almost double than those with 20% casein and 5% tannic acid. Neither 1% choline hydrochloride nor 1.5% methionine had any effect on growth rate when given with 5% tannic acid.

Glick & Joslyn (1970b) also studied the effect of tannic acid and related compounds on the absorption of protein in the rat. Nitrogen in feces was estimated for male rats weighing 50 to 400 g. given diet containing 2 to 8% tannic acid, 2 to 8% gallic acid, 2% D-catechin, 5% ellagic acid or 5% condensed tannins from grape seed and quebracho for 12 to 53 days. N in feces was more than the control values with 2% tannic acid and continued to increase with larger amounts of tannic acid. Condensed tannins from grape seed and quebracho had little or no effect on N is feces. There was no difference in the recovery ^{14}C in the feces from rats given 5% tannic acid or controls given casein levelled with ^{14}C in the diet. The proteolytic enzyme activity of intestinal contents, compared with that of control rats, was increased three-fold by 5% tannic acid and four-fold by 5% grape seed tannin. Tannic acid did not affect the size or proteolytic activity of the pancreas.

Mitjavila et al (1970) reported the adverse effect of

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gallotannic acid on intestinal absorption of glucose and methionine in mice. Mice intestine was perfused with 10 ml. of perfusion medium consisting of glucose, methionine and butyric acid each at a concentration of 2 g. per litre and after that the perfusate was collected for 1 hour and the amounts absorbed were estimated. In the experiment the intestine was perfused for 3 consecutive hours, during the second hour, values obtained were taken as normal control and during the 3rd hour gallotannic acid was added in concentrations of 0.5, 1, 5, 10 or 25 mg. per litre. Gallotannic acid at a concentration of 1 mg. per litre significantly decreased absorption of glucose and methionine which was about 6 and 10% respectively less than the control values. The absorption of butyric acid was not significantly decreased under 1 mg. per litre but 10 mg. decreased it by about 7%.

Tamir & Alumot (1970) made comprehensive study on carob tannins growth depressing activity and level of insoluble nitrogen in the digestive tract of rats. Male albino rats were given for 150 days diets containing green carobs, ripe carobs, ripe carobs without sugar, a hot water extract of green carobs, tannins from green and ripe carobs, an ethyl acetate extract of green carobs, a control diet or the control with sucrose. The d=ecrease in growth of rats given green carobs or ethyl acetate soluble polyphenols was thought to be caused by decreased food intake. Tannins from ripe carobs also caused significant decrease in growth while those from green carobs had less effect. The feces and digestive tract contents from rats given tannins from green or ripe carobs or ethyl acetate extracts of carobs

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contained more nitrogen than control rats. The increase was in the insoluble - N fraction and were correlated with the degree of growth depression. The activities of digestive enzymes in the caecum were increased in all the experimental groups.

(III). 'ARTIFICIAL RUMEN' TECHNIQUES :

Two main lines of approach have been employed in the study of biochemical reactions in the rumen namely, 'In vitro' and 'In vivo' experiments. 'In vitro' experiments involve collection of rumen contents from rumen of slaughtered animals or from alive animals provided with rumen canula and the subsequent incubation of these contents with suitable substrates in the laboratory under conditions, as much as possible, simulating intact rumen. This approach is suitable for theoritical studies and in studies of applied nature such as, evaluation of forage quality. This is advantageous than 'In vivo' procedure owing to its simplicity and other factors which can be controlled in 'In vitro' conditions but not under normal rumen conditions. But there is always a danger that the results obtained in this way do not always apply in the rumen 'In vivo'. 'In vivo' experiment is confined to use of animals from which rumen contents can be obtained as required through the suitable canula. The advantage of 'In vivo' method is that the reactions occur in the natural environment but this method is subject to many disadvantages. The process is laborious and sampling on large scale is difficult and exposed to great errors. As the rumen contents are not stationary, it is difficult to study the kinetics of

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various biochemical reactions that occur in the rumen.

These limitations led the workers to adopt 'In vitro' method of study and as such various types of artificial rumen techniques have been described by various workers from time to time in an attempt to make the 'In vitro' conditions more closer to those in the intact rumen.

The early developments in 'In vitro' techniques for studying rumen microbial activities consisted primarly of incubating stained rumen liquor in glass vessels with various substrates and measuring microbial activities in several ways.

Wegner et al (1940) were very critical to the 'artificial rumen techniques' in the sense that there was no movement and diffusion in this method. They used whole rumen content in artificial rumen technique.

Quin (1943) incubated various carbohydrates with rumen fluid in gas tight flasks and measured gas production with manometer. Although the system was crude, it was concluded that in view of the close relations between ruminant digestion and bacterial activity, the nutrition of ruminants was vitally linked to various products derived from bacterial metabolic activities.

Elsden et al (1946) adopted the procedure of incubation of rumen contents of mixed microbial population to study rumen fermentation which added a new chapter in the field of rumen physiology. They used 'washed suspensions' of rumen

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micro-organisms for this purpose. Since then various improve-m-ents have been made in this field.

Marston (1948) improved the technique by making attempts to regulate the pH of the content with addition of suitable buffer solutions and maintaining anaerobic environment to make conditions more closer to 'In vivo' environment.

Louw et al (1949) further improved the technique by using semipermeable vessel which dialysed the fermentation products by diffusion simulating closer to the rumen conditions.

Further modifications were made by Huhtanen et al (1954) by using cellophane sac suspended in a 4 oz. screw cap jar containing a solution similar in mineral composition to sheep saliva. The sac contained substrate and rumen fluid and was held in position by screwing the cap on the sac. The whole artificial rumen was small enough to be placed in incubator. The outside bath consisted of approximately 100 ml. of Mc Dougall's artificial saliva.

Bentley et al (1954) carried out 'In vitro' fermentation experiments with rumen micro-organisms in litre low actinic erlenmyer flasks which were arranged in a series in a thermostatically controlled water bath (38° - 39°C) to permit individual gassing of the flasks with carbon dioxide. The flask stoppers were fitted with two tubes and one, which was used to admit CO_2, extended to the bottom of the flask. The other tube, which extended only through the stopper, served as vent. The length

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of fermentation period was 24 to 48 hours and no addition other than sodium carbonate, were made to medium during the experiments.

Warner (1956[a]) described an 'artificial rumen' which attempts to reproduce as closely as possible conditions in the rumen of living animals. It consisted of a cellophane dialysing sac, holding rumen fluid and substrate, suspended in a complex mineral solution based upon the composition of rumen liquor. Provision was made for a constant supply of 5% CO_2 in nitrogen and for periodic removal of samples and addition of substrate. The criteria which can be used to assess the normality of microbial behavier in such a system over a period of incubation were discussed. They included direct microscopic counts of protozoa and bacteria, records of motility, and rate of breakdown of cellulose, starch and casein. It has been claimed that the conditions in the said artificial rumen reflect normal rumen functions of the particular animal concerned over a period of 8 hours.

Protasenja (1956) devised a new method of studying digestion of carbohydrates, proteins and fats in animals with fistulae. An appratus was illustrated in which starch, egg, protein or fat can be prepared in the form of stiff sticks 3 cms. long. Such sticks were enclosed in a perforated capsule suspended in the digestive tract through fistula for digestion studies.

Fina et al (1958) adopted a new artificial rumen

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technique for studying rumen digestion 'In vivo'. A porcelain tube, permeable to VFA but not to bacteria, connected to a glass frothing tube and gas escape mechanism, was suspended in the rumen of steer with a fistula. Maintenance of anaerobiosis was assisted by oxygen free nitrogen through the connected manifold. The inoculum for the tube was prepared by straining rumen fluid, allowing particles to seperate out at 40°C, and using relatively particle free layer of liquid. Tubes containing cellulose but no inoculum showed no digestion, tubes containing 500 mg. cellulose and inoculum showed complete digestion in 48 hours. By adding successive increaments of cellulose, digestion at a continuous rate was maintained for 10 days.

Davey et al (1960) devised an improved artificial rumen designed for continuous control during prolonged operation and compared the findings with those of the rumen in the living animal. A stream of nitrogen and CO_2 was bubbled through the rumen contents in a dialysing sac, capacity about 1 litre, and then over the continuously changing physiological saline in the outer compartment. Tubes filled in the two compartments allow for the removal of samples, addition of substrates and recording of pH and temperature. The appratus was run for a period of 14 d=ays and the bacteriological and chemical analyses of the contents of the rumen sac were compared with those of the rumen contents of the cow from which the initial artificial rumen inoculum had been taken. The artificial rumen was "fed" daily on the same hay, clover or leucerne as the cow from which the rumen inoculum had been taken and with amounts proportional to

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the relative volumes of the natural and artificial rumen, a proportional volume of artificial saliva was also added. The counts of bacteria, amounts of VFA and dry matter digestion coefficients were similar in the cow and artificial rumen. In artificial rumen pH values were some what lower. It was concluded that in all essentials the artificial rumen closely simulated the activity of the natural rumen.

El-shazaly et al (1960) made a comparative study on the efficiency of all glass, semipermeable membrane, and continuous flow types of appratus for 'In vitro' rumen fermentations. On the basis of cellulose degradation and production of total VFA and ammonia during 30 hours, the all glass appratus of Bentley et al (1954), a semipermeable type of Wrner (1956) and an appratus like the last, but with dialysis in a continuously flowing mineral solution, were compared. It was observed that there was little difference between them, the all glass type was preferred for its simplicity.

Donefer et al (1960) adopted an 'In vitro' fermentation technique in which 90 ml. centrifuge tubes were used as fermentation vessels. 700 mg. of substrate and 50 ml. of an inoculum nutrient buffer solution containing urea, glucose, biotin, para aminobenzoic acid, casein hydrolysate and valeric acid were used. To this solution 20 ml. of phosphate buffer extract was added per tube. Screw caps were used on each tube for adjusting the flow rate of CO_2 to 150 bubbles per minute and the sample was fermented in the water bath at 39°C.

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Tilley et al (1960) developed an 'In vitro' technique in which 1 gm. sample of substrate in 75 ml. centrifuge tubes fitted with gas release valves were used. Inoculum nutrient buffer solution consisted of 40 ml. Mc Dougall's artificial saliva and 10 ml. of strained rumen fluid per tube. The tube contents were flushed with CO_2 and immediately stoppered and fermented at 39°C in an incubator.

Stewart et al (1961) used continuous culture as method for studying rumen fermentation. The fermentation vessel consisted of an 8 litre glass tank with a plastic lid, inlet and outlet tubes, sampling tube and stirrer. Substrate, ground hay or hay with concentrate, suspended in artificial saliva, was added intermittently by a solenoid operated valve and culture was removed through a second valve which operated when the volume reached a predetermined point. The dilution rate was about 10% of the culture volume per hour. Operations were carried out for about 24 hours. The appratus was initially furnished with CO_2, but was not completely air tight and gas formation by the culture was relied on to keep conditions anaerobic. The production of VFA and the numbers of oligotrich protozoa and coliform were studied. The results suggested that normal rumen conditions were simulated in the appratus and that it could be used for more detailed investigations.

Baumgart et al (1962) developed a more simplified artificial rumen for the repeated estimates of forage nutritive value. The artificial rumen consisted of a 125 ml. conical

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flask capped with a rubber stopper and equipped with a bunsen valve. Mc Dougall's artificial saliva was used during their 'In vitro' studies. This system was compared to the semipermeable membrane system and no appreciable difference was found in cellulose digestion or VFA patterns.

Gray et al (1962) described an artificial rumen for fermentation studies. A gauze container, gentley shaken in a glass vessel held at 40°C, held rumen contents and artificial saliva was added continuously to the vessel at the same rate as fluid was drawn off. A mixture of 95% N_2 and 5% CO_2 was passed through the gas space. Regenerated cellulose tubing was wound around the gauze container and a dialysing medium, physiological saline, was pumped through at an appropriate rate. To test the system acetic, propionic and butyric acids labelled with ^{14}C were introduced into the rumen of a sheep and 2 hour later a sample of rumen contents was withdrawn and placed in the apparatus. During the next three hours the percentage decline and the specific activity of each acid was about the same 'In vivo' as in 'In vitro' but fermentation appeared to be slower in 'In vitro'.

Tilley & Terry (1963) have used a two stage technique for 'In vitro' digestion of forage crops in which they have combined a microbial digestion by rumen micro-organisms with an acid pepsin digestion and found high correlation (r = +0.97) between digestibility estimated 'In vitro' and 'In vivo' for a wide range of forages.

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Barnes et al (1964) made a comparative study on different 'In vitro' rumen fermentation methods for measuring rate of cellulose digestion. The study consisted of two experiments; the first involved four 'In vitro' methods described by different workers and three substrates fermented over four time periods. The second experiment involved three 'In vitro' methods utilising alfalfa meal substrate studied over five fermentation periods. Differences were found between fermentation periods and an interaction between periods and methods occured. The interaction was primarily the result of changing rates of cellulose digestion between time periods. It was suggested that the primary factors contributing differences between methods were size of substrate, nutrient media, preparation of inoculum, maintenance of anaerobiosis and type of fermentation vessel.The variability between duplicate samples decrease as the length of the fermentation period increased. Differences were also found to exist between methods in the magnitude of the within trial variability.

Looper et al (1966) described an 'In vitro' procedure for studying starch digestion by rumen micro-organisms. Washed cell suspensions of rumen micro-organisms in a buffered nutrient medium at pH 6.8 were incubated with purified water insoluble maize starch for 8 hours at 39°C. Incubations were in pyrex tubes with CO_2 bubbled continuously through the medium. Starch was estimated gravimetrically by filtering the contents of the fermentation tubes through Gooch crucible with asbestos fibre pads and estimating loss on ignition after drying.

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Brent (1967) described a continuous flow 'In vitro' fermentation system and used in the study of utilisation of protein and several NPN sources by rumen micro-organisms. A system of the dialysing, continuous flow type, designed to sample automatically both fermentation liquor and dialystate was used in the study of the utilisation of soya protein and various NPN sources and a control to which no NPN was added. Of these nitrogenous sources only soya protein and 1, 3 dimethyl urea appeared to be hydrolysed to an extent sufficient to be useful to the ruminant. They claimed that this system duplicates closely in quality and quantity reactions 'In vivo' and end products and also maintains an active protozoal populations throughout the five day fermentation period.

Aafjes & Nijhof (1967) devised a simple artificial rumen for VFA production. The appratus was a glass vessel, volume 18 litres, with a plastic lid. Through a hole in the lid a plunger operated at a speed 5 r.p.m. was attached to a nylon gauze filled with hay. The glass vessel was fitted with outflow and kept in a constant temperature water bath at 40°C. There were also inlet holes for gas and introduction of artificial saliva which was automatically controlled by a titrator connected to a pH meter. When pH dropped below 6.7, artificial saliva was introduced. At the start of the experiment the vessel was fitted with 10 litres of solutions containing 26.6 g. Na_2HPO_4 and 117.6 g. $NaHCO_3$. The bag attached to the plunger contained 400 g. hay. When temperature equilibrium reached another bag of nylon gauze containing rumen contents from a cow consuming

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similar hay was introduced and placed at the bottom of the vessel. The artificial rumen was fed twice daily at 9 A.M. and 5 P.M. The bags remained there for two days after which they were removed after pressing out as much as possible of the rumen content. This experiment lasted for 7 weeks. The VFA production was normal but the protozoal population was lower than in the natural rumen. More than 50% of the hay dry matter was digested in artificial rumen. The VFA production was estimated to be 200 g. per 24 hours. The concentration of acetic acid in the artificial rumen was lower and that of propionic and butyric was higher than in the normal rumen content.

Griffiths (1967) described an improved artificial rumen technique for the study of rumen fermentation. The results obtained with a permeable artificial rumen was compared with measurements 'In vivo' with a cow. The pH and production of ammonia, VFA and lactic acid were measured. The artificial rumen was found to be satisfactory for measuring treatment differences but was not accurate enough for quantitative measurements. There were significant correlations between the molar preparations of VFA's found by both methods but there was no significant correlation between 'In vivo' and 'In vitro' methods for pH and ammonia production.

Czerkawski (1967) described an improved appratus for the incubation of rumen liquor in semipermeable sacs inside the bovine rumen. It was found practicable, using one animal, to incubate simultaneously samples of stained diluted rumen liquor

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in three identical vessels, each of 100 to 120 ml. capacity for periods upto 23 hours. The pH value of the reaction mixture varried little compared with pH changes that occured inside the rumen and the sampling technique was satisfactory. The microbial growth was greatest when the rumen liquor contributed about 20% of the reaction mixture and when the protozoa and large food particles were removed. He claimed that the appratus was capable of giving reproducible results and could be adopted for investigations on different aspects of fermentation in the rumen. The appratus provided results in conditions similar to those obtained 'In vivo' and yet did not suffer the difficulties associated with other types of studies of fermentation inside the rumen namely, poor sampling and flow of the reaction medium. It could be possible, with this appratus, to study the kinetics of many reactions occuring in the rumen under conditions approaching those existing 'In vivo'.

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C H A P T E R - III

MATERIALS AND METHODS.

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MATERIALS AND METHODS

Feeds used in the experiment:

The following feeds were used in the present experiment :-

(i) Salseed (decorticated).

(ii) Banana leaves.

(iii) Groundnut cake.

(i) Salseed:

Sal fruits were collected from the sal forest in Ranchi area in the month of May, 1971 when the fruits were mature a sample of which has been illustrated in Photograph No.1. The shells of the fruits were separated and seeds were collected from inside it. The seeds did not contain any covering on it. The seeds were oven dried at 105° - 110°C to a constant weight and powdered in bulk for future use.

(ii) Banana leaves:

Green banana leaves were collected from the Veterinary College campus and were chopped in to small pieces and then oven dried at 105°- 110°C to a constant weight. The dried leaves were finely powdered and stored for the experiment.

Preparation of tannin extracted feed samples:

About 20 g. powdered dried feed material was boiled in

PHOTOGRAPH No. 1

PHOTOGRAPH SHOWING MATURED SAL FRUITS USED IN THE EXPERIMENT.

distilled water each in a proportion of 1 : 40 (W/V) for half an hour. It was cooled and strained with muslin cloth and washed 2 to 3 times with about 100 ml. of distilled water and then collected in a petri dish and dried in oven to a constant weight.

(iii) Groundnut cake :

Decorticated groundnut cake sample of good quality was dried in oven and finely powdered and kept in container for experimental use.

Proximate analysis of feed samples :

Proximate analysis of the salseed and banana leaves was done as recommended in A.O.A.C. method (1960).

Estimation of 'Tannins' in feed :

Tannin in salseed and banana leaves sample was estimated by the A.O.A.C. method of tannin estimation in tea leaves (A.O.A.C., 1960). The detailed procedure of estimation is as follows :

Exactly 5 g. of feed sample was boiled with 400 ml. of distilled water for 30 minutes. It was then cooled and the infusion was filtered through glass wool and transferred in a 500 ml. volumetric flask and was diluted upto the mark and thoroughly mixed.

10 ml. of the infusion was taken in one litre capacity conical flask and to it was added 25 ml. of Indigo carmine

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solution and about 750 ml. of distilled water. The whole content was titrated against N/10 $KMnO_4$ solution. A little N/10 $KMnO_4$ was added in the content from the burette at a time and thouroughly stirred with a glass rod. The same was continued till colour changed from blue to light green and then $KMnO_4$ solution was added drop by drop till colour changed to bright yellow. The volume of N/10 $KMnO_4$ utilised was designated as "a".

100 ml. of infusion was transferred to a stoppered bottle and to it 50 ml. of gelatin solution, 100 ml. of the acid sodium chloride solution and 10 g. of powdered kaolin were added and the whole content was shaken several minutes. After allowing the mixture to settle, the supernatent was decanted and filtered through ordinary filter paper.

25 ml. of the filtrate was taken in 1 litre conical flask and to it 25 ml. of Indigo carmine solution and about 750 ml. of distilled water were added and titrated with N/10 $KMnO_4$ solution as before till bright yellow colour appeared. The volume of N/10 $KMnO_4$ used was noted as "b" (25 ml. of the filtrate represented 10 ml. of infusion, because 100 ml. of infusion was diluted to 250 ml. as mentioned above).

<u>Calculations</u> :

The number of ml. of N/10 $KMnO_4$ used in "b" substracted from that of "a" gives the volume of N/10 $KMnO_4$ solution required to oxidise tannins in 10 ml. of the infusion i.e.(a-b).

Since 500 ml. of infusion was prepared from 5 gm of

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feed sample, 10 ml. of the infusion represented $\frac{5 \times 10}{500} = 0.1$ g. of the feed sample.

Now, $\because$ 1 ml. of N/10 $KMnO_4 \equiv$ 1 ml. of N/10 oxalic acid and

1 ml. of N/10 oxalic acid $\equiv$ .00416 g. of tannin (gallotannic acid).

Therefore, the percentage of tannin in the feed sample =

$$(a-b) \times .00416 \times 10 \times 100.$$

<u>Strained Rumen Liquor (S. R. L.) used for 'In vitro' Incubation</u>:

Rumen contents used for 'In vitro' incubations were collected from goats slaughtered at butcher shop situated adjacent to the Bihar Veterinary College, Campus. A wide mouth jar of 1 litre capacity with bakelite cap was first flushed with carbon dioxide gas from a gas cylinder and stoppered immediately in the laboratory for collection of rumen content. Rumen was slit open with a sharp scalpel immediately after slaughtering and the rumen contents were mixed thouroughly and then jar full of the content were collected directly from rumen to the jar and immediately stoppered. The content was immediately brought to the laboratory. In the laboratory the content was again flushed with carbon dioxide gas and the jar was placed in a water bath at 39°C for a few minutes. The rumen content was then strained with double folds of muslin cloth in 600 ml. conical flask already flushed carbon dioxide gas. The strained liquid, thus obtained was called "strained rumen liquor" and was used for incubation immediately.

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'In vitro' Incubation in the Artificial Rumen :

'In vitro' incubations were carried out in 100 ml. conical flask. The flasks were stoppered with rubber cork (no. 5A size) having two holes. Through the holes glass tubes were passed, one for the inlet and the other for the outlet of carbon dioxide gas. The inlet tube extended much below in the conical flask but above the level of incubation medium to ensure perfect anaerobiosis in the flask with carbon dioxide gas. The outlet tube extended only upto the lower end of the cork. The conical flasks were connected together in series with rubber tubing and arranged in two rows. The outlet of one flask was connected with the inlet tube of the next flask in the series. The inlet tube of first flask in each row was connected to one end of 'Y' tube and 'Y' tube was connected with an empty conical flask of 600 ml. capacity. The 600 ml. conical flask in turn was connected to carbon dioxide gas cylinder with pressure tubing. The inlet and outlet ends of each row were provided with Mohr's screw clips. The 100 ml. flasks with incubation medium were kept in an electrically operated, thermostatically controlled water bath at a constant temperature (39° - 40°C). The carbon dioxide was passed at a slow rate until it came out from the outlet tube in sufficient amount to turn barium hydrooxide solution milky or to extinguish a lighted match stick. The conical flasks were made air tight and were tested by putting water at the junction of rubber cork and the flask and also at the junction of inlet and outlet tubes. The inlet and outlet ends of

each row were closed with Mohr's screw clips when sufficient amount of gas was passed to ensure anaerobiosis. The flasks were gently shaken at an interval of half an hour. The carbon dioxide gas was passed at hourly interval to expel the gas produced during fermentation which might inhibit the activities of rumen microbes.

The flasks were previously charged with 2 ml. of M-phosphate buffer solution to maintain a pH of 6.5 and with other materials depending upon the treatments. Then 20 ml. of strained rumen liquor was pipetted after its preparation and were put in water bath for incubation. The time consumed for starting incubation after bringing the rumen contents in the laboratory did not exceed 15 minutes.

Details of Incubation work :

Three different series of incubation experiments were carried out with the three feed stuffs under investigation viz. salseed, banana leaves and groundnut cake.

The incubation experiments followed a general pattern with minor variations in certain cases depending upon the tannin content of the feed stuffs.

Degradation of proteins of salseed, tannin extracted salseed and extracted salseed with added tannic acid at 8 hours of 'In vitro' incubation (Experiment - I) :

The following is the arrangement of the incubation flasks in this experiment, each flask being in duplicate :

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(a) 20 ml. strained rumen liquor + 2 ml. M-phosphate buffer + 3 ml. distilled water (control).

(b) Control + 66.5 mg. tannic acid.

(c) Control + 500 mg. powdered salseed.

(d) Control + 332 mg. powdered tannin extracted salseed.

(e) Control + 332 mg. powdered tannin extracted salseed + 58.2 mg. tannic acid.

1. 332 mg. extracted salseed (in flasks d & e) was taken against 500 mg. salseed (c) to equate the level of protein in both the samples which was calculated on the basis of their crude protein percentage shown in Table No. 5.1, Chapter- V.

2. In column (e) of the above design 58.2 mg. of tannic acid was taken to equate the normal amount of tannin present in 500 mg. normal salseed in flask (c). The extracted salseed sample still contained 2.5% of tannin as shown in Table No. 5.2, Chapter - V, and accordingly, 332 mg. of extracted salseed contained 8.3 mg. tannin. Thus, total amount of tannin present in (e) comes to 66.5 mg. (58.2 mg. + 8.3 mg.) which is equal to amount of tannin present in 500 mg. normal salseed in flask (c).

<u>Protein degradation of banana leaves and tannin extracted banana leaves with and without added amounts of tannic acid at 8 hours of 'In vitro' incubation (Experiment-II)</u>:

The following is the arrangement of the incubation flasks (in duplicate) in this experiment :

(a) 20 ml. strained rumen liquor + 2 ml. M-phosphate

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buffer + 3 ml. distilled water (control).

(b) Control + 12.5 mg. tannic acid.

(c) Control + 62.5 mg. tannic acid.

(d) Control + 500 mg. powdered bannna leaves.

(e) Control + 500 mg. powdered banana leaves + 50 mg. tannic acid.

(f) Control + 500 mg. extracted powdered banana leaves.

(g) Control + 500 mg. extracted banana leaves + 12.5 mg. tannic acid.

(h) Control + 500 mg. extracted banana leaves + 62.5 mg. tannic acid.

1. Since tannin extracted sample of banana leaves sample contained approximately same amount of crude protein as in normal leaves, shown in Table No. 5.1, Chapter- V, the amount of extracted and unextracted leaves taken was kept same i.e., 500 mg. in both cases.

2. Five times more tannic acid than normal 2.5% tannin in banana leaves was adjusted in column (e) and (h) with a view to study the influence of high level of tannins (or tannic acid) on banana leaves protein degradation. In case of column (e) the amount actually added was 50 mg. tannic acid because 12.5 mg. of tannin was already present in 500 mg. banana leaves whereas 62.5 mg. (five times of normal tannin content) of tannic acid was added in (h) as tannin extracted banana leaves sample did not contain any amount of tannin as shown in Table No. 5.2,

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Chapter- V.

Protein degradation of groundnut cake without and with added amounts of tannic acid at 4 hours of 'In vitro' incubation (Experiment - III):

The following is the arrangement of incubation flasks (in duplicate) in this experiment :

(a) 20 ml. strained rumen liquor + 2 ml. M-phosphate buffer + 3 ml. distilled water (control).

(b) Control + 50 mg. tannic acid.

(c) Control + 100 mg. tannic acid.

(d) Control + 500 mg. powdered groundnut cake.

(e) Control + 500 mg. powdered groundnut cake + 50 mg. tannic acid.

(f) Control + 500 mg. powdered groundnut cake + 100 mg. tannic acid.

In all the above three experiments, NPN was estimated at the begining (0 hour) and at the end of incubation. Increase in the NPN level was taken as guide line to indicate the extent of protein degradation. The unincubated samples served as 0 hour sample of which 10 ml. in each case was at once deproteinized by Somogy's (1930a, b) method for NPN estimation. The same method was also adopted in all the samples after the end of incubation.

Estimation of Non-protein Nitrogen (NPN) :

NPN estimation in the samples was done as follows:

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(a) Deproteinization:

Somogy's method (1930a, b) of deproteinization, usually used for blood samples, was adopted as advocated by Drori & Loosli (1961) for rumen liquor. This method has the following advantages :-

(i) Deproteinization is done at the neutral pH.

(ii) The action is this case is instantaneous.

(iii) The reagents are cheap and readily available.

10 ml. of strained rumen liquor was pipetted in a 250 ml. conical flask. It was diluted with 70 ml. of distilled water and mixed. Then 10 ml. of 10% zinc sulphate solution was added to it and thouroughly mixed up by vigourous shaking. Then 10 ml. of 0.5 N sodium hydroxide solution was added drop by drop with constant shaking. The precipitate thus, formed was filtered through whatman No. 42 filter paper after few minutes standing and filtrate was used for NPN estimation.

(b) Digestion:

Micro kjeldahl digestion by Arnold-Gunning method was adopted. 50 ml. of the filtrate obtained, was pipetted in a 100 ml. conical flask and 1 ml. of concentrated sulphuric acid (A.R.) was added to it. The volume was reduced to about 5 ml. by gentle evaporation. It was then quantitatively transferred to a micro kjeldahl digestion flask by twice rinsing conical flask with distilled water. 3 ml. of concentrated sulphuric acid (A.R.),10 drops of 5% copper sulphate solution and about

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1 gm potassium sulphate (A.R.) was added to it. Few glass beads were also put into it to avoid bumping during digestion. The Micro kjeldahl flask with content was then placed on the micro kjeldahl digestion apparatus. The heating was continued till the mixture became clear i.e. light Greenish blue colour and no trace of black or yellow colour. It was then allowed to cool for distillation.

(c) Distillation of ammonia and titration :

The method of Meeker & Wagner (1940) was followed which involves absorption of liberated ammonia into boric acid solution. This method is based on the principle that 1% boric acid solution has a pH of approximately 4.8, which can serve as an accurate end point with the indicater used. When ammonia is distilled into boric acid solution, the solution of boric acid at once turns alkaline to methyl red. N/28 sulphuric acid is added drop by drop to turn the colour of the solution to original colour, light pink. The amount of standard sulphuric acid consumed is equivalent to the amount of ammonia distilled into boric acid solution. The advantages in this method are as follows :

(1) Only one standard solution (sulphuric acid) is needed and in this case need of standard NaOH solution, which changes slightly its strength on keeping for long time in the glass container, is eliminated.

(2) The errors of titration by difference is avoided, especially when small amount of ammonia is distilled.

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(3) There is no danger of exhausting the standard solution in the receiver by neutralisation with ammonia in this method.

The procedure adopted is noted below :

After completion of digestion the content in the kjeldahl flask was transferred quantitatively into Markham's distillation apparatus using distilled water. 20 ml. of 4% boric acid solution containing Meeker's indicator (consisting of methyl red and methylene blue) was taken in a 100 ml. conical flask, with two marks at 50 ml. and 80 ml. The tip of the condenser of Markham's distillation apparatus was always kept dipped in the boric acid solution. Steam was then passed in to the distillate at a very slow rate and 40% sodium hydroxide (A.R.) solution was added slowly until a blackish precipitate appeared. Then steam was allowed to pass through the digesta at a moderate speed. When distillate was collected upto the 50 ml. mark to ensure the complete distillation of all the ammonia present, the receiver was lowered down and the tip of the condenser was s washed with distilled water. The receiving flask was then kept for titration. The distillation was stopped, and the inner chamber of the Markham's distillation apparatus was washed with distilled water which was allowed to pass through back suction in the outer chamber and finally removed out through the lower outlet of the outer chamber. In this way the Markham's set was cleaned and made ready for the next distillation. The set of incubation, digestion and distillation has been shown in Photo

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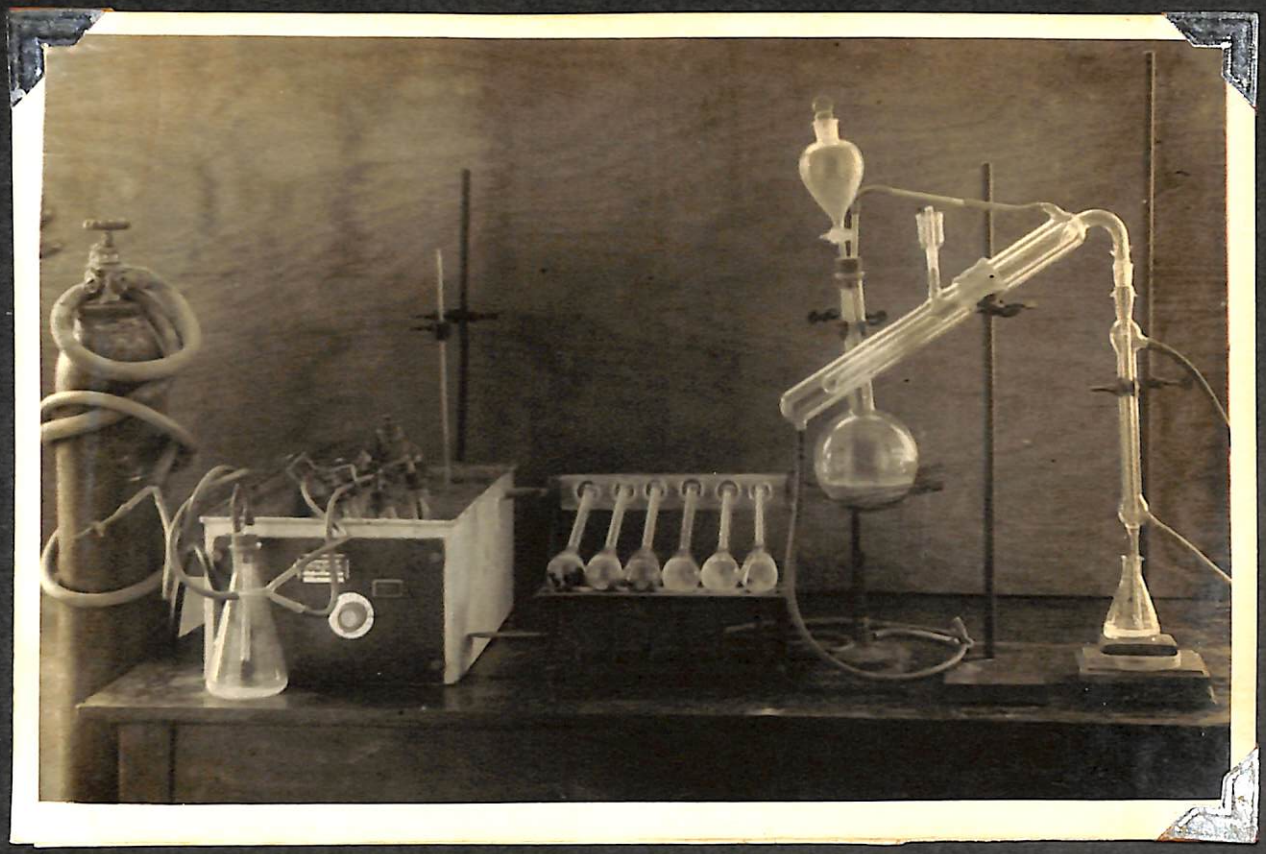

PHOTOGRAPH No 2.

PHOTOGRAPH SHOWING SETS OF INCUBATION, DIGESTION AND DISTILLATION USED IN THE EXPERIMENT.

graph No. 2.

The distillate collected in the conical flask was diluted with distilled water to the 80 ml. mark. Then the whole distillate was titrated against N/28 H_2SO_4 solution with the help of a 10 ml. micro-burette with graduation of 0.05 ml. The end point of the titration was judged by comparing the colour with another flask containing 20 ml. of boric acid with indicator diluted to 80 ml. with distilled water.

Blank titrations were also done to check the presence of ammonia as impurity in the reagents used but no ammonia in traceable amount was detected.

(d) Calculations for Non-protein Nitrogen :

Since 10 ml. of incubation medium was diluted to 100 ml. with distilled water and deproteinizing agents and only 50 ml. of the filtrate was taken for NPN estimation, the original amount of incubation medium used for NPN estimation was 5 ml.

25 ml. of incubation medium contains 20 ml.of rumen liquor.

Therefore, 5 ml. of incubation medium contains $\frac{20 \times 5}{25}$ = 4 ml. of rumen liquor.

5 ml. of incubation medium = 'A' ml. of N/28 H_2SO_4

$\equiv$ 'A' x 0.5 mg. NPN

(Since 1 ml. of N/28 H_2SO_4 $\equiv$ 0.5 mg. of N)

or 4 ml. of rumen liquor (S.R.L.) = A x 0.5 mg. NPN.

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Therefore, 100 ml. of rumen liquor (S.R.L.) $= \frac{'A' \times 0.5 \times 100}{4}$ mg. NPN

$= A \times 12.5$ mg. NPN

The number of ml. of N/28 H_2SO_4 solution consumed was multiplied with 12.5 to give the exact amount of NPN in milligrams (mg.) present per 100 ml. of rumen liquor (S.R.L.).

Extent of overall protein degradation from different feeds on incubation:

The extent of increase in NPN after incubation was regarded as an estimate of protein degradation. The increase in NPN value of each incubation flask after end of the incubation (8 hours or 4 hours as may be the case) was obtained by substracting the NPN value of incubated flasks from that of the corresponding unincubated flasks (0 hour).

REAGENTS USED :

I. For tannin estimation :

(1) N/10 Oxalic acid solution - 1.575 g. of oxalic acid (A.R.) crystals dissolved in 250 ml. of glass distilled water.

(2) N/10 $KMnO_4$ solution - 1.333 g. of potassium permanganate (A.R.) dissolved in one litre of distilled water and standardised against N/10 oxalic acid solution (freshly prepared).

(3) Indigo carmine solution - 6 g. of indigo carmine (sodium indigotine disulfonate) dissolved in 500 ml.

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distilled water by heating. It was cooled and then 50 ml. of concentrated sulphuric acid was added to it and volume was made up to 1000 ml. and filtered through ordinary filter paper (freshly prepared).

(4) Gelatin solution - 25 g. of pure gelatin was soaked in saturated sodium chloride solution for an our. Then it was heated until gelatin was dissolved. It was then cooled and diluted with saturated sodium chloride solution to 1 litre.

(5) Acid sodium chloride solution - 975 ml. of saturated sodium chloride solution was acidified with 25 ml. of concentrated sulphuric acid.

(6) Kaolin powder pure.

II. For incubation and NPN estimation :

(1) M-Phosphate buffer (pH 6.5) - 113.8 g. Na_2HPO_4 12 H_2O (A.R.) and 92.8 g. KH_2PO_4 (A.R.) per litre of distilled water.

(2) Zinc sulphate 10% solution : - 100 g. zinc sulphate (A.R.) in 1 litre of distilled water.

(3) 0.5 N sodium hydroxide solution - Solution No. 2 and 3 were so related that when 10 ml. of zinc sulphate solution was titrated with sodium hydroxide solution, 10.8 to 11.2 ml. of the alkali solution was required to produce a permanent pink colour in the present of phenolphthalein. 10 ml. of zinc sulphate solution was dilu-

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-ted with 50 to 70 ml. of distilled water before the titration and the alkali was added slowly with constant shaking.

(4) Copper sulphate 5% solution - 5 g. copper sulphate (A.R.) dissolved in 100 ml. distilled water.

(5) Boric acid 4% solution - 40 g. boric acid (A.R.) dissolved in some hot distilled water and volume was made upto a litre.

(6) Meeker's Indicator - 25 mg. of methylene blue and 100 mg. of metyl red in 100 ml. of absolute alcohol.

2 ml. of meeker's indicator was added per litre stock solution of 4% boric acid.

(7) Standard sulphuric acid (A.R.) - N/28 H_2SO_4 solution.

(8) Strong Alkali (Approx.40%) - Saturated sodium hydroxide (A.R.) solution was prepared which is approx. 40% in concentration.

STATISTICAL METHODS ADOPTED:

Analysis of variance (Snedecor, 1946) was done to know whether the difference in protein degradation by rumen micro-organisms between different treatments were statistically significant. The following set up of the analysis was adopted:

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Sources of variation	d.f.	S.S.	M.S.	F.
Between treatments	n - 1	W	$\frac{W}{n-1}$ =A	A/C
Between samples.	p - 1	X	$\frac{X}{p-1}$ =B	B/C
Error.	(N-1)-(n-1) -(p-1)	Y-(W+X)	$\frac{Y-(W+X)}{(N-1)-(n-1)-(p-1)}$ = C	
Total:	N-1	Y		

Where,

d.f. denotes degree of freedom.

S.S. denotes corrected sum of squares.

M.S. denotes mean square.

N. denotes total number of observations.

n. denotes total number of treatmentss

p. denotes total number of samples.

W. denotes corrected sum of squares between treatments.

X. denotes corrected sum of squares between samples.

Y. denotes total corrected sum of squares.

A. denotes mean square between treatments.

B. denotes mean square between samples.

C. denotes mean square of Error.

The analysis of variance table provides a ready means of testing the significance of the difference between class means. A comparision of the mean square between treatments with

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that of the error provides a test of significance of difference arising from the different treatments. Similarly a comparision of the mean square between samples with that of error provides the test of significance of the difference arising from the samples. The comparision is made by finding the ratio of the mean squares concerned to the error mean square. This ratio is denoted by 'F'. If calculated value of 'F' exceeds the 'F' value obtained from the standard table the difference between treatments is considered significant. It is denoted significant or highly significant on the basis of its significance at 5% level or 1% level respectively.

Critical difference :

Where ever the 'F' was significant, the difference between any two of the treatments was tested by the critical difference. The critical difference (C.D.) was calculated both at 5% level and 1% level by the following formulae :

$$C.D.\ 5\% = t_{5\%}\ (\text{Error d.f.})\ \sqrt{S^2\left(\frac{1}{r_1} + \frac{1}{r_2}\right)}$$

$$C.D.\ 1\% = t_{1\%}\ (\text{Error d.f.})\ \sqrt{S^2\left(\frac{1}{r_1} + \frac{1}{r_2}\right)}$$

Where,

$t_{5\%}$ denotes 't' table value with error d.f. at 5% level.

$t_{1\%}$ denotes 't' table value with error d.f. at 1% level.

S^2 denotes Error mean square.

r_1 & r_2 denotes number of replicates of the treatments.

The 'critical difference' value was then compared

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with the difference between averages of the two treatments. If the difference between averages exceeded the 'critical difference' value, it was denoted significant or highly significant according to 5% level or 1% level.

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CHAPTER - IV

ESTIMATION OF ERRORS IN VARIOUS EXPERIMENTAL PROCEDURES.

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ESTIMATION OF ERRORS IN VARIOUS EXPERIMENTAL PROCEDURES

Efficiency of A.O.A.C. (Lowenthal-procter) method of Tannin estimation :

The recovery of tannic acid from its known solution was tried for this purpose. A known volume (10 ml.) of standard solution of pure tannic acid (5%) was taken and volume was made upto 500 ml. in a 500 ml. volumetric flask. 10 ml. of this aliquot (diluted solution) was taken for the estimation of tannic acid as described in the chapter of "materials and methods." The amount of tannic acid recovered was expressed in terms percentage of the actual amount present as shown in Table No. 41 below :

TABLE NO. 4.1

Table showing recovery of tannic acid in standard tannic acid solution (%).

No. of observations	Amount of tannic acid present in 10 ml. of 5% tannic acid solution.	Amount of tannic acid recovered.	Recovery % ± S. E.
3	500 mg.	488.8±6.0 mg.	97.76 ± 1.2

The result mentioned above shows a high accuracy of A.O.A.C. method of estimation of tannin adopted in this work, as recovery was almost complete.

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Efficiency of markham's distillation appratus in nitrogen estimation :

A known amount of ammonium chloride (A.R.) in solution was distilled in markham's distillation appratus to find out the amount of distillate to be collected for complete distillation of ammonia. 20 ml. of distillate was collected in first instance, followed by two successive 10 ml. distillates in separate conical flaks, each of the flasks containing 20 ml. of 4% boric acid with indicator. Each of the three distillates were diluted u=pto 80 ml. mark in 100 ml. conical flask and titrated against N/28 H_2SO_4 making the end point matching the colour with another conical flask containing 20 ml. of 4% boric acid and diluted to 80 ml. with distilled water. The result is shown in Table No. 4.2 below :

TABLE NO. 4.2

Table showing the recovery of nitrogen in successive fractions of distillate (%).

No. of observations.	I Fraction 20 ml.	II Fraction 10 ml.	III Fraction 10 ml.
3	98.21 ± 0.17	1.72 ± 0.11	Nil

It is apparent from the above table that the IIIrd fraction of 10 ml. distillate invariably did not contain any nitrogen and that about 99.9% of nitrogen was recovered in first 30 ml. fraction.

It is also clear from the table that the major portion of ammonia was distilled in to the first 20 ml. fraction, but to ensure complete distillation, 10 ml. more distillate was collected as a routine procedure.

Efficiency of Somogy's method of deproteinization for rumen liquor:

It has been observed by Pearson & Smith (1943a) that the estimated value of NPN differs in rumen liquor with trichloroacetic acid, alcohol and sodium tungstate in sulphuric acid as protein precipitant. So the method which gives consistant results with varying concentration of NPN in the rumen liquor is the precipitant of choice.

In the present investigation only Somogy's method (1930a, b) of deproteinization was adopted and its efficiency was tested.

For this, recovery of NPN was done after addition of different concentrations of standard ammonium chloride solution (prepared from A.R. NH_4Cl) in equal volume of S.R.L. Results obtained has been summerised below in Table No. 4.3.

TABLE NO. 4.3

Table showing recovery of added ammonium chloride nitrogen in rumen liquor.

I	II	III	IV	V	VI
Particulars	No. of observations	Amount of NH_4Cl-N added (mg.)	NPN estimated mg.	Amount of NH_4Cl-N recovered (mg.) ± S.E.	Recovery % ± S.E.
2.5 ml.S.R.L.	3	-	0.40	-	-

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Table No. 4.3 cont'd.

I	II	III	IV	V	VI
2.5 ml.S.R.L. +2.5 ml. NH_4Cl (1.0%) solution.	3	6.542	6.90	6.500±0.087	99.35 ± 1.33
2.5 ml.S.R.L. +2.5 ml. NH_4Cl (0.5%) solution.	3	3.271	3.66	3.260±0.067	99.68 ± 2.05

After addition of NH_4Cl solution to the S. R. L., it was immediately deproteinized by Somogy's reagents and NPN was estimated. The result obtained in Table No. 4.3 show a high accurecy of Somogy's method of deproteinization as recovery of NH_4Cl-N was almost complete indicating complete precipitation of proteins of rumen liquor.

Moreover, this process simultaneously tests the efficiency of both digestion and distillation, after deproteinization.

A close agreement between duplicates of the incubation medium in the experimental flasks after incubation during the course of present investigation also points to the accuracy of various analytical steps involved in this work. The agreement between duplicates, for instance, is shown below in Table No. 4.4 below which has been obtained during the course of present study with salseed.

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TABLE NO. 4.4

Table showing agreement between duplicates of same samples in NPN estimation of salseed, IIIrd incubation (ml. of N/28 H_2SO_4/ 4 ml. S.R.L.)

...

Flask description	ml. of N/28 H_2SO_4	
	0 hour	8 hour
20 ml. S.R.L. + 2 ml. M-phosphate buffer + 3 ml. distilled water (control).	2.05	3.15
-do-	2.00	3.00
Control + 500 mg. salseed.	2.15	2.30
-do-	2.15	2.25
Control + 500 mg. extracted salseed.	2.10	2.85
-do-	2.10	2.85

It is apparent from the table presented above that there are slight variations between the duplicates which may be ascribed both to experimental error and slight biological variation in the incubation media.

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C H A P T E R - V

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Our knowledge on various aspects of ruminant's metabolism is based on 'In vivo' and 'In vitro' studies carried out by different workers. 'In vitro' studies have been generally favoured due to their simple approach to the problem with control over wide range of variations and also on economical ground. In the present investigation 'In vitro' incubations with Goat's rumen micro-organisms were carried out to study the effect of natural tannins and tannic acid on protein degradation in rumen.

Results obtained in the present study have been presented under following headings :

I. (a) Proximate composition of salseed, banana leaves and their extracted samples.

(b) Estimation of tannins in salseed, banana leaves and their extracted samples.

II. Protein degradation of salseed, extracted salseed and extracted salseed with added tannic acid at 8 hours of 'In vitro' incubation.

III. Protein degradation of banana leaves and extracted banana leaves without and with added tannic acid at 8 hours of 'In vitro' incubation.

IV. Effect of added amounts of tannic acid on the degradation of easily fermentable sources of proteins like groundnut cake at 4 hours of 'In vitro' incubation.

I. (a) Proximate composition of salseed, banana leaves and their extracted samples:

The proximate composition of feed samples under study has been estimated by appropriate A.O.A.C. (1960) methods as indicated in the chapter of 'Materials and Methods'. The results are presented in Table No. 5.1.

TABLE NO. 5.1

Table showing proximate composition of salseed and banana leaves and their extracted samples.

	Moisture %	Crude protein %	Ether extract %	Crude fibre %	Nitrogen free extract %	Total Ash %
1. Salseed (R.M. basis)	9.75	7.30	15.25	4.71	60.82	2.17
-do- (D.M. basis)	-	8.00	16.71	5.16	67.75	2.38
2. Extracted salseed (R.M. basis).	8.90	11.30	17.60	2.60	58.09	1.51
-do- (D.M. basis)	-	12.40	19.32	2.85	63.77	1.66
3. Dried banana leaves (R.M. basis)	10.91	13.19	7.40	16.85	38.42	13.23
-do- (D.M. basis)	-	14.80	8.31	18.91	43.13	14.85
4. Extracted banana leaves (R.M. basis).	9.85	13.28	8.50	18.96	36.76	12.65
-do- (D.M. basis)	-	14.73	9.43	21.03	40.78	14.03

A study of Table No. 5.1 shows that salseeds contain a moderate amount of C. P., a low crude fibre and high ether extract and N.F.E. contents. Our results are very similar to those of Saxena (1967) whose findings on D. M. basis are : C.P. 9.20, E. E. 10.00, C. F. 4.80, N. F. E. 73.20 and total ash 2.80 percent and Nayak et al (1967) who obtained on R. M. basis a composition of : Moisture 5.23, C. P. 6.16, E. E. 16.77, C.F. 4.81, N. F. E. 63.25 and total ash 3.78 percent.

Proximate composition of banana leaves indicates that it is fairly rich in protein and mineral contents. Our results are similar to those obtained by Johri et al (1967) on D.M. basis as follows : C. P. 12.18, E. E. 8.06, C. F. 25.04, N. F. E. 38.64 and Total Ash 15.54.

A comparison of the composition of salseed and extracted salseed indicates that extracted sample is higher in C. P. and E. E. contents and lower in C. F., N. F. E. and Total Ash contents. Thus, it appears that the process of extraction removed some amounts of soluble ash and N.F.E. along with tannin as a result of which the C.P. content showed corresponding increase. The Ether Extract content, however, did not show similar trend because, as was observed during extraction, small droplets of fat globules were also extracted during the process. As for extracted and unextracted banana leaves, no appreciable difference in proximate composition was observed because of the low amount of tannin present in the leaves removed during extraction.

(b) Estimation of tannin in salseed and banana leaves:

Tannin in feed samples was estimated by Lowenthal - Proctor (A.O.AC., 1960) method as described in 'Materials and Methods' chapter and the results obtained have been presented in Table No. 5.2.

TABLE NO. 5.2

Table showing amounts of tannin present in salseed, banana leaves and their extracted samples.

Feeds.	Tannin %(pooled sample)			Tannin %
	I	II	III	Mean ± S.E.
Salseed.	12.89	13.31	13.70	13.30 ± 0.23
Extracted salseed.	2.50	2.91	2.08	2.50 ± 0.23
Banana leaves.	2.50	2.29	2.70	2.50 ± 0.12
Extracted banana leaves.	Nil	Nil	Nil	Nil

Table No. 5.2 shows that tannin concentration is fairly high in salseed and low in banana leaves. It is interesting to note that the tannin content was removed completely from banana leaves and over 80 percent from salseed samples by the simple method of extraction procedure adopted as described in the 'Materials and Methods' chapter. Apparently the tannins present in these two substances appear to exist in rather simple form and not in complex combination with cell constituents.

Tannin content in salseed was found to be 3.5% by Panda et al (1969). They have also shown that soaking with water (1:4) for 24 hours or boiling for 30 minutes removed about 60% of the tannin from salseed. The great differences observed in the tannin concentration from our results could possibly be due to different strains of the salseed or due to difference stages of maturity when the salseeds were collected. Another possible reason for the same may be the different physical state of the salseed from which extraction was done. In our experiment salseed in the form of fine powder was extracted while Panda et al (Loc. cit) have not detailed that whether the extraction was made from intact decorticated seeds or in the form of powder.

II. Protein degradation of salseed, extracted salseed and extracted salseed with added tannic acid at 8 hours of 'In vitro' incubation:

It appears from Table No.5.2 that salseed contain a fairly high amount of tannin. Earlier reports have shown that although the salseed is a rich source of energy along with moderate amount of protein, its utilisation as a feed for ruminents is limited owing to deletarious effect of tannin present in it. The present work has been under taken to know whether the tannin at the level of its presence in salseed interfere with the protein breakdown and also to know if there is any benefit of the extracted sample over unextracted on its utilisation for ruminents.

As described earlier in 'Materials and Methods' chapter, powdered salseed and extracted powdered salseed samples,on

equi-protein level basis were incubated with rumen liquor obtained from slaughtered goats in different incubation flasks. The detailed set up of the experiment has been mentioned in 'Materials & Methods' chapter. The results obtained has been shown in Table No.5.3. Each of the readings are mean of observations of two incubation flasks in each treatment.

TABLE NO. 5.3

Table showing protein degradation of salseed, extracted salseed and extracted salseed with added tannic acid at 8 hours of incubation(mg. of NPN/100 ml.of S.R.L.).

Treatments.	Samples of rumen liquor											
	I			II			III			IV		
	Hours of incubation		Increase in NPN on 8 hours of incubation.	Hours of incubation		Increase in NPN on 8 hours of incubation.	Hours of incubation		Increase in NPN on 8 hours of incubation.	Hours of incubation		Increase in NPN on 8 hours of incubation.
	0 hour	8 hours		0 hour	8 hours		0 hour	8 hours		0 hour	8 hours	
20 ml.S.R.L.+ 2 ml.M-phosphate buffer + 3 ml. distilled water (control)	26.56	53.44	26.88	16.25	36.59	20.34	25.31	38.44	13.13	15.62	27.50	11.88
Control +66.5 mg. tannic acid.	26.56	40.31	13.75	16.25	26.87	10.62	25.31	33.12	7.81	15.62	22.81	7.19
Control +500 mg. salseed.	27.81	29.06	1.25	19.06	20.00	0.94	26.87	28.44	1.57	19.06	20.00	0.94
Control +332 mg. extracted salseed.	26.87	45.31	18.44	18.44	27.19	8.75	26.25	35.62	9.37	18.44	25.00	6.56
Control + 332 mg. extracted salseed+ 58.2 mg. tannic acid.	26.87	29.69	2.82	18.44	22.19	3.75	26.25	27.50	1.25	18.44	21.87	3.43

Increase in NPN as a result of protein degradation in different treatments has been presented in Table No. 5.4 for the sake of comparison.

TABLE No.5.4.

Table showing increase in NPN with salseed, extracted salseed and extracted salseed with added tannic acid at 8 hours of incubation (mg. of NPN/ 100 ml.S.R.L.).

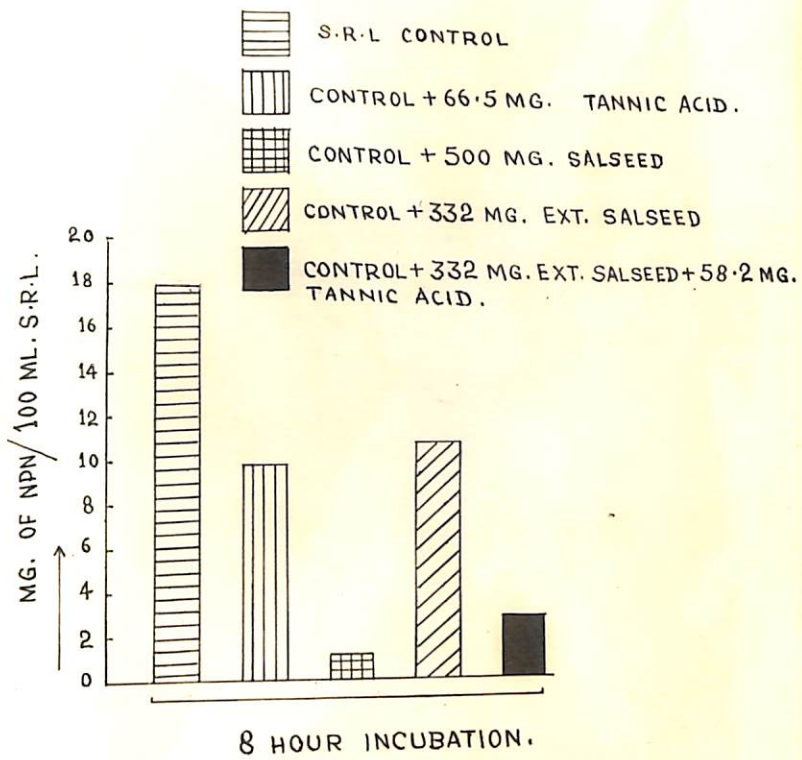
Treatments	Samples of rumen liquor				Average increase in NPN
	I	II	III	IV	Mean ± S.E.
20 ml.S.R.L.+ 2 ml.N-phosphate buffer + 3 ml distilled water (control).	26.9	20.3	13.1	11.9	18.0 ± 3.5
Control + 66.5 mg. tannin acid.	13.7	10.6	7.8	7.2	9.8 ± 1.5
Control + 500 mg. salseed.	1.2	0.9	1.6	0.9	1.1 ± 0.5
Control + 332 mg. extracted salseed.	18.4	8.7	9.4	6.6	10.8 ± 2.6
Control + 332 mg. extracted salseed + 58.2 mg. tannic acid.	2.8	3.7	1.2	3.4	2.8 ± 0.5

N.B.:- All results have been presented upto 1st decimal place only/ Keeping in view the expected accuracy in such experiments.

A study of Table No. 5.3 and 5.4 shows varying extent of protein breakdown with different treatments with almost all samples of rumen liquor at 8 hours of incubation.

PHOTOGRAPH No. 3.

DIAGRAM SHOWING THE EXTENT OF PROTEIN BREAKDOWN OF SALSEED, TANNIN EXTRACTED SALSEED AND EXT. SALSEED WITH ADDED TANNIC ACID DURING 8 HOURS OF INCUBATION.



The increase in NPN after end of incubation has been regarded as the sole guide for protein degradation. The variations in the extent of protein breakdown in different samples of rumen liquor with and without added substrates may be due to different levels of protein-N and soluble carbohydrates in the rumen liquor with different microbial populations, as there was no control over the diet of animals slaughtered. Experimental errors and natural biological variation of such type of experiments may also account for the such variations. However, the constant close agreement between duplicate incubation flasks have considerably added confidence to the result obtained in each incubation.

Table No. 5.4 and Photgraph No. 3 reveals the following facts :

1. Addition of 66.5 mg. tannic acid to control depressed the extent of NPN increase to almost half as compared with the control alone.

2. Addition of salseed (500 mg.) containing (13.3%) tannin and extracted salseed plus added tannic acid to make it at the same level as in unextracted sample greatly reduced the NPN level as compared with that of extracted salseed containing 2.5% tannin. The increase in NPN level were 1.1 and 2.8 mg./100 ml.S.R.T. for salseed and extracted salseed plus tannic acid respectively and 10.8 mg./100 ml.S.R.T. for extracted salseed.

3. Great difference in the extent of NPN increase has been observed between salseed and extracted salseed the amounts being 1.1 mg./100 ml. S.R.L. in former and 10.8 mg./100 ml. S.R.L. in latter.

4. Difference in the extent of NPN increase is also observed between salseed (1.1 mg./100 ml. S.R.L.) and extracted salseed plus added tannic acid to same level (2.8 mg./100 ml. S.R.L.).

To know whether the apparent differences observed, between different treatments are statistically significant, the mean data of Table No. 5.4 have been subjected to 'F' test by means of analysis of variance as shown in Table No. 5.5.

TABLE NO. 5.5

Analysis of variance of protein hydrolysis during 8 hours of incubation of salseed samples.

Sources of variation	d.f.	S. S.	M. S.	F.
Between treatments.	4	737.28	184.32	17.77**
Between samples.	3	134.61	44.87	4.33*
Error.	12	124.43	10.37	
Total:	19	996.32		

N.B.:- * denotes significant at 5% level.

** denotes significant at 1% level.

Table No. 5.5 clearly indicates that variations between different treatments are highly significant. The variations between samples of rumen liquor has also been found to be significant which is quite natural as the rumen liquor samples were collected from different animals of variable age without any controlled feeding.

Since variations between treatments were found significant on the basis of analysis of variance, a comparison between the extent of protein degradation between any two different treatments was made by critical difference test shown in Table No. 5.6.

TABLE NO. 5.6

Comparison of protein hydrolysis between different treatments during 8 hours of incubation.

Sl. No.	Treatments	Differences between averages.	Critical difference	
			5% level	1% level.
1.	20 ml.S.R.L. + 2 ml.M-phosphate buffer + 3 ml.distilled water (control).	-	4.96	6.95
2.	Control Vs. control + 66.5 mg. tannic acid.	8.2**		
3.	Control Vs. control + 332 mg. extracted salseed.	7.2**		
4.	Control + 66.5 mg. tannic acid Vs. control + 500 mg. salseed.	8.7**		
5.	Control + 66.5 mg. tannic acid Vs.control +332 mg.extracted salseed +58.2 mg.tannic acid.	7.0**		

Cont'd. Table No. 5.6

Sl. No.	Treatments	Differences between averages	Critical difference 5% level	Critical difference 1% level
6.	Control + 500 mg. salseed Vs. control + 332 mg. extracted salseed.	9.7**		
7.	Control + 500 mg. salseed Vs. control + 332 mg. extracted salseed + 58.2 mg. tannic acid.	1.7 N.S.		
8.	Control + 332 mg. extracted salseed Vs. control +332 mg. extracted salseed + 58.2 mg. tannic acid.	8.0**		

N.B.:- ** denotes significant at 1% level.

N.S. denotes non-significant.

It is evident from Table No. 5.6 that difference between any two treatments are highly significant excepting difference between salseed Vs. extracted salseed plus tannic acid.

It has already been described that the amounts of salseed and extracted salseed has been taken on equi-protein level basis for incubation. But under this situation the amount of soluble carbohydrates differ in the two, the amount being 304 mg. in former and 192.5 mg. in latter. The difference of 111.5 mg. of soluble carbohydrates exists between the two treatments. It has also been described that total amounts of tannin have been kept same between 500 mg. salseed and 332 mg. of extracted salseed plus 58.2 mg. of tannic acid (8.3 mg. salseed

tannin + 58.2 mg. tannic acid) although the sources of tannins are different.

With regard to the effect of natural tannins or tannic acid on protein degradation, it is clear from the comparison between control and control + 66.5 mg. tannic acid that the added tannic acid has greatly reduced the extent of protein breakdown. It is clear that the substrates were otherwise same in the rumen liquor in both cases. As the difference between the above two treatments is highly significant, tannic acid can be taken as the sole factor responsible for the inhibitory effect on rumen liquor protein breakdown. Such inhibiting effect has been observed in all the four rumen liquor samples presented in Table No. 5.4.

Addition of 58.2 mg. of tannic acid to 332 mg. of extracted salseed has also greatly reduced the protein breakdown from it (Sl. No. 4 and 5 of Table No. 5.4). It is also important to note that in this case too, the amounts of all organic and inorganic nutrients in the two cases are equal. This furnishes another support of the view that added tannic acid has greatly inhibited the protein breakdown during incubation.

The extent of NPN increase between salseed and extracted salseed has also been found to be highly significant. The amount of tannin present in two treatments were 66.5 mg. and 8.3 mg. respectively and the amount of soluble carbohydrates in two treatments were 304 mg. and 192 mg. respectively.

The significant difference in the NPN levels at the

end of incubation in the flasks with unextracted and extracted salseeds may be due to the combined effect of the following two opposing factors viz:

(i) The inhibitory effect of the larger amount of tannin (66.5 mg.) in the unextracted salseed on its protein degradation.

(ii) Increased synthesis of microbial protein from NPN with unextracted salseed which contained 304 mg. of soluble carbohydrate as compared to 192 mg. in extracted salseed.

Of these two factors the first one seems to be of decidedly greater importance as the level of tannin between the two is very wide whereas the level of soluble carbohydrate in the two flasks although different might have been in excess in both.

Our findings on the inhibitory effect of feed tannins find supports from observations of Volcani et al (1963), Leroy et al (1964), Tagari et al (1965), Delort and Zelter (1968) and Zelter et al (1970).

III. Protein degradation of banana leaves and tannin extracted banana leaves without and with added tannic acid at 8 hours of 'In vitro' incubation:

From Banana growing areas of the state it has been learnt that ruminants do not consume banana leaves with choice. It was thought that probably, the tannin present in them imparted an astringent taste which limited its use as cattle feed in bulk. This work has been undertaken to ascertain whether the

tannin present in banana leaves at the level of its presence affects the breakdown of its protein which is fairly high in amount as shown in Table No. 5.2.

Powdered dried banana leaves and tannin extracted banana leaves with and without added amounts of tannic acid on equi-protein basis were incubated with goat's rumen liquor as described in "Materials and Methods" chapter. The detailed set up of the experiment has also been described in "Materials and Methods" chapter. The results obtained are presented in Table No. 5.7. Each of the readings are mean of two duplicate observations.

TABLE NO. 5.7

Protein degradation in banana leaves and tannin extracted banana leaves without and with added tannic acid at 8 hours of incubation (mg.of NPN/100 ml.S.R.L.).

Treatments	Samples of rumen liquor								
	I			II			III		
	Hours of incubation		Increase in NPN during incubation.	Hours of incubation		Increase in NPN during incubation	Hours of incubation.		Increase in NPN during incubation
	0 hour	8 hours		0 hour	8 hours		0 hour	8 hours	
20 ml.S.R.L.+ 2 ml. M-phosphate buffer + 3 ml.distilled water (control).	17.81	25.00	7.19	25.31	35.94	10.63	21.56	30.31	8.75
Control + 12.5 mg. tannic acid.	17.81	23.75	5.94	25.31	33.44	8.13	21.56	28.75	7.19
Control + 62.5 mg. tannic acid.	17.81	21.56	3.75	25.31	30.31	5.00	21.56	25.62	4.06
Control + 500 mg. dried banana leaves.	24.69	29.69	5.00	32.50	40.31	7.81	28.75	35.62	6.87
Control + 500 mg. dried banana leaves +50 mg.tannic acid.	24.69	27.50	2.81	32.50	36.56	4.06	28.75	32.50	3.75
Control + 500 mg. extracted banana leaves.	20.31	26.25	5.94	27.19	35.62	8.43	23.44	30.31	6.87
Control +500 mg.extracted banana leaves +12.5 mg.tannic acid.	20.31	25.00	4.69	27.19	34.06	6.87	23.44	29.37	5.93
Control +500 mg.extracted banana leaves + 62.5 mg.tannic acid.	20.31	21.56	1.25	27.19	31.56	4.37	23.44	27.19	3.75

Increase in NPN in various incubation flasks during 8 hours of incubation has been summerised in Table No. 5.8.

TABLE NO. 5.8

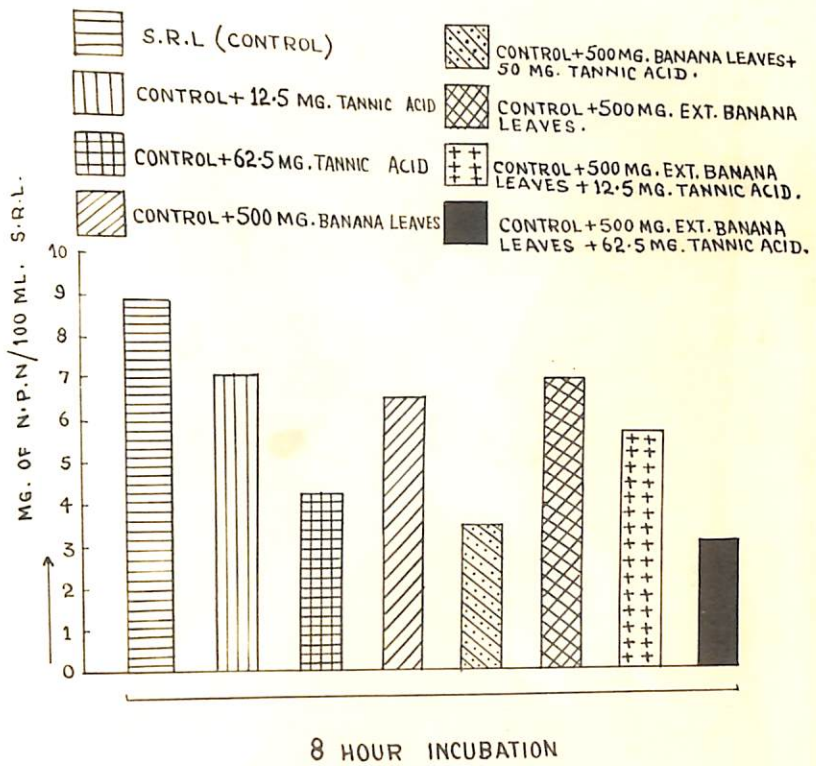
Increase in NPN in banana leaves and extracted banana leaves without and with added tannic acid at 8 hours of incubation (mg. of NPN/100 ml. S.R.L.).

Treatments.	Samples of rumen liquor I	II	III	Average increase in NPN Mean ± S.E.
20 ml.S.R.L. + 2 ml. M-phosphate buffer + 3 ml. distilled water (control).	7.2	10.6	8.7	8.8 ± 1.0
Control + 12.5 mg. tannic acid.	5.9	8.1	7.2	7.1 ± 0.6
Control + 62.5 mg. tannic acid.	3.7	5.0	4.1	4.3 ± 0.4
Control + 500 mg. banana leaves.	5.0	7.8	6.9	6.6 ± 0.8
Control + 500 mg. banana leaves + 50 mg.tannic acid.	2.8	4.1	3.7	3.5 ± 0.4
Control + 500 mg. extracted banana leaves.	5.9	8.4	6.9	7.1 ± 0.7
Control + 500 mg. extracted banana leaves + 12.5 mg.tannic acid.	4.7	6.9	5.9	5.8 ± 0.6
Control + 500 mg. extracted banana leaves + 62.5 mg. tannic acid.	1.2	4.4	3.7	3.1 ± 0.9

N.B.:- All results have been present upto first decimal place only, keeping in view the expected accuracy in such experiments.

PHOTOGRAPH No 4.

DIAGRAM SHOWING THE EXTENT OF PROTEIN BREAKDOWN OF BANANA LEAVES AND TANNIN EXTRACTED BANANA LEAVES WITH AND WITHOUT ADDED TANNIC ACID DURING 8 HOURS OF INCUBATION.



A study of Table No. 5.7 and 5.8 reveals a varying extent of protein breakdown in different flasks without and with added substrates, the tendency being the same as observed with salseed incubation experiment. The reasons for the same can be explained on the same line as it has been discussed in the case of salseed incubation experiment.

A study of Table No. 5.8 and Photograph No. 4 reveals the following facts :

1. Addition of 12.5 mg. of tannic acid to control reduced the protein breakdown in control from 8.8 to 7.1 mg. of NPN/100 ml. S.R.L., and addition of tannic acid to control at higher level of five times more (62.5 mg.), reduced the extent of protein breakdown to almost half than the control alone.

2. Addition of 50 mg. of tannic acid to 500 mg. banana leaves also reduced the extent of protein breakdown to almost half as compared with 500 mg. banana leaves during incubation.

3. A small reduction in protein breakdown has also been observed when tannic acid was added at low level (12.5 mg.) to 500 mg. extracted banana leaves. Protein breakdown was greatly inhibited when a high level of tannic acid (62.5 mg.) was added to 500 mg. extracted banana leaves, the average values being 5.8 and 3.1 mg. of NPN/100 ml. S.R.L. with low and high levels of tannic

acid respectively against 7.1 mg./100 ml. S.R.L. from extracted banana leaves with no tannic acid.

To know whether the differences observed between different treatments are statistically significant the mean data in Table No. 5.8 were subjected to 'F' test by means of analysis variance and the same has been presented in Table No. 5.9.

TABLE NO. 5.9

Analysis of variance of protein hydrolysis during 8 hours of incubation of banana leaves samples.

Sources of variation	d.f.	S.S.	M.S.	F
Between treatments.	7	83.42	11.92	54.18**
Between samples.	2	22.03	11.01	50.04**
Error.	14	3.09	0.22	
Total:	23	108.54		

N.B.:- ** denotes significant at 1% level.

Table No. 5.9 clearly shows that difference between treatments is highly significant. The difference between samples of rumen liquor has also been found highly significant which is quite natural because the rumen liquor samples were collected from different animals of variable age and under unknown feeding regime prior to slaughter resulting in varying microbial population and varying level of substrates in the

rumen liquor samples.

Since variation between treatments are highly significant on the basis of analysis of variance a comparison of the extent of protein hydrolysis between two different treatments was made by critical difference test which has been shown in Table No. 5.10.

TABLE NO. 5.10

Comparison of protein hydrolysis between different treatments during 8 hours of incubation.

Sl. No.	Treatments.	Difference between averages.	Critical difference at 5% level	Critical difference at 1 % level
1.	20 ml.S.R.L. + 2 ml. M-phosphate buffer + 3 ml. distilled water(control).	-	0.82	1.14
2.	Control Vs. control + 12.5 mg.tannic acid(TA).	1.7**		
3.	Control Vs. control + 62.5 mg. TA.	4.5**		
4.	Control Vs.control + 500 mg.extracted banana leaves.	1.7**		
5.	Control + 12.5 mg. TA Vs. control + 62.5 mg. TA.	2.8**		
6.	Control + 12.5 mg. TA Vs. control + 500 mg. banana leaves.	0.5 N.S.		
7.	Control + 12.5 mg. TA Vs. control + 500 mg.extracted banana leaves + 12.5 mg.TA.	1.3**		

Cont'd. Table No. 5.10

Sl. No.	Treatments.	Difference between averages.	Critical difference at 5% level	Critical difference at 1% level
8.	Control + 62.5 mg. TA. Vs. control + 500 mg. banana leaves + 50 mg. TA.	0.80 N.S.		
9.	Control + 62.5 mg. TA. Vs. control + 500 mg.extracted banana leaves + 62.5 mg.TA.	1.2**		
10.	Control + 500 mg. banana leaves Vs. control + 500 mg. banana leaves + 50 mg. TA.	3.1**		
11.	Control + 500 mg. banana leaves Vs. control + 500 mg. extracted banana leaves.	0.5 N.S.		
12.	Control + 500 mg. banana leaves Vs. control + 500 mg. extracted banana leaves + 12.5 mg. TA.	0.80 N.S.		
13.	Control + 500 mg. banana leaves + 50 mg. TA. Vs. control + 500 mg.extracted banana leaves + 62.5 mg.TA.	0.4 N.S.		
14.	Control + 500 mg. extracted banana leaves Vs. control + 500 mg. extracted banana leaves + 12.5 mg. TA.	1.30**		
15.	Control + 500 mg. extracted banana leaves Vs. control + 500 mg. extracted banana leaves + 62.5 mg. TA.	4.0**		
16.	Control + 500 mg.extracted banana leaves + 12.5 mg.TA. Vs. control + 500 mg. extracted banana leaves + 62.5 mg. TA.	2.7**		

N.B.:- ** denotes significant at 1% level.
N.S. denotes non-significant.

Critical difference test in Table No. 5.10 shows that treatment with low (12.5 mg.) and high (62.5 mg.) levels of tannic acid to control significantly reduced the protein breakdown. Since the amount of nutrients are same in all these three treatments, it can be concluded that added tannic acid at both levels has produced highly significant inhibiting effect on protein degradation in rumen liquor. The difference between treatments with 12.5 mg. and 62.5 mg. of tannic acid are also highly significant and thus, it indicates that higher level of tannic acid has produced more severe inhibition to protein degradation than the lower.

Further support of this view is obtained from a comparison of extracted banana leaves with those of extracted banana leaves with added levels (low and high) of tannic acid. Here, again the amounts of nutrients are equal in all the three treatments. At both levels of tannic acid the protein degradation has been significantly reduced. In this case too, the high level of tannic acid has produced a more severe inhibition on protein breakdown than low level, the difference between two being highly significant.

A comparison of banana leaves and banana leaves with 50 mg. added tannic acid also shows that the added tannic acid has produced a marked inhibition on protein degradation in banana leaves.

A comparison of banana leaves and extracted banana leaves shows that the presence of 2.5% of tannin in unextracted

banana leaves did not produce any significant inhibition on protein degradation, although addition of tannic acid at the same level to extracted banana leaves has produced significant inhibition on protein breakdown. The possible reason for the same could be different degrees of inhibitory effect of banana leaves tannin and tannic acid.

Incidently, it also appears from Table No. 5.8 that the average increase in NPN in control + 12.5 mg. tannic is at the same level as in control + 500 mg. extracted banana leaves. This accidental coincidence does not seem to have any significance since the substrates were different in them as also the latter did not contain any tannic acid.

From the above discussions it is quite clear that the added tannic acid at low and high levels significantly inhibit the protein breakdown and that the natural tannin of banana leaves does not seem to have any significant inhibitory effect at the level in which it is present. Thus, as far as tannin in banana leaves is concerned, it does not appear to be a limiting factor in its protein utilisation for the ruminants.

IV. <u>Effect of added amounts of tannic acid on the breakdown of groundnut cake protein at 4 hours 'In vitro' incubation</u>:

It has been established that the G.N. cake protein is degraded in the rumen with a rate which greatly exceeds the rate with which the micro-organisms are capable to utilise liberated NH_3 and other NPN for the synthesis of their body protein.

This results in an influx of NH_3 in the rumen which is largely absorbed in the blood through the ruminal wall and ultimately excreted in urine as urea and goes waste. In this experiment attempt has been made to restrict the rapid degradation of G.N. cake protein with tannic acid for the better ultimate utilisation of undegraded protein in the lower tract and also better utilisation of liberated ammonia at a slower rate for the microbial protein synthesis.

Powdered decorticated G. N. cake without and with added amounts of tannic acid was incubated in 'artificial rumen' for 4 hours with goat's rumen liquor as described in the chapter "Materials and Methods". The detailed setup of the experiment has also been described in the same chapter. The results obtained have been presented in Table No. 5.11. Each of the readings are mean of two duplicate observations.

TABLE NO. 5.11

Degradation of Groundnut cake protein without and with added amounts of tannic acid at 4 hours of incubation (mg. of NPN/100 ml. S.R.L.).

Treatments	Samples of rumen liquor								
	I			II			III		
	Hours of incubation		Increase in NPN during incubation.	Hours of incubation		Increase in NPN during incubation.	Hours of incubation.		Increase in NPN during incubation.
	0 hour	4 hours		0 hour	4 hours		0 hour	4 hours	
20 ml.S.R.L. + 2ml. M-phosphate buffer + 3 ml.distilled water (control).	12.50	22.50	10.00	40.00	44.69	4.69	5.94	13.44	7.50
Control + 50 mg. tannic acid.	12.50	17.81	5.31	40.00	42.19	2.19	5.94	10.00	4.06
Control + 100 mg. tannic acid.	12.50	15.94	3.44	40.00	41.50	1.50	5.94	8.44	2.50
Control + 500 mg. powdered G.N.cake.	21.25	51.87	30.62	50.31	74.37	24.06	14.69	32.50	17.81
Control + 500 mg. powdered G.N.cake + 50 mg. tannic acid.	21.25	32.81	11.56	50.31	58.44	8.13	14.69	22.19	7.50
Control + 500 mg. powdered G.N.cake +100 mg. tannic acid.	21.25	25.62	4.37	50.31	54.06	3.75	14.69	17.81	3.12

Increase in NPN in various incubation flasks during 4 hours of incubation has been summarised in Table No. 5.12.

TABLE NO. 5.12

Increase in NPN in Groundnut cake without and with added amounts of tannic acid at 4 hours of 'In vitro' incubation (mg. of NPN/100 ml. S.R.L.).

Treatments	Samples of rumen liquor			Average increase in NPN
	I	II	III	Mean ± S.E.
20 ml.S.R.L. + 2 ml. M-phosphate buffer + 3 ml. distilled water (control).	10.0	4.7	7.5	7.4 ± 1.5
Control + 50 mg. Tannic acid.	5.3	2.2	4.1	3.9 ± 0.9
Control + 100 mg. tannic acid.	3.4	1.5	2.5	2.5 ± 0.6
Control + 500 mg. G.N. cake.	30.6	24.1	17.8	24.2 ± 3.7
Control + 500 mg. G.N. cake + 50 mg. tannic acid.	11.6	8.1	7.5	9.1 ± 1.3
Control + 500 mg. G. N. cake + 100 mg. tannic acid.	4.4	3.7	3.1	3.7 ± 0.4

N.B.: - All results have been presented upto first decimal place only, keeping in view the expected accuracy in such experiments.

A study of Table No. 5.11 and 5.12 shows that there is a varying extent of protein breakdown in different rumen

PHOTOGRAPH No. 5.

liquor samples with or without added substrates during 4 hours of incubation. This variation can also be explained on the same line as it has been discussed in case of salseed incubation experiment.

There is a clear indication from the study of Table No. 5.12 and Photograph No. 5 that with addition of tannic acid at two levels (50 mg. and 100 mg. or 2 and 4 mg./ml. incubation media) to the rumen liquor samples with or without G.N. cake, there is a sharp decline in the extent of protein breakdown. This effect is more highly marked with G. N. cake than without. Higher (100 mg.) level of tannic acid has more severe effect than lower (50 mg.) level.

Addition of 50 mg. tannic acid to control has declined the NPN level from 7.4 mg. to 3.9 mg./100 ml. S.R.L. on average and addition of 100 mg. of tannic acid to control has further declined the level to 2.5 mg./100 ml. S.R.L.

There is a sharp rise in the NPN level of G.N. cake flask indicating, as expected, an extensive breakdown of G.N. cake protein by rumen micro-organism during 4 hours of incubation. The extent of increase in NPN level after incubation is 24.2 mg./100 ml. S.R.L. Our observations on groundnut cake are in agreement with the findings of a number of workers that soluble proteins are rapidly degarded in the rumen (Pearson and Smith, 1943a; Mc Donald, 1948, 1952; Chalmers et al, 1954; Chalmers & Synge, 1954; Annison et al, 1954; Annison, 1956, Elshazly, 1958; Blackburn & Hobson, 1960; Jasiorowski, 1961; Pagari

et al, 1962; Hogan, 1964 and Brochers, 1965).

Addition of 50 mg. (2 mg./ml. of incubation media) tannic acid to 500 mg. G.N. cake along with control has depressed the protein breakdown from 24.2 mg. to 9.1 mg. NPN/100 ml. S.R.L. and 100 mg. (4 mg./ml. of incubation media) tannic acid declined the level to 3.7 mg. NPN/100 ml. S.R.L. Thus, the protein degradation is reduced to about 1/3rd with lower and to about 1/6th with higher level of tannic acid during incubation. That the two levels of added tannic acid did not inhibit the protein degradation of G.N. cake completely will be evident from the comparison with the levels obtained from rumen liquor alone.

To ascertain whether the differences discussed above are statistically significant, the mean data of Table No. 5.12, were subjected to 'F' test by means of analysis of variance which is presented in Table No. 5.13.

TABLE NO. 5.13

Analysis of variance of protein degradation during 4 hours of incubation of Groundnut cake.

Sources of variation	d.f.	S.S.	M. S.	F.
Between treatments.	5	981.85	196.37	32.84**
Between samples.	2	53.57	26.78	4.48*
Error.	10	59.77	5.98	
Total:	17	1095.19		

N.B.:- ** denotes significant at 1% level.
* denotes significant at 5% level.

Table No. 5.13 makes it quite clear that the differences between treatments are highly significant. The differences between rumen liquor samples are also significant which are quite natural as they were obtained from slaughtered goats of variable age and under uncontrolled feeding régime.

Since between treatment variations are highly significant, a comparison of the rate of protein hydrolysis between different treatments by critical difference test has been made as shown in Table No. 5.14 below :

TABLE NO. 5.14

Comparison of protein hydrolysis between different treatments during 4 hours of incubation.

Sl. No.	Treatments.	Difference between averages.	Critical difference at 5% level	Critical difference at 1% level
1.	20 ml.S.R.L. + 2 ml. M-phosphate buffer + 3 ml. distilled water(control).	-	4.45	6.32
2.	Control Vs. control + 50 mg.tannic acid (TA).	3.5 N.S.		
3.	Control Vs. Control + 100 mg. TA.	4.9*		
4.	Control Vs. control + 500 mg. G.N. cake.	16.8**		
5.	Control + 50 mg. TA. Vs. control + 100 mg. TA.	1.4 N.S.		
6.	Control + 50 mg.TA.Vs. control + 500 mg. G.N. cake + 50 mg. TA.	5.2*		

Cont'd. Table No. 5.14.

Sl. No.	Treatments.	Difference between averages.	Critical difference at 5% level.	Critical difference at 1% level.
7.	Control + 100 mg. TA. Vs. control + 500 mg. G.N. cake + 100 mg.TA.	1.2 N.S.		
8.	Control + 500 mg. G.N. cake Vs. control + 500 mg. G.N. cake + 50 mg. TA.	15.1**		
9.	Control + 500 mg. G.N. cake Vs. control + 500 mg. G.N. cake + 100 mg. TA.	20.5**		
10.	Control + 500 mg. G.N. cake + 50 mg. TA. Vs. control + 500 mg. G.N. cake + 100 mg. TA.	5.4*		

N.B: - ** denotes significant at 1% level.
* denotes significant at 5% level.
N.S.denotes non-significant.

A study of Table No. 5.14 shows that although the difference of control Vs. control + 50 mg. tannic acid is non-significant there is a decline in the NPN to the extent of 3.5 mg. of NPN/100 ml. S.R.L. with addition of 50 mg. tannic acid to control. Addition of 100 mg. of tannic acid to control has caused a significant decrease in the extent of protein breakdown. The difference of the two treatments is 4.9 mg. NPN/100 ml. S.R.L. It is evident that the three treatments - control, control + 50 mg. tannic acid and control + 100 mg. tannic acid,

are identical excepting the added levels of tannic acid in the last two. Thus, the inhibition of the rumen liquor protein breakdown in the last two treatments mentioned above can solely be regarded due to the presence of added tannic acid. It is also clear that 100 mg. level has caused a significant inhibition to protein breakdown.

Addition of 500 mg. of G.N. cake to control has caused a sharp rise in NPN level and its difference with control is highly significant. This confirms that G.N. cake protein is rapidly degraded in the rumen.

A sharp decrease in NPN level with addition of 50 and 100 mg. (2 and 4 mg./ml. of incubation media) tannic acid to G.N. cake was observed. Higher level more strongly inhibited the protein breakdown. The levels being 9.1 and 3.7 mg. of NPN/100 ml. S.R.L. with two levels of tannic acid respectively, against 24.2 mg./100 ml. S.R.L. with G.N. cake (without tannic acid). In other words, 50 mg. (2 mg./ml) produced about 60% and 100 mg. (4 mg./ml.) about 87% depression on protein breakdown in G. N. cake. The depressive effect with two treatments are highly significant. The difference between two levels of tannic acid with G.N. cake is also significant. As the nurtients in these three treatments are qualitatively and quantitatively same the decline in the NPN level in the last two treatment could only be ascribed to the added amounts of tannic acid.

A comparison of control plus 100 mg. tannic acid with control plus G.N. cake plus 100 mg. tannic acid shows a non

significant difference but it is quite clear from this that the tannic acid, even at the level of 4 mg./ml. of incubation media did not completely prevent breakdown from groundnut cake.

Our results on the effect of tannic acid (tannins) on G. N. cake are in close agreement with the findings of earlier workers (Leroy et al, 1964; Murty and Khan, 1969 and Zelter et al, 1970).

DISCUSSION

'In vitro' incubation technique:

'Artificial rumen technique' was employed in this work to study the effect of feed tannins and added tannic acid on protein degradation in the rumen. 'In vitro' techniques have been usually favoured in the study of rumen metabolism due to their simple approach with control over wide range of variations. All glass type of Bentley et al (1954) with only modification that in our case 100 ml. conical flasks were used instead of 1 litre flaks was adopted in our experiment due to its simplicity and reliability. El-shazly (1960) compared the all glass, semi permeable and continuous flow types of apparatus for 'In vitro' rumenfermentations and found no major differences between types of apparatus. They favoured all glass system of Bentley et al (1954) because of its simplicity.

All the incubation flasks in different experimental setup were in duplicate. Close agreement of the duplicates as

a rule, indicated in Table No. 4.4 for a single incubation, is indicative of the efficiency and reliability of this technique. A further evidence on the score is obtained from the similarity in the trends of the results between different incubations of the experiments.

Addition of mineral solution of Burroughs et al (1950) was avoided in 8 hours incubation experiments with salseed and banana leaves due to presence of large amount of inorganic nitrogen in its composition. Although addition of such solution is favoured for longer hours of incubation for the normal microbial activity, but in our experiment additional source of nitrogen other than rumen liquor and substrates was bound to complicate the results. Moreover, a period of 8 hours for incubation was not considered long enough to make it essential to add mineral solution as is the case with cellulose digestion which is carried on usually for periods of over 12 hours.

Choice of incubation period:

The factor guiding the period of incubation is naturally the rate of protein degradation by rumen liquor to give a sizeable reading. The period of incubation has varied from 4 hours to few days in the hands of various workers depending upon their objectives, the periods being usually much longer in studies with digestion of cellulose. For protein degradation studies much shorter periods varying from 4 to 8 hours have generally been used (Annison, 1956; Reis and Reid, 1959;

Phillipson et al, 1962).

In a preliminary trial it was observed that, as expected, the increase in NPN was more at 8 hours of incubation than those of 4 and 6 hours of incubation with salseed and banana leaves. Thus, assuming that the proteins of salseed and banana leaves were relatively slowly degraded the period of incubation for these two feeds was fixed at 8 hours. On the other hand, period of incubation with G.N. cake as substrate was fixed at 4 hours owing to its known rapid degradation in the rumen (Annison et al, 1954; Annison, 1956; Murty and Khan, 1969).

As our studies were limited to the protein degradation the highest period of incubation did not exceed 8 hours.

Initial level of NPN in rumen liquor samples:

In all, ten rumen liquor samples were used in incubation work and analysed for initial NPN as shown in Table Nos. 5.3, 5.7 and 5.11. A study of these tables for the 0 hour NPN value in the rumen liquor (control) alone reveal a varying extent of NPN level in different rumen liquor samples, ranging from 5.9 to 40.0 mg./100 ml. of S.R.L. These variations are quite natural as the rumen liquor samples were obtained from slaughtered goats of variable age and under unknown feeding regime and at unknown intervals after feeding. Similar type of results with great variations in NPN value in rumen liquor of different species has been observed in the past by many workers.

The values of NPN in rumen liquor of sheep before feeding have been obtained by Tomas et al (1961) to range from 28.84 to 59.94 mg./100 ml. S.R.L. The values ranged from 8.1 to 35.0 mg./100 ml. S.R.L. (Khan, 1966) of goats, 3.5 to 38.0 mg./100 ml.S.R.L. (Srivastava, 1967) and 10 to 41.25 mg./100 ml. S.R.L. (Tripathi, 1970) of buffaloes. Generally low values observed by the last three workers mentioned above are due to their samples being obtained from slaughter house animals who are expected to be kept on no feed for varying periods before slaughter.

Protein hydrolysis in rumen contents:

A comparison of NPN content of the rumen liquor samples before and after 8 hours of incubation showed an increase in NPN ranging from 7.2 to 26.9 mg./100 ml. S.R.L. (Table Nos. 5.3 and 5.7) and those at 4 hours of incubation from 4.7 to 10.0 mg./100 ml. S. R.L. (Table NO. 5.11). Both the minimum and maximum values of NPN were nearly double in 8 hours of incubation as compared to 4 hours value. A point of interest that was observed, was that the extent of increase in NPN in rumen liquor (4 or 8 hours of incubation) apparantely did not seem to have any relation with their respective initial values (0 hour level). For example the increase in NPN values in three rumen liquor samples were 10.00, 4.69 and 7.50 mg./100 ml. S.R.L. respectively at 4 hours of incubation when their initial 0 hour levels were 12.50, 40.00 and 5.94 mg./100 ml. S.R.L. respectively. However, another interesting point emerged from the fact that samples of rumen liquor producing higher NPN in incubation

also produced higher amounts with substrates as well. The variations in NPN increase in different samples of rumen liquor could be due to combined effects of varying levels of protein-N, soluble carbohydrates and other organic and inorganic constituents, nature of the injested protein and nature and size of microbial population. A low level of NPN after incubation may be due to higher level of soluble carbohydrates promoting increased biosynthesis from NPN to microbial protein (Pearson and Smith, 1943b, Mc Donald, 1952; Annison et al, 1954; Warner, 1956b; Lewis and Mc Donald, 1958; Lewis, 1962; Henderick and Martin, 1963; Hoshino, 1965; Susova, 1968; Devuyst et al, 1968 and Kumar and Murty, 1970). Nature of injested protein is also an important factor, the extent of its breakdown is largely dependent on its solubility in rumen fluid. It has been established that the microbial population of the rumen fluctuates seasonally and that the variation in number and proportion of various species of the micro-organisms is due to the variation in the amount, composition and nutritive value of the diet (Pounden and Hibbs, 1948; Gall et al, 1949; Mason, 1950; Moir, 1951 and Blaxter, 1954).

Role of rumen micro-organisms in the utilisation of dietary nitrogen by ruminants:

Ammonia has been shown to be a major end product of protein degradation of several different proteins and NPN substances in rumen. The amount of ammonia formed has been shown to be dependant both upon the nature of the dietary protein and proportion of carbohydrates in the diet (Mc Donald, 1948a,b and

1952). The changes occuring in the rumen by intervention of rumen micro-organisms are advantageous to the animal when its diet contains mainly poor quality protein or a NPN material and are disadvantageous when large amount of dietary nitrogen consists of good quality protein owing to its extensive degradation to ammonia, a major portion of which goes waste ultimately as urea through urine. The merits and demerits of such variation, as they affect the nitrogen economy of the animal as a whole, has been extensively studied and attempts have been made to modify the process for the benefit of the animal. Thus, when the dietary nitrogen comprises mainly of poor quality protein or NPN sources, the conversion of dietary protein to microbial protein is an advantage to the host because of its superior biological value. Inhibition on degradation of poor quality protein by any physical or chemical agents can, therefore, limit the utility of such proteins. On the other hand, it appears to be advantageous to avoid the extensive degradation of good quality protein like G.N. cake by means of various physical or chemical agents, such as, heat treatment (Chalmers et al, 1954; Blaxter and Martin, 1962; Little and Mitchell, 1967), treatment with tannins (Leroy et al, 1964; Delort et al, 1968; Murty and Khan, 1969) or formaldehyde (Ferguson et al, 1967), provided they do not adversely affect other metabolic activities in the rumen.

Effect of feed tannins and added tannic acid on protein degradation:

The results of our study with the three feeding stuffs have clearly demonstrated that naturally occuring

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tannins, as in salseed and added tannic acid significantly depressed the extent of protein degradation. However, the tannin of banana leaves at the level of 0.5 mg./ml. of incubation media did not produce a significant depression. Our results also show that protein breakdown in rumen liquor, with and without added substrates, was inhibited even at a minimum level of 0.5 mg./ml. of tannic acid, the extent of inhibition generally increasing with increasing levels of tannic acid ending in almost complete inhibition at a concentration of 4 mg./ml. tannic acid. Tannin in salseed at the level of 2.66 mg./ml. of incubation media strongly inhibited protein degradation during 8 hours of incubation. With G.N. cake, addition of tannic acid at 2 mg./ml. and 4 mg./ml. of incubation media brought about inhibition by about 62 and 87% respectively during 4 hours of incubation.

Our results are in general agreement with the findings of many workers who have also reported the inhibitory effect of various tannins and tannic acid on protein breakdown.

Volcani et al (1963) have reported that carbb pod meal (a tannin rich substance) and its various extracts inhibited deamination 'In vitro'.

Leroy et al (1964) has shown that the groundnut and Soyabean cakes soaked with tannic acid solution produced less NH_3 than untreated samples in 'In vitro' incubation with sheep rumen micro-organisms. They also concluded that tannins prevented bacterial deamination in the rumen.

Tagari et al (1965) have reported that the carob pod tannin at the level of 1 mg./ml. incubation media completely inhibited proteolysis with casein as substrate on 'In vitro' incubation. However, they have also shown that the gallotannic acid at the same level of concentration produced very slight inhibition on proteolysis. They have found that the carob pod tannins were more determental to protein biosynthesis and that the deamination was not affected to the same extent.

Our findings differ partly with the results of Tagari et al (1965) that in our study the added tannic acid has also produced significant inhibition on protein breakdown. Although the source of tannin is different in our case but added tannic acid at the level of 4 mg./ml. of incubation media did not completely inhibited the protein breakdown in G.N. cake during incubation.

Delort and Zelter (1968) observed that treatment with tannins strongly inhibited the deamination of many proteins, without adversely affecting the digestibility of the diet, rumen microflora cellulotic activity and VFA production.

Murty and Khan (1969) observed better overall N-retention and less ammonia-N level 'In vivo' and 'In vitro' when G.N. cake was fed or incubated with equal amount of salseed cake than when given alone.

Zelter et al (1970) studied 'In vitro' the behavior of various proteins tanned with tannin from chestnut wood or

with some aldehydes. They concluded that the minimum amount of each tanning agent which would prevent protein degradation depended on the original physico-chemical properties and heat treatment applied to the protein.

Indirect evidence of inhibitory effect of various tannins on protein degradation is also available from the digestibility trial experiments of Hawkins (1955), Oslage and Becker (1958), Mc Ginty (1969), Pal (1969) in cattle and Bissel and Weir (1957), Oslage and Becker (1958) in sheep who have reported that the tannins reduced the digestibility of crude protein.

Mechanisms of inhibition on protein breakdown by tannins and tannic acid are yet to be elucidated. It is not known that whether the inhibition is produced by precipitation of protein in rumen or inhibition of bacterial enzymes concerned with protein breakdown or inhibition on growth and multiplication of micro-organisms or other mechanisms are involved.

Efficacy of the method of tannin extraction:

The method adopted for tannin extraction from salseed and banana leaves was on the line of Lowenthal-Proctor Method (A.O.A.C., 1960) for tannin estimation in tea leaves. Although data are not available to show that boiling for 30 minutes removes completely the tannin content from tea leaves, this procedure was followed on the presumption that the process ensures complete extraction for the valid results obtained by

this method. It is evident from our results in Table No. 5.2 that the tannins of banana leaves could be completely extracted following this method of extraction but with salseed, which contained a high level of tannin, extraction could not be complete. The process, however, cannot be blamed for the left amount because it was observed during extraction that the salseed powder after boiling for 30 minutes was changed into thick pasty mass and it is possible that the extracted tannin could not be completely washed out after repeated washings with water. However, it was possible to extract another 2.5% of tannin from the dried pasty mass obtained after the first extraction by repeating the same process indicating that this amount could also perhaps be removed in the first extraction process if it could be made more effective.

On the basis of our findings it is concluded that -

1. The salseed tannins and tannic acid exert inhibitory effect on the breakdown of protein in rumen.

2. The wastage of G.N. cake nitrogen due to its extensive degradation in rumen could possibly be restricted with addition of tannic acid to it.

3. The salseed could possibly be better utilised as a subsidiary feed to ruminants after its tannin extraction.

4. Small amount of tannin present in Banana leaves does not seem to have any inhibitory effect on its protein degradation.

CHAPTER - VI

SUMMARY

S U M M A R Y

1. Analysis of salseed, banana leaves and their tannin extracted samples were done to estimate their proximate composition by appropriate A.O.A.C. (1960) methods.

2. Tannin content was estimated by Lowenthal-Proctor (A.O.A.C., 1960) method. Tannin extraction from salseed and banana leaves was done by boiling the feeds for 30 minutes in water (1:40, w/v). Average tannin content in salseed and banana leaves were 13.3% and 2.5% respectively and that in their extracted samples were 2.5% and nil respectively.

3. Separate 'In vitro' incubations were carried out with strained rumen liquor (obtained from slaughtered goats) and three different test materials, viz., salseed (unextracted and extracted), banana leaves (unextracted and extracted) and groundnut cake, to study the effect of natural tannins and/or added tannic acid on protein degradation. The period of incubation was fixed at 8 hours for salseed and banana leaves and 4 hours for G.N. cake because of rapid degradation in latter.

4. Non-protein nitrogen of the incubation media were estimated both before and after incubation. The extent of protein degradation was indicated by increase in NPN during incubation.

5. Probable experimental errors in various procedures adopted were estimated and found to be negligible.

6. Both low and high level (0.5 mg./ml. and 4 mg./ml.) of tannic acid significantly inhibited protein breakdown in rumen liquor, the extent of inhibition generally increasing with increasing levels of tannic acid.

7. Tannin of salseed at the level of its presence (13.3% or 2.66 mg./ml. of incubation media) produced a strong inhibitory effect on protein degradation. A highly significant increase in protein breakdown was observed with tannin extracted salseed. Almost a similar degree of inhibition was also brought about by bringing the level of tannin in extracted sample to 2.66 mg./ml. of incubation media with added amounts of tannic acid, as was observed with unextracted salseed.

8. Tannin of banana leaves at the level of its presence (2.5% or 0.5 mg./ml. of incubation media) did not seem to have inhibitory effect on protein breakdown, but added tannic acid at the same level (0.5 mg./ml.) produced significant inhibition in extracted banana leaves. Added tannic acid at high level (2.0 mg./ml. and 2.5 mg./ml. of incubation media) produced strong inhibitory effect in both unextracted and extracted banana leaves.

9. Added amounts of tannic acid (10% and 20% of the substrate or 2 mg./ml. and 4 mg./ml. of incubation media) to G.N. cake produced about 62% and 87% inhibition on protein breakdown respectively.

10. The similarity in the trends of the effect of added tannic acid in different incubations with a particular substrate gives confidence in the results so obtained.

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