STUDIES ON THE INFLUENCE OF TANNINS ON NUCLEIC ACID AND PROTEIN SYNTHESES IN RUMINANTS

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Certified that the thesis entitled "Studies on the Influence of tannins on Nucleic acid and Protein syntheses in Ruminants" submitted by Sri. K.P. Kalaiyandan embodies the work of the candidate himself. He had worked under my supervision for thirty months commencing from the date of his application. He had put in the required attendance of over 900 days in my department during this period.

Karnal,
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LIST OF ABBREVIATIONS

DCP	Digestible crude protein
DM	Dry matter
DNA	Deoxyribonucleic acid
IVDMD	*In vitro* dry matter digestibility
N	Nitrogen
NPN	Nonprotein nitrogen
OM	Organic matter
RNA	Ribonucleic acid
rpm	Revolutions per minute
SRL	Strained rumen liquor
TCA	Trichloracetic acid
TDN	Total digestible nutrients
TVFA	Total volatile fatty acids
VFA	Volatile fatty acids

CHAPTER I

INTRODUCTION

Due to the prevalent acute scarcity of livestock feeds, incorporation of agricultural and industrial by-products in livestock rations has become obligatory. Hence such by-products as salseed meal, oak kernals, tree leaves, neem seed meal etc. are being advocated for livestock feeding. Most of these contain tannins and the tannin content of salseed meal, an important agro-industrial by-product, ranges from 3.5 to 13.3%. Tannins when present in small quantities, protect proteins and lipids from microbial attack in the rumen. The use of protected protein may improve ruminant productivity (Farguson, 1972).

Tannins have the property of entering into chemical combination with proteins and to a much lesser extent with cellulose. A large number of phenolic groups in the tannin molecule provide many points of attachment with the peptides of adjacent chains through hydrogen bonding to form protein-tannin complexes. Tannins can precipitate proteins of both food and body origin resulting in decrease in the digestion and absorption of proteins. These may also damage the intestinal mucosa by precipitating the constituent proteins, resulting in decrease in feed intake, its digestion and absorption. This in turn results in impaired growth rate and negative nitrogen balance and wider feed : gain ratios.

In the rumen tannins may adversely effect microbial multiplication either by the process of adsorption on their surface by or by penetrating into the bacterial cell and reacting with its components. Inhibition of cell wall synthesis is one of the early effects exerted by tannins on sensitive bacteria (Henis et al., 1964). The inhibitory effect to growth may also be effected through interference with energy metabolism of bacteria (Watson, 1975).

Tannins are absorbed through gastro-intestinal tract, detoxicated in the liver by O-methylation and decarboxylation. Then the by-products such as 4-O-methylgallic acid, pyrogallol, pyrocatechol, syringic acid and 3,4 dimethoxy - 5 - hydroxy - benzoic acids are excreted in urine. If detoxification is not complete, it may lead to partial inhibition of RNA and protein synthesis in the liver cells and result in necrosis of liver (Badawy et al., 1969).

Once the tannins are absorbed into the body, the toxic effects produced in ruminants and monogastrics may be the same. Many studies with rats (Glick and Joslyn, 1970 a, b; Mitjavila et al., 1970, 1971 a, b, c and 1973, and Correra et al., 1973) show that tannins interfere with digestion and absorption resulting in decrease in feed consumption and impaired growth rate. But there are reports which show that tannins can be nearly harmless. Handler and Baker (1944) observed that gastro-intestinal tract was impermeable to tannic acid. Glick and Joslyn (1970 a) reported partial adaptation to tannic acid diet after a few days. It may be interesting to investigate the influence of dietary tannins on synthesis of RNA, DNA and protein in the liver tissue of rats or other species.

Sal seed meal is a by-product obtained after the extraction of oil from sal seed for industrial use. Sal trees are extensively found in the sub-Himalayan regions from Punjab to Assam in the forests of eastern India, Bihar, West Bengal, Orissa, Madhya Pradesh and Uttar Pradesh. The gross production potential of sal seed meal has been estimated to be 46,78,000 tonnes annually (Sethi and Saini, 1969); even though the collection at present may be a mere fractional of the total. Sal seed is a good source of hard fat which can be used as a substitute extender to cocoa butter in soap industry.

A few experiments conducted to evaluate the nutritive value of oilseed meal reveal that its incorporation upto 40% in concentrate mixture may not have deleterious effects on growth. Its level upto 30% may not affect the digestive coefficients of dry matter, organic matter, crude protein, nitrogen, calcium or phosphorus balances.

On the toxicity side, Mudgal and Jaspath (1969) reported flocculi appearing in the urine of experimental bullocks.

Synthetic processes rather than degradative processes in the rumen are more important due to several reasons. It has been reported that the end-products of microbial fermentation might not be the same on different feeding regimes, but nitrogen retention in the animal was different (Weir and Somers, 1957); because protein synthesis in the rumen was a function of microbial activity and their multiplication.

Determination of nucleic acids in the rumen is one of the methods of assessment of protein synthesis (Smith and McAllan 1970a). Usually nucleic acid : protein ratios differ considerably in different microbial populations. Hence it is imperative to determine the ratio to facilitate better estimate of synthesized protein.

The following studies were conducted to investigate the influence of tannins in rats and ruminants. The major aims were:

(1) to investigate the toxic effect of tannins in the diet of rats,

(2) to ascertain the influence of incorporation of oilseed meal and tannins in the rationof ruminants on rumen metabolism and

(3) to find out the effect of tannins on ^{32}P uptake by rumen microbes as an index of microbial activity.

CHAPTER II

REVIEW OF LITERATURE

The literature has been reviewed under five major headings, keeping in view the direction of research work conducted.

I. INFLUENCE OF DIETARY TANNINS IN MONOGASTRIC ANIMALS

1. Metabolic effects of dietary tannins

Booth et al. (1959) studied the metabolic fate of tannic acid and related compounds. They found that O-methylation resulting in the formation in 4-O methyl gallic acid accounted for the major metabolite in the urine of rats and rabbits ingesting gallic acid, propyl gallate, lauryl gallate or tannic acid. Pyrogallol was formed as a result of decarboxylation in rats and rabbits receiving gallic acid. Syringic acid and 3, 4 dimethoxy - 5 - hydroxybenzoic acid were identified as urinary metabolities of rats receiving 3-O methylgallic acid.

Potter and Muller (1968) studied the fate of dietary tannic acid and gallic acid in chickens and found gallic acid, 4-O methyl gallic acid pyrogallol and pyrocatechol in the urine. Tannic acid was apparently hydrolysed to gallic acid and large part of this material was O-methylated and excreted in the urine as 4 - O - methyl gallic acid.

Das and Griffiths (1969) estimated the radioactivity in the urine of both rats and guinea pigs after administering ^{14}C - catechin and found that the total radioactivity over a four day period of urine collection, accounted to 50-60% of the administered dose. Only less than 1.5% was excreted in the faeces over a two day period of collection.

Milic and Stojanovic (1972) studied the metabolites of 0.5 and 4.0% of lucerne tannin isolate fed to mice in an artificial diet. They detected

3-methoxy-4-hydroxy benzoic acid, 5-O-methylgallic acid, (-)-epigallocatechin gallate, (+)-gallocatechin and (-)-epicatechin in greater amounts and 3, 4 dihydroxycinnamic acid, 3-4 dimethoxy-5-hydroxybenzoic acid, m-digallic acid, m-hydroxycinnamic acid, m-hydroxyhippuric acid and (-)-gallocatechin gallate in smaller amounts in the feces of mice.

2. Mode of tannin action

Feeny (1969) observed that casein was almost completely protected from hydrolysis at pH 7.6 when complexed with oak leaf tannin. In the midgut of the larvae of winter moth Operophtera brumata, the inhibition was more marked with condensed type than with hydrolysable oak leaf tannin. The larval growth was inhibited by oak leaf tannin which could be correlated with decrease in casein digestibility.

According to Van Buren and Robinson (1969) tannin and protein interacted to form soluble and insoluble complexes and the latter were favoured by a pH near the isoelectric point of the protein and excess of tannic acid.

Gupta and Bilgami (1979) found that decoctions of the barks of Acacia arabica, Casurina equiseptifolia, Emblica officianalis and Saraca Indica and heartwood of Acacia catechu inhibited the production and activity of cellulolytic enzymes of certain fungi. The decoctions contained tannins or phenolic compounds.

Strumeyer and Malin (1970) reported that unlike typical protein, yeast invertase was able to tolerate the denaturing action of condensed tannin isolated from sorghum.

Mitjavila et al. (1970) observed that intestinal absorption of glucose and methionine was decreased while perfusing the mouse small intestine with

gallotannic acid at a concentration of 1mg/litre of perfusate. The absorption decreased by about 6 to 10% as compared to control values. The absorption of butyric acid was not significantly decreased by concentration under 10 mg/litre; but 10 mg/litre decreased it by 7%.

Gladwell et al. (1971) observed that tannic acid when applied on frog sartorius muscle caused depolorisation. Higher concentrations produced a more rapid and greater fall in membrane potential. Membrane resistance showed an initial rise and subsequently fell. These effects, according to the authors, were the result of action of tannic acid on Cl^- permeability of the muscle membrane.

Cope et al. (1971) found that aqueous leaf extracts of stems of Sericea lespedeza contained a fraction of tannin which inhibited l-pectinase and cellulase. The inhibitor concentrationof a given date or genotype appeared to be relatively independent of accumulation of astringent tannin or leucoanthocyanidin fraction.

Diwan (1972) reported that tannic acid selectively inhibited a phosphate dicarboxylate exchange mechanism in rat liver mitochondria. In contast to the inhibitory effects of tannic acid on influx of anionic solutes, it stimulated the rate of influx of potassium under energised conditions.

Luciani (1973) observed that tannic acid inhibited succinate oxidation in rat liver mitochondria, probably by preventing the operation of the carrier of dicarboxylic acids on the mitochondrial membrane.

3. Tannic acid toxicity

Booth et al. (1961) fed rats a low methionine, low choline diet

containing 1% gallic acid and observed that they developed fatty livers. Added choline or methionine prevented the increase in liver fat. Pyrogallol in contrast did not cause fatty livers.

Dollahite et al. (1962) observed that, in rabbits, the single oral median lethal dose (LD 50) respectively of pyrogallol, gallic acid and tannic acid were : 1.6g/kg, 5.0g/kg, 5.0g/kg. Tannic acid was absorbed from the gastro-intestinal tract into the blood stream in rabbits, sheep and goats. In rabbits it was rapidly detoxified or excreted.

Rabbits, administered a gallotannin from oak, had haemorrhagic gastritis and results of microscopic study of hepatic tissue indicated hepatic necrosis with nuclear alterations. The poisioning was associated with increase in serum transaminase and blood urea-nitrogen (urea-N) values, haemoconcentration and less serum proteins (Camp et al., 1967).

Sadawy et al. (1969) observed in rats that intraperitoneal administration of tannic acid resulted in inhibition of nuclear RNA and protein synthesis and production of necrosis in the liver.

Studies conducted by Glick and Joslyn (1970a) revealed that 4 or 5% tannic acid depressed feed intake and growth and at 8% caused 90% mortality in weanling rats. Rats weighing 130 and 200 g tolerated large amounts of tannic acid better than weanling rats and they adapted partly to tannic acid after a few days. Growth depression thereafter was caused by factors other than reduced feed intake.

Glick and Joslyn (1970b) found that N in feces was more than control values with 2% tannic acid and continued to increase with larger amounts of tannic acid. Condensed tannins from grape seeds and quebracho had similar effects. Gallic acid, D-catechu and ellagic acid had little or no effect

on N in feces.

Mitjavila et al. (1971a) observed a considerable decrease in lipid deposits in rats subjected to tannic acid. The overall biological utilisation of food was decreased by the effect of tannic acid at the level of intestinal absorption. Mitjavila et al.(1971b) reported slow growth rate with tannic acid without meaningful effect upon the rate of ingestion. In liver, the functional status did not alter, nor was there any fluctuation in the triglyceride ratio or in the activity of few enzymes involved in the oxidising process. Tannic acid did not clear the intestinal barrier under most conditions.

Mitjavila et al.(1971c) observed that hepatic necrosis caused in rats by intravenous injection of tannic acid (20mg/kg body weight) was accompanied by a decrease in the level of plasma purification of hepatocytes which was measured by bromosulphalein clearance. The intervention of reticulo-endothelial system during regeneration of the necrosed zone was confirmed by the hyperphagocytic response noted during the study of the clearance of colloidal calcium particles.

Bataleski and Stanislav (1971) intrarectally injected baryta with addition of tannic acid in rats. Tannic acid had toxic effects on the hepatic cells, which were dependent on the concentration of the preparation and the time after application.

Correra et al. (1973) administered a solution of tannic acid (250 or 500 mg/kg body weight) in rats 14 hours prior to feeding vitamin B_{12}. This resulted in the increased excretion of this vitamin. They concluded that inhibition of absorption of the vitamin by tannic acid resulted from its binding with tannic acid and gastric mucin which made it unavailable to intrinsic factor.

Mitjavila et al. (1973) observed that the administration of a single dose (300mg/kg body weight) of tannic acid had no influence on the secretory capacity of the stomach, but inhibited the peptic activity of gastric juice and partly precipitated the mucin. Tannic acid administered at repreated dosages increased the max secretion of pepsin and free acidity. It also reduced the concentration of mucin in the gastric juice, but no case of gastric ulceration was detected.

Feaslee and Einhellig (1973) observed reduced fecundity in mice on tannic acid diet. White mice fed a continuous diet of 8% tannic acid produced litters of smaller numbers, slower growth rate, and lower body weight. Females on tannic acid required longer time to become impregnated.

Bochar and Friedman (1974) concluded that barium enemas containing 1% tannic acid did not cause liver damage in adults, provided that colon lesion was neither extensive nor severe and there was no evidence of preceeding liver damage.

Gaillard et al.(1974) observed that enzyme activity of liver microsomes in groups of 16 female rats was not affected by gallic acid, ethylgallate, syringic acid, veratric acid, vanillic acid or 3,4,5 - triuethoxy benzoic acid. The results indicated a change at sub-cellular level. When 500mg tannic acid/kg body weight was given by mouth daily for four days, there was no change in the activity of microsome enzymes.

Molina et al.(1974) fed rats coffee pulp containing 2.4% tannins. The feed efficiency ratio given a basal diet alone or with 20 and 30% dried pulp was 2.13, 4.08 and 5.61 respectively. There was a high correlation between mortality and tannin, chlorogenic acid and total caffeic acids; but not caffeine content of pulp.

4. Influence of certain tannin rich ingredients on metabolism

Tamir and Alumot (1970) found that tannins in carobs decreased growth in rats and digestive tract contents contained more N than the control rats. The increase in insoluble N fraction was correlated with the degree of growth depression.

Maxson et al. (1973) observed in rats that tannin content in sorghum grain was significantly and negatively correlated with dry matter (DM) disappearance; but not with lysine concentrations or protein efficiency ratios.

May and Nelson (1973) fed six variation of milo varying in tannin content and amino acid availability as determined with chicks. They observed a correlation of 0.38 between digestible energy and tannin and 0.39 between metabolizable energy and tannin. The results indicated that tannin content from milo had not influenced the utilizable energy content of milo in rats.

Schaffert et al. (1974a) used high and low tannin content sorghum with supplementation of four levels of soybean meal in growing rats. Average net weight gains from high tannin genotypes differed significantly from low tannin weight gains at 0,5 and 10% of any soybean supplemented diets respectively. The gains from 15% soybean diets were not significantly different.

Dreyer and Niekerk (1974) studied growth suppressing and related effects of unextracted and ethanol extracted grains of sorghum in rats. Cultivars differed significantly, mainly in their effect on growth rate and protein digestibility. Lipid content of liver was not influenced by tannin in diet.

Tannins have been found to exert various harmful effects in birds also.

Connor et al. (1969) found that addition of tannic acid in diets depressed feed intake, fat content of liver and weight of testes in growing chickens. The retardation could be partially alleviated by supplementation

with large amount of methionine and choline.

Rayudu et al. (1970a) found that tannic acid depressed growth less than its metabolites like gallic acid, pyrogallol, pyrocatechol and digallic acid. At 2% level it caused no mortality in chickens. Fat content of livers of chickens given 10% gallic or tannic acid was increased, but was not influenced by 1% pyrogallol or 0.5% tannic acid. A diet based on maize, milo and soybean meal, containing tannic acid (0.5, 1 or 2%) consistently depressed growth in broiler chickens (Rayudu et al., 1970b).

Arcanious et al. (1973) studied the effect of methionine and choline on tannic acid and tannin toxicity in laying hen. They observed that 2% tannic acid diet was toxic and caused a decrease in egg production, egg weight, body weight and an increase in yolk mottling. Supplementing the diet with choline, methionine or both, partially alleviated toxic effects. High tannin grain sorghums had no significant effects on egg production, egg weight, but there was a trend towards a decrease in both factors.

Hughes (1973) reported production of green yolks in hen's eggs by ingestion of red gum sawdust litter. A dietary level of 0.05% tannins from saw dust resulted in mottled yellow yolks, while 0.5% tannins gave olive green yolks. Dietary tannic acid and tannin interfered with pigment metabolism.

Rostagno et al. (1973a) observed that the growth rate and feed efficiency were minimum with bird resistant sorghum and maximum with bird susceptible sorghum. Corn diets consistently exhibited superior weight gains, but weight gains and feed conversion were decreased on addition of tannic acid. Rostagno et al. (1973b) found that supplementation of protein free diet with 1.41% tannic acid resulted in a four fold increase in endogenous amino acid

excretion inhibited. Apparent and corrected amino acid digestibilities of sorghum of intermediate (4%) and high (2%) tannin contents were lesser than low tannin sorghum plus tannic acid equivalent to the high tannin sorghum(6%).

Nelson et al. (1975) studied the effect of tannins in sorghum grains on the energy utilization and biological availability of amino acids in chicks. The correlation coefficients between tannin content and amino acid availability (0.82) was highly significant. The correlation coefficients between tannin content and kcal of metabolizable energy per gram, percent of gross energy (-0.62 for both) utilized were also significant.

Quard et al.(1970) observed some beneficial effects of tannins in rats (40mg/kg body weight daily) on assimilation of dietary protein and liver reserve of vitamin A. It increased the absorption of the vitamin and also limited its needs of the animal.

5. Influence of tannins and tannin rich diets on nutrient utilization

Smart Jr. et al. (1961) observed that a water soluble substance or substances, probably polyphenolic in nature, in the leaves of Lespedeza inhibited enzymatic hydrolysis of soluble cellulose. The reduction in cellulase activity was proportional to the concentration of the inhibitor present.

Lorrey et al. (1964) found that enzyme solubility of N from maize either ground nut or soya cake with 6% tannin was less than with untreated cake. According to them, tannins prevented bacterial deamination of feed without upsetting digestive processes.

Tagari et al. (1965) observed that inhibitory effects of carob pod extract upon cellulolysis and deamination were correlated mainly with its sugar content rather than its tannin components. On the other hand, proteolytic activity and protein biosynthesis were significantly affected

by tannin fraction. In contrast to the tannin fraction of carob pod extract, gallotannic acid inhibited cellulolytic activity.

In *in vitro* studies, Driedger and Hatfield (1972) observed that treatment of soybean meal with 10% tannins resulted in a 90% decrease in deamination. Pepsin digestion of tanned soybean meal was not affected by level or type of tannin used, but pancreatic digestion of the protein decreased significantly with increasing levels of tannin. Average daily gains, feed efficiencies and N balances were greater in lambs receiving soybean meal tanned with 10% tara tannin. Efficiency of N utilization appeared to have enhanced due to tannin treatment.

II. INFLUENCE OF TANNINS IN RUMINANTS

1. Influence of tannins on in vitro dry matter digestibility and solubility

Lyford Jr. *et al.* (1967) observed inhibition of cellulose hydrolysis by a purified preparation of water extractable inhibitor from fresh sericea forage both *in vitro* and *in vivo*. When casein was added to the cellulose rumen fluid mixture containing purified sericea inhibitor considerable increase in activity was offered to the cellulose degradation system. But at higher levels of inhibitor, casein had no effect.

Donnelly and Antony (1970) determined DM digestibility by the *in vivo* nylon bag technique on two cuttings of low and high tannin sericea plants. Low tannin sericea was higher in DM digestibility than normal or high tannin sericea.

Zelter *et al.* (1970) complexed proteins from various sources with different tanning agents in an artificial rumen. The minimum amount of each tanning agent which would prevent protein degradation depended upon the original physicochemical properties and heat treatment applied to the protein. The original values for enzymic solubility of the protein were

reduced by 5% chestnut extract, but had no negative effect on the cellulolytic activity of rumen inoculum. Reduction in DM and crude protein (CP) digestibility by tannins had been observed by Donnelly and Antony (1973) also.

Saba et al. (1972) studied the influence of processing on Arkansas (AKS) 614 (bird resistant sorghum grain) by determining the gas produced per gram DM incubated and in vitro DM digestibility (IVDMD). When the grains were steam processed and flaked, the gas production was more. Tannic acid addition to both processed and nonprocessed AKS 614 and red grain significantly depressed gas production and DM disappearance. This effect was not as large when the additions were made to steam processed flaked grains.

Burns et al. (1972) observed that on feeding tannin containing crownvetch, Sericea lespedeza and alfalfa to dairy heifers, volatile fatty acids (VFA) concentrations in the rumen fluid appeared to be associated only with structural constituents of forages. IVDMD was depressed about equally by structural differences and by tannin and phenol differences.

Bhargava et al. (1973) examined 28 samples of ground nut cake for max protein solubility and ammonia production. The level of tannic acid as low as 6% decreased protein solubility of ground nut cake by 76%.

Schaffert et al. (1974b) observed that IVDMD of low and high genotypes after 48 hour fermentation was 72.5 and 46% respectively. The major factor limiting the utilization of high tannin sorghum was apparently the availability of protein. Undigestible protein and tannin complex might account for more than half of the difference in IVDMD between low and high tannin genotypes.

Tripathi (1974) determined inhibitory effect of ~~in~~ banana leaf - tannin and added amounts of tannic acid on protein breakdown, by in vitro studies using goat's rumen liquor. Tannins of banana leaves (2.5%) did not produce a significant inhibition on protein breakdown. On the other hand, added tannic acid at the same level (2.5%) exerted significant inhibitory effect on protein breakdown. Tannic acid at a level of 12.5% produced a severe degree of inhibition.

Orsan(1974) demonstrated an almost perfect negative correlation between IVDMD and tannin content in sorghum grain . Arora and Ludhra (1974) also got similar results. Digestibility of sorghum was reduced by 6.36% with each unit increase in tannin content of the grain.

Armstrong et al. (1974a) observed that two non-bird resistant sorghums exhibited higher in vitro protein digestibility values than two bird resistant varieties. Extraction of tannins from a bird resistant variety resulted in increased in vitro protein digestibility, whereas the extraction process had little influence on the protein digestibility of a low tannin non-resistant sorghum grain.

Burns and Cope (1974) studied the nutritive value of crownvetch forage as influenced by structural constituents and phenolic and tannin compounds. Total phenol and tannin fractions of leaflets were negatively associated with IVDMD. Total phenols accounted for 39% of the variation and tannins 30%. In another experiment, Burns and Cope (1976) observed that IVDMD was depressed to well under 60% at total phenol levels of 6 to 7%.

2. Influence of tannins on rumen micro-organisms

Kenis et al. (1964) observed that carob pod extract and tannic acid exerted strong antimicrobial activity towards Cellvibrio fulvus and Clostridium cellulosolvens at 15/~~ug~~/ml, to Sporocytophaga myxococcoides at 45/~~ug~~/ mg/m

and to Bacillus subtilis at 75 μg/ml. The inhibitory tannic acid concentrations were found to be 12, 10, 45 and 30 μ g/ml respectively. Although the growth of some of the micro-organisms was only slightly affected, the morphology was drastically changed in the presence of sub-inhibitory concentrations of carobpod extracts of tannic acids. It was suggested that the site of action of tannins was presumably on cell envelopes.

Watson (1975) demonstrated a substance, probably a polyphenol, inhibitory to growth and fermentation of Lactobacillus leichmanii and Saccharomyces cerevisiae. The inhibition was probably through energy metabolism of bacteria.

3. Effect of tannins on feed consumption, digestibility, weight gain and gas production

Hawkins (1955) fed powdered gallotannin added to alfalfa hay raising its tannin content equal to that of Lespedeza sericea. He observed that the calves consumed more of alfalfa hay with tannic acid than the sericea hay. Addition of tannins to alfalfa hay did not affect the consumption of DM and water or the gains in body weight. Also apparent digestibility of DM and CP in alfalfa hay was not significantly affected. He came to the conclusion that any effect of tannin on the quantity of forage consumed would be due to the astringent taste and that any effect on digestibility would be associated with the property of tannin to precipitate protein.

Driedger et al. (1969) observed that average daily gains of steers receiving urea, soybean meal or tannic acid treated soybean meal as supplemental source of N were 1.42, 1.56 and 1.62 kg respectively. Gain : feed ratios were 0.163, 0.167 and 0.179 respectively. Average daily gain of lambs receiving three N supplements were 112, 177 and 217 g for urea, soybean meal and treated soybean meal respectively. N retained

(% of intake) for urea, soybean meal and treated soybean meal were 29.2, 34.2 and 35.8 respectively for one trial and 32.8, 33.6 and 42.3 respectively for another trial. Apparent N and DM digestibility did not differ amongst treatments.

Leroy and Meitor (1970) fed adult sheep diets with ground nut meal or soybean meal, each tanned with chestnut wood tannin. The tanned protein was 70% of total protein. In rumen contents, tanned protein reduced concentration and total amounts of ammonia and nonprotein-N (NPN). Rumen concentration and total of C_2 and C_3 fatty acids were unchanged, except butyric acid which was less. Digestibilities of organic matter (OM), crude fibre and nitrogen free extract were not affected except protein which was nine units less for tanned proteins.

Donnelly et al. (1971) evaluated Sericea lespedeza varieties for low and high tannin (3.7 and 6.5%) to find a relationship between tannins and digestible DM, digestible crude protein (DCP) and daily intakes of DM. The bullocks grazing high tannin sericea ate 0.98 kg more DM than those grazing low tannin sericea, but there was no significant difference. The crude protein and DM of low tannin sericea were better digested than those in high tannin sericea.

Delfort - Laval et al.(1972) treated skim milk powder with a tannin substance extracted from chestnut wood and observed that the treatment reduced 89% solubility both in artificial rumen and in sheep with a permanent rumen cannula. When included in a balance experiment, in diet of adult sheep, the tanned protein caused a small decrease in N digestibility (68.2 Vs 72.0). There was a significant improvement in N retention (25.6 Vs 16.1) and practical utilization (16.1 Vs 11.6%). Tannin had no significant effect on digestibility of OM or production of total volatile fatty acids (TVFA) in the rumen.

Nishimuta et al. (1973) conducted a digestion and N balance trial with lambs to compare the effect of heat, formalin and tannic acid treatment of soybean meal on nutrient utilization. Cellulose digestibility was significantly reduced but N retention was highest in lambs consuming heated soybean meal. No differences in cellulose, DM and CP digestibilities or N retention were found between tannic acid treated and normal soybean meal.

Katiyar et al. (1975) found that feeding of oak-kernal (tannin content 8.8%) with oat hay in 40:60 ratio of DM intake decreased the proteolysis and cellulolysis in sheep significantly. The availability of total digestible nutrients (TDN) from oak hay decreased on oak-kernal feeding when compared with oak hay ration due to appreciable adverse effect of kernals on the digestibility of both protein and fibre fractions. The deleterious effect of oak tannin was however evident on its long term feeding in this species.

Bhargava and Ranjhan (1974) elucidated the effect of feeding tannin treated groundnut cake with wheat straw impregnated with urea molasses on rumen fermentation pattern in lambs. There was significantly less rumen ammonia concentration in the treated group . No effect of treatment on bacterial N concentration or TVFA was noticed.

With a basal diet of wheat straw and concentrate mixture of ground nut cake (untreated or treated with tannic acid), maize and wheat bran in lambs, it was observed that protein solubility decreased by 79%. But there was no change in TVFA concentration (Verma et al., 1974).

Nishimuta et al. (1974) observed that tannic acid treatment had no effect on increasing either ruminal by-pass of dietary protein or the quantity of amino acids presented to the abomasum in steers.

III. SAL[illegible] SEED MEAL FEEDING TO LIVESTOCK AND RUMINANTS

Panda and Pradhan (1967) found out the composition of salfruit by chemical analysis and concluded that decorticated and undecorticated sal fruit could be roughly compared with wheat and oat respectively. They also suggested its possible use as a substitute for cereal grains in poultry mash.

Mithra and Mishra (1967) reported the amino acid composition of processed salseed meal as follows: arginine-6.8, histidine-4.8, isoleucine and leucine-12, lysine-5.7, phenylalanine-7.8, tryptophan-3.4 and threonine-7.8%.

According to Murthy and Khan (1969) addition of salseed meal to the rations containing ground nut cake significantly reduced the ruminal ammonia production which inturn gave lower excretion of urinary-N and thus high positive N balance.

Hudgal and Sampath (1969) attributed the occurrence of mucus threads in the urine of bullocks due to salseed meal. On the contrary Rao et al. (1970) reported that the inclusion of 10-20% salseed meal in the concentrate mixture had no deleterious effect.

Panda et al.(1969) standardised a method of removal of tannic acid from salseed meal by boiling the decorticated salseed for 30 minutes or by soaking the product for 24 hours in water. They recommended its use in poultry ration to a level of 5-7% in starter feed and about 7-10% in the layer feed.

Singh (1971) made in vitro studies on the effect of pipal leaves and salseed and added tannic acid on VFA production in goats. He concluded

that the VFA production was not affected in goats even at a considerably higher tannin concentrations.

Dash and Misra (1972) observed that large number of dairy cattle suffered from primary indigestion as a result of sudden change of feed by replacing rice bran with decorticated oilseed in the ration.

Incorporation of oilseed cake alongwith groundnut cake enhanced DM intake from 1.53 to 2.24kg/100kg body weight with subsequent decrease in DM and ether extract digestibilities in calves (Pal et al., 1973). Poor palatability and gradual decline in consumption was observed when oilseed meal was fed as a sole supplement. Urine of pH of animals fed concentrate mixtures consisting of oilseed meal (20 to 30 parts) was alkaline and had considerable amounts of sediments. Microscopic examination revealed more phosphate and a few oxalate crystals (Anon., 1972).

Paul et al.(1972) fed one group of chicks containing 30% maize as control and two groups containing 10 and 15% oilseed meals as replacement of maize. During 12th week of age control group had maximum weight and 15% oilseed meal group minimum. The results showed that 10% replacement of maize by decoiled oilseed cake did not have any adverse effect on growth of chicks.

Pathak and Ranjhan (1973) studied the influence of replacement of maize by decoiled oilseed meal from the finisher rations of pigs. The replacement of maize by decoiled oilseed meal did not affect significantly the rate of gain of body weight in gilts. The digestibility of CP was depressed by 12 and 27% in diets containing 20 and 40% decoiled oilseed meal and digestible energy by 9 and 15% respectively. The digestible energy of oilseed meal was 2.28 Kcal of digestible energy/kg decoiled oilseed meal.

Nagpaul et al. (1973) showed in bull and buffalo calves weighing about 200 kg that feeding of 1.3 kg of salseed meal to animals did not produce any deleterious effect. On the other hand Shukla and Talpada (1975) observed that feeding salseed meal alone as concentrate ration to adult animals affected body weight and the digestibility of protein and ether extract adversely. Feeding of salseed meal at 40, 30 and 20% level in concentrate ration to adult animals had no harmful effect on the body weight.

Vijayan and Katiyar (1975) conducted a study to observe the chemical nature of tannins in decoiled salseed meal. The total percentage of tannins in decoiled salseed meal was 6.2% of which 64% was hydrolysable.

At Nanmathy, salseed meal feeding caused no deleterious effect on the urinary system of animals. Samples of semen collected from animals on both 20 and 30% salseed meal rations revealed no abnormality on biometric examination. Postmortem examination of two animals from 30% salseed meal group revealed no gross lesions in the internal organs. Histopathology of the kidneys revealed no abnormalities attributable to salseed meal toxicity (Anon., 1975).

Sonwane and Nudgal (1974) observed no statistical difference among the groups A (control), B (fed with 20% salseed meal), C (fed with 30% salseed meal) and D (fed with 30% salseed meal and 2% urea) in digestibility coefficients of DM, OM, CP, crude fiber, ether extract and ni-trogen free extract. No statistical difference was observed among the different groups in digestibility coefficients of DM, OM, CP and crude fibre and nitrogen free extract when salseed meal with biuret was fed to growing heifers (Kurar and Nudgal, 1974).

Panda et al. (1975) replaced maize in the ration with 5 levels of salseed and sal oil meal varying from 2.25 to 45% in two experiments. The chicks of control group were much heavier than those of other groups fed salseed or salseed meal in the ration. There was inverse relationship between the levels of salseed or sal oil meal and the body weights attained. Supplementation of extra protein counteracted growth depression brought about by salseed or sal oil meal. No significant difference was noticed between salseed and sal oil meal when they replaced 100% maize in the ration. Mortality of chicks was very heavy in groups fed higher levels of salseed and sal oil meal. Both salseed and sal oil meal lowered the feed efficiencies.

Tripathi (1975) observed that tannin from salseed meal (1.13%) produced a strong inhibitory effect on protein breakdown in an in vitro system.

IV. RUMEN RNA, DNA AND PROTEIN SYNTHESIS AND THEIR INTERRELATIONSHIP

Ellis and Pfander (1958) investigated the relationship between the rumen microbial protein and nucleic acid synthesis to determine if microbial nucleic acid synthesis could serve as an index to microbial protein synthesis. A highly significant correlation was found to exist between increase in microbial protein and increase in RNA-phosphorus at a particular period of time.

Ellis and Pfander (1965) reported that microbial polynucleotide-N represented 5.0 to 7.6% of the ingesta - N from the sheep fed wein and 8.4 to 13.3% of the ingesta - N from sheep fed urea and amino acids. The data suggested that a significant proportion (from 5.0 to 13.3%) of the ruminant dietary - N is converted to microbial polynucleotide - N.

Smith et al. (1968a) investigated the nutritional importance of conversion of dietary - N to microbial - N, with particular reference to the production and utilisation of nucleic ac acids. The degradation products of added pure RNA and DNA had disappeared four hours after the addition of nucleic acids. Nucleic acid - N comprised 17 - 24% of the total - N in some strains of bacteria commonly found in the rumen. Thus by determining the nucleic acid - N in samples of digesta and taking a mean value of 20 for its total - N, it was possible to estimate the proportion of microbial - N in the samples and the extent of conversion of food - N to mi microbial -N.

Smith et al.(1968b) fed calves either a N free synthetic diet and straw or barley, fish meal and straw, or flaked maize and hay, or flaked maize alone or pasture. In the rumen fluid of any one calf there was a strong direct correlation between concentration of nucleic acid - N (range 1.9 - 47 mg/100 ml) and total - N (range 15 to 320 mg/100 ml). There were marked calf to calf variation and in four calves the proportion of nucleic acid - N to total - N varied from 9.5 to 15.5% (mean 12.7%). Nucleic acids in the rumen fluid were largely microbial and micro-organisms separated from rumen fluid by centrifuging contained a fairly constant proportion (about 19%) of their total - N in the form of nucleic acids, irrespective of N content of the diet given to calves.

Smith (1969) reported that bacteria contained about 75 to 85% of their total N in the form of proteins, peptides or free amino acids so that these substances and nucleic acids together appeared to account for nearly all the bacterial - N. It appeared that for every four parts of dietary - N converted to bacterial protein or amino acids approximately one part was converted to nucleic acids. About 40-50% of the microbial nucleic acid-N produced in the rumen was not absorbed from the gut and the rest which wasabsorbed, was excreted in the urine as allantoin. Most of the remainder

entered into urea pool. It appeared to be of limited value to the animal.

Smith and McAllan (1970a) observed in calves given a low - N basal diet (flaked maize and straw) supplemented with isonitrogenous amounts of different N sources, RNA concentrations in rumen fluid fell slightly immediately after feeding, presumably due to dilution, but in the next 4-5 hours approximately doubled, suggesting a comparable increase in total microbial N. With other supplements (fish meal, decorticated ground nut meal or maize gluten) the RNA-N to total - N ratios in rumen fluid remained fairly constant, suggesting the N for the microbial growth was provided mainly by progressive dissolution of N from the supplement.

According to Smith and McAllan (1970b) relative amounts of RNA and DNA differed considerably in the different bacterial preparations, but RNA-N : total-N ratios varied little within the samples from calves and agreed with the main value for the bacteria grown in pure cultures. This value could be used to obtain a valid estimate of total microbial - N in digesta samples from calves. Such an estimate although subject to some error from small diurnal variations in bacterial composition and the different values shown by protozoa, had marked advantages over estimation by other available means.

Smith and McAllan (1970c) found that ratios of RNA to DNA in rumen fluid were smaller to those in rumen bacteria and were not related to those in diets. Pure nucleic acids added to rumen were rapidly degraded. In most experiments with diets of different N contents, nucleic acid-N formed a fairly constant percentage (8-15 for different animals) of the total non-ammonia - N in the rumen fluid. This suggested that N entering the rumen fluid limited the microbial growth. Comparison of nucleic acid-N : total N ratios in rumen fluid and bacteria suggested that 55-60% and 40-50% of non-ammonia-N in the rumen fluid was of microbial origin for calves and cows respectively.

Smith and McAllan (1971) observed that there was little net change in amount of RNA or DNA between rumen and duodenum, but there was a marked increase in the amount of total - N. In duodenal digesta for any one animal given most diets, nucleic acid - N formed a fairly constant percentage (8-11%) of the total non-ammonia-N for different animals. This value was lower (by about 3) than the corresponding percentage in the rumen fluid. Comparison of nucleic acid - N : total - N ratios in duodenal contents and bacteria suggested that, for these diets, about 40-55% of the total non-ammonia-N in duodenal contents was of microbial origin. During the passage of digesta from the duodenum to ileum the mean percent disappearance of total - N, RNA and DNA - N were estimated to be 67, 85 and 75 % respectively.

McAllan and Smith (1972) observed that in 29 samples of mixed bacteria, separated by centrifuging rumen contents taken 4-6 hours after feeding of calves (14-42 weeks), ratios of RNA-N : total - N and DNA - N : total - N were 0.114 $\pm$ 0.003 and 0.066 $\pm$ 0.004 respectively. Corresponding ratios were much lower in comparable groups of bacterial samples obtained from eight sheep (0.076 $\pm$ 0.003 and 0.051 $\pm$ 0.004) and nine cows (0.070 $\pm$ 0.004 and 0.051 $\pm$ 0.003). The striking differences in properties of bacterial populations between the groups were due, not to the different species of animals, but due to animals reared in different environments. Within each group, deviations amongst individual RNA-N : total bacterial - N ratios were half of those for corresponding DNA ratios. It was concluded that nucleic acids, and more particularly RNA concentrations were suitable for estimating the contribution of total microbial - N in rumen or duodenal digesta samples.

McAllan and Smith (1973a) observed that nucleic acid introduced into the rumen of calves, or incubated with calf, sheep or cow rumen contents

in vitro were rapidly destroyed. Cell-free preparations from calf rumen fluid contained enzymes which converted RNA and DNA into ultrafiltrable oligonucleotides. When ground hay was incubated with whole rumen contents, the nucleic acids from hay were degraded to a mixture of nucleotides, nucleosides and bases almost as easily as pure nucleic acids.

McAllan and Smith (1973b) studied degradation of nucleic acid derivatives by rumen bacteria. They observed that when purine or pyrimidine bases, nucleosides or nucleotides were incubated *in vitro* with whole rumen contents, all derivatives bearing a side amino group were deaminated to varying extents. All nucleotides and nucleosides were rapidly degraded to the parent base. Little or no breakdown was observed when any derivative was incubated *in vitro* with cell free preparations.

Smith and McAllan (1974) gave sheep, cows and calves fitted with rumen cannulas, diets mostly containing 10-16 g N/kg DM and consisting of roughage and cereals. Mixed bacteria were separated from samples of their rumen contents. Bacteria from calves with no contact with adult animals contained more dextran, less total N and higher nucleic acid - N : total - N ratios than similar bacteria from calves reared in contact with adult sheep. Calves with no contact with adult animals were devoid of ciliate protozoa. But when inoculated or placed in contact with adults, they developed a normal protozoal population. Mixed bacteria taken just before feeding had significantly lower RNA - N : total - N ratios and slightly higher DNA - N : Total - N ratios as compared to bacteria taken 4-6 hour after feeding.

V. INFLUENCE OF TANNINS ON ^{32}P UPTAKE BY RUMEN MICRO-ORGANISMS

Taylor (1946) established through standard chemical methods the amount and distribution of phosphorus in bacteria of B strain of *E. coli*.

Approximately 2.72% ~~of~~ of the dryweight of organism from 6 hour broth culture was phosphorus, of this 66% was in ribose and 19% in deoxyribose nucleic acids. Out of the remaining, 12% occured in phospholipids and 3% in unidentified compounds.

Smith et al. (1955) observed that $^{32}PO_4$ entered therumen via saliva and even larger quantities directly through rumen wall. A rapid increase in radioactivity of the omasal contents indicated phosphorus secretion into that organ. The principal site of endogenous phosphorus secretion seemed to be the rumen and very little entered the intestines.

Bucholtz and Morgan (1973) studied phosphate uptake into intracellular inorganic phosphorus and cellular phopholipids and the relationship between cell growth and phospholipid synthesis. There was a highly significant relationship between phospholipid synthesis and cell growth. By this method it was found that in a 4 litre rumen, 16.1 g of protein was synthesised per 100 g of CH digested. This value was higher than the theoretical upper limit proposed by Hungate (1966). But it agreed with the values reported by Hogan and Weston (1971), Hume et al. (1970) and Hindsay and Logan (1972) from direct measurements.

Literature regarding influence of tannins on ^{32}P uptake by rumen microorganisms is not readily available.

CHAPTER III

INFLUENCE OF TANNIN ON FOOD CONSUMPTION, GROWTH AND NUCLEIC ACID AND PROTEIN CONTENTS IN THE LIVER OF RAT

MATERIALS AND METHODS

Thirty weanling rats weighing on an average 37.8g were randomly distributed into three groups of 10 each(groups A to C) and housed in individual cages. They were kept in the laboratory at room temperature. The woodencages they were washed with boiling water followed by permanganate solution for disinfection. The cages and feed dishes were marked with identification numbers for each rat for estimation of growth rate and feed consumption. Filter paper was placed on trays used for collection of feces for the absorption of urine.

The experimental diet for the rats is given in table - 1.

Table - I

The composition of basal ration		
Maize	:	20
Black gram	:	10
Linseed oil*	:	15
Fish meal	:	7
Wheat bran	:	10
Vitamin mixture**	:	2
Wheat flour	:	35
Yeast powder	:	1
Total	:	100

Linseed oil was added at 5% to each diet. Vitamix** - 10 g/100 kg diet

**Proprietary preparations

Novimix contains vitamin A 50,000IU and D_3 5,000 IU/g.

All ingredients were ground in milling machine and mixed before feeding. Enough material required for one week was mixed in one lot.

The diet was offered for 3 day periods as 20 g to each rat for the first six days, followed by 30 g for the next nine days and 40 g for the next 15 days. The rats were allowed to drink water ad libitum. They were weighed every 4th day upto the end of the experiment, and the data were used to calculate regression of growth rates. Simultaneously on each weigh day, the feed residue and feces were collected from each rat for estimation of DM and N contents. These data were used to calculate feed : gain ratios and DM digestibility for the corresponding growth period. On 24th, 27th and 30th day equal number of rats from each group were sacrificed for liver samples which were weighed and kept in ice for further processings.

Rats in group A were fed basal ration and those of groups B and C with 2.5 and 5% tannic acid added to the basal ration.

For determination of DM in the feces total collections were used. The DM and N in the feces were determined by standard procedure (A.O.A.C., 1970). 0.5 g liver was taken for estimating N content by Micro-Kjeldahl method (A.O.A.C., 1970).

The data were statistically analysed according to the methods described by Snedecor and Cochran (1968).

Analytical procedure for preparation of RNA and DNA extracts from tissue homogenate and their determination

RNA and DNA were estimated by the methods of Schmidt - Thannhauser - Schneider as described by Volkin and Cohn (1954).

The liver was removed and it was blotted free of blood. After weighing, it was kept in deep freeze till further processing. 0.2 g of liver was homogenized with 1 ml of distilled water in tissue homogeniser for preparing RNA and DNA extracts.

Tissue homogenate was mixed with 2.5 ml of cold 10% trichloroacetic acid (TCA) and centrifuged. The precipitate was suspended in 2.5 ml of 10% TCA and centrifuged. The combined extract which constituted the acid soluble phosphorus fraction was discarded.

The tissue residue was suspended in 1.0 ml of water with 4.0 ml of 95% ethanol and centrifuged. The residue was suspended in 5.0 ml of ethanol and centrifuged. It was then extracted three times with three portions of 3 : 1 alcohol – ether mixture at room temperature, with brief stirring. The combined extract which constituted the phospholipid fraction was discarded.

The residue was treated for 18 hours at 37° C with 2.0 ml of 1 N KOH, which resulted in the solution of the tissue. The solution was neutralised with 6 N HCl. DNA and protein were precipitated by adding 2.0 ml of 5% TCA. The centrifuged precipitate was washed with 5.0 ml of TCA. The extracts were combined to give the RNA fraction. Volume was made upto 10 ml with 5% TCA.

The residue was suspended in 5.0 ml of 5% TCA at 90° C, cooled and centrifuged. The residue was resuspended in 2.5 ml of 5% TCA and centrifuged. The extracts were combined to give DNA fraction. Volume of extract was made upto 10 ml by adding 5% TCA. The residue thus left was protein fraction.

To 2.0 ml of RNA extract plus 3 ml distilled water in a test tube,

5.0ml of 0.02% $FeCl_3$ in concentrated HCl and 0.3 ml of 10% orcinol solution in alcohol were added and mixed. The tubes were immersed in boiling water for 20 minutes. The change in colour from yellow to green was noted. The tubes were cooled and the content was diluted to 15 ml. Optical density was measured against blank in Spectronic-20 at 650 mμ. The values were read on the standard curve prepared from standard yeast RNA.

DNA was estimated by diphenylamine reaction as modified by Burton (1956). Diphenylamine reagent was prepared by dissolving 1.5 g of steam distilled diphenylamine in 100 ml of redistilled acetic acid and adding 1.5 ml of concentrated H_2SO_4. The reagent was stored in the dark. Just before use, 0.5 ml of aqueous acetaldehyde (16 mg/ml) was added for each 100 ml of the required reagent. 2.0 ml of DNA extract was mixed with 4.0 ml of diphenylamine reagent and the blue colour was developed by incubating at 30^0 C for 18 hours. The optical density at 600 m was measured against blank and compared with the values with standard DNA from calf thymus gland.

RESULT AND DISCUSSION

There was a significant depression in feed intake in tannic acid groups as compared to control (table - 2). But the difference in feed intake between group B (2.5%) and group C (5% tannic acid) was not significant. The rate of gain per 3 day per rat in groups A, B and C were : 7.87, 5.69, and 4.53 g respectively (table -2). The results indicated that the tannic acid depressed gain in weight significantly on the levels studied in this experiment. Because of poor rate of gain in groups B and C, the feed : gain ratios were correspondingly wider (table -2).

TABLE 2

Influence of tannic acid on feed consumption, weight gain and feed : gain ratios

Group	Treatment (% tannic acid)	Feed consumption/ 3 days	Weight gain/3 days (b values)	Feed : gain ratios
A	0	20.05 ± 7.6	7.87 ± 0.41	2.73 ± 0.05
B	2.5	16.66 ± 6.0	5.69 ± 0.35	3.14 ± 0.07
C	5.0	16.11 ± 5.4	4.53 ± 0.21	3.80 ± 0.11

Analysis of variance

Source of variation	df	MS	
		Weight gain/ 3 days	Feed : Ratio ratio
Between Blocks	9	1.877	0.08
Between treatments	2	28.70*	3.20 *
Error	18	0.7944	0.06
Critical difference		0.833	0.229

*p < 0.05 **p < 0.01

continued on next page

TABLE 2 (continued)

Influence of tannic acid on feed consumption

Analysis of variance

Source of variation	df	ms Feed consumption/ 3 days
Between Blocks	9	83.56*
Between Treatments	2	363.76**
Between Interval	7	77.95*
Treatment x interval	14	7.908
Error	207	6.125
Critical difference for treatment		0.767

*p < 0.05 **p < 0.01

It was further evident from the results that there were no significant differences in DM digestibility between different treatments. The DM digestibility values in groups A, B and C were 78.56, 78.62 and 78.82% respectively (table -3); N digestibility in groups A, B and C were : 78.29, 73.50 and 69.67 respectively. The differences were highly significant($p < .01$) among the groups indicating adverse influence of tannins on N - digestibility.

Earlier reports with tannins had also shown the same trend in different species (Connor et al., 1969; Mitjavila et al., 1970; Tamir and Alumot, 1970; Glick and Joslyn, 1970 a, b; Bayudu et al., 1970 a,b; Mitjavila et al., 1971 a, b; Arnanious et al., 1973; Bostagno et al., 1973 a, b; Molina et al., 1974; Schaffert et al., 1974 a; Dreyer and Hiekerk, 1974 and Armstrong et al., 1974).

Tannins have got the ability to enter into a chemical combination with proteins, because the large number of phenolic groups in the tannin molecule provide many points of attachment for linkages through hydrogen bonding with peptides of adjacent chains to form protein - tannin complexes. This property of tannins leads to the precipitation of proteins of food and body origin resulting in overall decrease in the digestibility (Chang and Fuller, 1964 and Tamir and Alumot, 1970).

Negative correlation between tannin content in the diet and decrease in growth rate could be attributed to less protein digestibility in this experiment. Similar results were reported earlier by Chang and Fuller (1964) where sorghum with a high tannin content resulted in growth retardation in chicken similar in magnitude to that caused by proportionate levels of tannic acid. Vohra et al. (1966) and Connor et al.(1969)

TABLE 3

Influence of tannic acid on dry matter and nitrogen digestibilities

Group	Treatment (% tannic acid)	Dry matter digestibility (%)	Nitrogen digestibility (%)
A	0.0	78.56 ± 0.44	78.28 ± 0.56
B	2.5	78.62 ± 0.64	73.50 ± 0.86
C	5.0	78.82 ± 0.52	69.97 ± 0.75

Analysis of variance

Source of variation	df	ms	
		DM digestibility	N digestibility
Between blocks	9	3.10	3.00
Between Treatments	2	0.105	185.88**
Error	18	2.805	6.32
Critical difference		—	4.55

**$p < 0.01$

concluded that tannins in the diet reduced feed intake and N retention in growing chickens. Increase in endogenous N excretion as reported by Glick and Joslyn (1970b) may be another factor.

The depression in feed intake might be another important factor for the reduced growth rates of rats fed tannin containing diets. In the present experiment feed consumption per three day interval in group A was 20.05 g while that of group B was 16.66 g. Thus there was a 20% reduction in feed intake. When tannic acid content in the diet was two fold in group C, it caused only a slight and negligible further decrease in feed intake (16.11 g). Hence further decrease in growth rate might be due to further lowering in N digestibility and utilization of other dietary nutrients. Tamir and Alumot (1969) showed that digestive enzymes were denatured due to non-specific protein-tannin binding. Lindgren (1975) also observed a strong correlation between low digestion coefficients of CP and tannin content in the diet of hens.

The liver weights of the sacrificed rats were : 3.61, 2.85 and 2.44 g in groups A, B and C respectively. The difference between groups A and C was significant ($p < 0.05$) (table -4). Corresponding tannin levels in the diets caused a decrease in the liver weights along with less live weight gains.

Protein contents per gram of liver tissues were : 198.7, 199.9, and 208.2 mg and total protein contents in the whole liver on calculation were found to be 717.34 $\pm$ 4.76, 569.68 $\pm$ 4.31 and 507.95 $\pm$ 2.55 mg (table 4) which were significantly ($p < 0.01$) different from each other except between groups B and C. Dietary tannic acid did not affect protein content per gram of tissue, but due to differences in liver weights, total protein contents varied between different groups.

Table 4

Influence of tannic acid on weight, protein, RNA and DNA contents of liver

Group	Treatment % tannic acid	Weight of liver (g)	Protein content (mg)		RNA content (mg)		DNA content (mg)	
			Per g	Total	Per g	Total	Per g	Total
A	0.0	3.61 ± 0.21	198.69 ± 3.31	717.3 ± 4.76	5.93 ± 0.16	21.42 ± 1.41	1.48 ± 0.05	5.36 ± 0.41
B	2.5	2.85 ± 0.23	193.89 ± 5.14	569.7 ± 4.31	5.68 ± 0.16	16.40 ± 1.60	1.49 ± 0.06	4.29 ± 0.45
C	5.0	2.44 ± 0.01	208.16 ± 2.52	507.9 ± 2.55	5.66 ± 0.03	13.79 ± 0.59	1.38 ± 0.04	3.34 ± 0.16

Analysis of variance

Source of variation	df	ms						
		Liver weight	Protein/g	Total protein	RNA/g	Total RNA	DNA/g	Total DNA
Between Blocks	9	0.8514	1.62	34101.86*	0.237	37.15**	0.0288	2.85**
Between treatments	2	3.536*	3.14	115763.73**	0.105	150.38**	0.0350	10.16**
Error	18	0.9760	1.26	6854.34	0.241	7.31	0.0257	0.56
Critical difference		0.93	—	77.37	—	2.542	—	0.2211

*$p < 0.05$ **$p < 0.01$

The RNA contents per gram of tissue were : 5.93 ± 0.16, 5.68 ± 0.16, 5.66 ± 0.03 mg for groups A, B and C respectively whereas DNA contents per gram were : 1.48 ± 0.05, 1.49 ± 0.06 and 1.38 ± 0.04 mg in the corresponding groups (table -4). The differences among treatments were not statistically significant for both the parameters though they varied significantly ($p < 0.001$) on total value basis. The values for whole liver were : 21.42 ± 1.41, 16.40 ± 1.60 and 13.79 ± 0.58 mg for RNA and 5.66 ± 0.41, 4.29 ± 0.45 and 3.34 ± 0.16 mg for DNA in groups A,B and C respectively (table -4).

The protein : RNA ratios for the treatments were : 3.2, 35.4 and 36.9 and those for protein : DNA were 134.9, 136.7 and 153.7 for treatments A,B and C respectively. Corresponding body weight : liver weight ratios were : 29.0, 30.3 and 31.0 (table-5).

According to Chesco and Leblond (1962) DNA is constant within a single diploid cell in any species and hence it is possible to calculate the number of diploid cells in a given organ at any time by analysing the total organ DNA and dividing with DNA per cell (6.2 µµ g in the rat). In the present studies, the total RNA, DNA and protein in the liver tissue weresignificantly less in groups B and C as compared to group A; but their contents per gram of tissue among different treatments were more or less the same. Nevertheless, the protein : DNA ratio in group C was wider which meant that per unit weight of DNA, the protein content was much higher. This observation might be attributed to hypertrophy of individual cells. Srivastava et al.(1974) reported that malnutrition imposed at various periods of life in female rats also caused reduction in organ weights, total DNA, RNA and protein contents, and an increase in cell weight and

TABLE 5

Influence of tannins on body weight : liver weight,

Protein : RNA and protein : DNA ratios.

Group	Treatment (% tannic acid)	Body weight/ liver weight	Protein/ RNA	Protein/ DNA
A	0.0	29.0 ± 2.23	33.2 ± 1.01	134.9 ± 5.04
B	2.5	30.3 ± 2.45	35.4 ± 1.22	136.7 ± 5.75
C	5.0	31.0 ± 2.66	36.9 ± 1.01	153.7 ± 4.17

Analysis of variance

Source of variation	df	ms		
		Body weight/ liver weight	Protein/ RNA	Protein/ DNA
Between Blocks	9	163.3800**	32.1727**	365.0934
Between Treatments	2	12.8005	17.4480	910.1706*
Error	18	8.5337	8.7226	197.8721
Critical difference (5%)	—	—	—	13.167

*$p < 0.05$ **$p < 0.01$

the RNA and protein content per cell in the various organs of the young.

Normal growth of individual organs are due initially to the multiplication of cells (hyperplasia) as well as increase in size of individual cells (hypertrophy). Before weaning, all organs grow primarily by cell division. In rats, after 65 days, growth in all organs is primarily due to cell enlargement (Winick and Noble, 1966). Cell division may be interrupted by malnutrition at any time. In this regard, Srivastava et al. (1974) reported that restriction of protein in the diet of female rats during gestation and lactation periods caused an irreversible retardation in the growth and development of offspring. Kenny et al. (1968) observed that the spleen of adult rats fed a low protein diet contained less RNA and had fewer number of cells.

No decrease in protein and RNA per gram of tissue was observed in the tannic acid groups (table -4). But in acute cases of tannic acid poisoning, Horvath et al. (1960) and Badawy et al. (1969) demonstrated a decrease in protein and RNA contents in liver cells of tannic acid poisoned rats. This discrepancy can be explained, if route of administration of tannic acid and duration of experimental period are taken into account. Badawy et al. (1969) administered tannic acid intraperitoneally. In the present studies, relatively smaller doses were mixed with the food and hence much of the toxic action of tannic acid might be evaded due to relative impermeability of gastro-intestinal tract to tannic acid as reported by Handler and Baker (1944). Partial adaptation to tannic acid diet after a few days might also prevent toxicity (Glick and Joslyn, 1970a). Mitjavila et al. (1970) observed that intestinal absorption of glucose and methionine was decreased by perfusing the mouse small intestine with

gallotannic acid. According to them the overall biological utilisation of the diet was decreased at the level of intestinal absorption. Hence the decrease in liver weight without change in protein and RNA contents per unit weight, observed in this experiment, might be mostly due to general and protein malnutrition. Shortage in the availability of nutrients simply decreased hyperplastic changes in the liver as a long term effect.

The ratios of liver weights to total body weights were more or less the same (table -5) indicating that the toxicity was uniform in all tissues of the body which affected the hyperplasia of cells adversely. The doses of tannic acid in the diet of rat were such that classical symptoms of tannic acid poisoning such as staring coat, catarrhal and sometimes haemorrhagic discharge from the eyes and mouth, constipation followed by diarrhoea and disinclination to move and staggering gait were not seen. All the rats in all the groups survived the total experimental period of 24 to 30 days.

CHAPTER IV

IN VITRO TRIALS

MATERIAL AND METHOD

EXPERIMENT I. EFFECT OF FEEDING ON RUMEN LIQUOR VOLUME AND DRY MATTER DIGESTIBILITY

Four fistulated, adult, non-pregnant female Murrah buffaloes were selected as donors of rumen liquor for the in vitro trials. The animals were fitted with permanent Perspex glass rumen cannulae having screw caps which were always kept in position except the time of drawing samples.

The composition of the concentrate ration is given in table 6.

TABLE 6

Percent composition of concentrate ration

Maize	:	50
Ground nut cake	:	21
Wheat bran	:	26
Aquamineral	:	2.5
Salt	:	0.5
Total	:	100.00
DCP	:	13.97
TDN	:	72.65

Bovimix was given @ 5 g/animal once in a week.

The concentrate ration was fed to each animal @ 2 kgs per day along with 8 kg wheat straw for a period of 20 days prior to the collection of rumen liquor. The time schedule of sampling was as follows:

Sample collection : 8.45 A

Concentrate feeding : 9.00 AM

Watering : 10.00 AM

Feeding of wheat straw: 11.00 AM

Watering : 4.00 PM

Rumen liquor samples from the fistulated animals were collected with the help of 1 cm diameter hard polythene tube inserted through the rumen cannula, 30 cm deep into the rumen into a thermoflask kept at 39°C. The samples were collected to the brim of the flask to exclude all the air. After bringing to the laboratory, the samples were strained through two layers of muslin cloth and immediately used to charge the *in vitro* system.

The nitrogen solubility was determined by the method described by Peter *et al.* (1971).

The material used for the *in vitro* system was the same ration given under table 6, excluding the minerals. The ingredients were separately ground (40 mesh size) and then mixed together.

There were five treatments in duplicate with different quantities of tannic acid. The finely ground material (1 g each) was suspended in 20 ml McDougall's buffer (McDougall, 1948) in 150 ml bottles fitted with rubber corks with Bunsen valve apparatus. The required quantity of 5% tannic acid solution for each treatment (0, 0.25, 0.5, 1 and 1.5 ml to get 0, 1.25, 2.5, 5.0 and 7.5% tannic acid treatments) was added to each bottle. The bottles were then allowed to stand for two hours at room temperature. Then 30 ml each of McDougall's buffer was added to each bottle, mixed and kept at room temperature for 24 hours. The samples were then centrifuged at 1750 x g to remove insoluble particles. Soluble N was

determined by Kjeldahl method.

The solubility was expressed as a percentage of total N present in the material. The experiment was repeated twice.

For determining IVDMD by microbes the method described by Burns et al. (1970) was followed.

The treatments were the same as described for N solubility. The rumen liquor samples were collected from buffalo No. 61. Substrate 0.5 g and McDougall's buffer (pH 6.8) 20 ml were taken and treated with tannic acid. After two hours of tanning, the bottles were kept in a constant temperature water bath maintained at 39 ± 1°C. Thereafter 20 ml McDougall's buffer and 10 ml of strained rumen liquor (SRL) were charged into each bottle and the bottles were flushed with CO_2 by passing the gas over the contents for 1 minute and corked immediately. After 24 hour fermentation period, 1 ml of saturated $HgCl_2$ solution was added to stop bacterial action. Afterwards 2 ml quantity of 1 N Na_2CO_3 (Smith et al.,1971), was added to aid sedimentation. The tubes were centrifuged at approximately 1,800 x g for 15 minutes and the supernatant was discarded.

The precipitate was dried at 80°C in hot air oven and weighed. From this the amount of material digested or disappeared was calculated and expressed in percentage.

EXPERIMENT 2 INFLUENCE OF TANNINS ON IN VITRO DRY MATTER, CELL WALL AND PROTEIN BY MICROBES

The animals and feeding management, collection and processing of rumen liquor were the same as described under experiment 1.

Individual animals were fed the experimental ration 20 days ahead of sample collections. After the collection of first sample from each

animal, the animals were continued under the same feeding regime till the collection of the second sample 10 days after the first sampling. Thus, rumen liquor samples from four fistulated animals were collected and used in succession.

The *in vitro* system consisted of 12 bottles, so as to have two bottles for each of the 6 treatments (control 1.25, 2.5, 5 and 7.5% tannic acid treatments and a negative control). The details of the *in vitro* system are given in table 7.

TABLE 7

The details of in vitro system

Cellulose	: 0.75 g	
Starch	: 0.25 g	
Ammonium sulphate	: 151 mg	(151 mg)
McDougall's buffer	: 40 ml	
RL	: 30 ml	

One gram of finely powdered substrate (40 mesh size) was suspended in 20 ml McDougall's buffer along with 0.25, 0.5, 1.0 and 1.5 ml of 5% tannic acid solution in bottles (in duplicate) and allowed to tan for two hours at room temperature. Substrate without tannic acid formed the control treatment. A negative control, into which 1.5 ml of saturated $HgCl_2$ solution was added at the beginning of incubation, was also kept.

After two hours of tanning, the bottles were transferred to constant temperature water bath (39 ± 1° C). McDougall's buffer, 20 ml and RL, 30 ml were added to each bottle. The contents were mixed by gentle agitation and flushed with CO_2 gas as described under experiment 1 and allowed to incubate for 24 hours. At the end of incubation period, the

bacterial activity was stopped by adding 1.5 ml of $HgCl_2$ solution and the material was used for further estimations.

The samples were analysed for protein - N, RNA and DNA. Out of the total, 10 ml of the liquid portion was strained through sintered glass crucible and filtrate was used for total VFA estimation (Barnet and Reid 1959).

Nucleic acids were determined according to McAllan and Smith (1969). 40 ml of the sample was mixed with 40 ml of 18% TCA (w/v) in ethanol and kept at 4 °C for atleast 1 hour for complete precipitation. The material was centrifuged at 21,000 rpm (MSE supercentrifuge) for 20 minutes. The supernatant was discarded and the residue was washed successively with 20 ml volumes of 9% (w/v) aqueous TCA (twice), ethanol saturated with sodium acetate, 25% chloroform in ethanol (twice), 25% ether in ethanol and finally with ether. Each washing was centrifuged at 21,000 rpm (approximately 30,000 x g) for 20 minutes.

The ether dried residue was ground, weighed and divided into two equal halves each for RNA and DNA estimations.

(a) RNA estimation

Ether dried sample was hydrolysed with 10 ml of 1 N KOH for 18 hours at 37 °C and centrifuged at 21,000 rpm for 20 minutes. The residue was extracted with 1 ml of 1N KOH. Alkaline hydrolysate was made acidic by adding 4.4 ml of 1N perchloric acid ($HClO_4$) and centrifuged at the same speed for the same time. The acidic soluble part of alkaline digests was neutralised with KOH to pH 7.0 and the filtrate was buffered with 15 ml of 0.05 M tris (Tris hydroxymethylaminomethane) at pH 7.8. The solution (about 30 ml) was added to a column (15 cm x 0.5 cm) of Dowex 1 x 8,

50-200 mesh ion exchange resin in Cl^- form. The column was washed with 0.025 m tris buffer (pH 7.8) and then eluted with 0.5 N HCl to collect 100 ml elute. The readings were taken at 260 mμ in spectrophotometer against blank and compared with the values of the standard RNA from yeast.

(b) DNA estimation

Ether dried sample was extracted twice with 5 ml of 1N $HClO_4$ for 20 minutes at 70°C and centrifuged at 21,000 rpm for 20 minutes each. The volume of the total extract was made upto10 ml by adding 1N $HClO_4$. In the extract DNA was estimated by diphenylamine method as modified by Burton (1956).

Protein – N

To 20 ml of the aliquot, 5.0 ml of 1.07N H_2SO_4 and 5.0 ml of 10% sodium tungstate were added and mixed thoroughly. After standing for a minimum period of four hours, the mixture was centrifuged at 2,000 x g for 20 minutes and the precipitate was washed twice in a solution with 4 volumes of water plus one volume each of 1.07N H_2SO_4 and 10% sodium tungestate solutions. The N in the precipitate was determined by A.O.A.C. (1970).

EXPERIMENT 3. INFLUENCE OF TANNIN ON ^{32}P UPTAKE BY RUMEN MICROBES

For taking SRL, buffalo No. 31 was kept under the same feeding and management conditions as reported under experiment 1. The substrate was prepared from glucose 600 mg, and ammonium sulphate 85 mg.

The rumen liquor was strained through four layers of muslin cloth and thereafter 240 ml of the sample was centrifuged in CO_2 filled tubes at low speed for a minute to remove feed particles. The supernatant was

again centrifuged at 10,000 x g for 20 minutes at 2°C. The sediment of protozoa and bacteria thus obtained was suspended in 480 ml of anaerobic buffer (Bueholts and Bergan, 1973) and used in the in vitro system.

TABLE 8

Composition of Bueholts and Bergan Buffer

K_2HPO_4 0.3%	:	7.5 ml
Solution P*	:	7.5 ,,
Water	:	82.34 ,,
12% Na_2CO_3	:	1.11 ,,
3% Cysteine HCl	:	0.56 ,,

*Composition of solution - P

KH_2PO_4	:	0.3%
Na_2SO_4	:	1.2%
NaCl	:	0.6%
$MgSO_4.7H_2O$	:	0.6%
$CaCl_2.2H_2O$	:	0.06%

The mineral solution was xxx heated to 95°C and gassed with CO_2, followed by the addition of Na_2CO_3 and cysteine, equilibrated under CO_2 at 39°C and then finally adjusted to pH 6.7 with 1N NaOH or 1N HCl.

From the total, 40 ml of the reconstituted fluid was transferred into each bottle (experiment 1). The buffer provided 3.654 mg P to the total medium. To each bottle 2.48 Ci of ^{32}P (0.062 mg) as $NaH^{32}PO_4$ in 2 ml quantities was added; thus making the ratio of ^{32}P : ^{31}P as 1 : 58.9 in the total medium. The tannic acid treatments were the same as described under experiment 2. The bottles were incubated at 39 ± 1°C for 24 hours. After the incubation period, the organisms were precipitated by adding 10 ml of 1.07 N H_2SO_4 and 10 ml of 10% sodium tungstate;

kept for four hours at room temperature and centrifuged at 2,500 x g for 20 minutes at room temperature. The precipitate was washed twice with 20 ml portions of a solution which was prepared by taking four volumes of water and one volume each of 1.07 N H_2SO_4 and 10% sodium tungstate solutions. The supernatants were collected in volumetric flasks and made upto 100 ml volume. The precipitate was reconstituted to 50 ml with 0.1 N NaOH. The 0.5 ml quantities of supernatants and precipitate fractions were spread and dried on planchets at 70° C and counted for radio-activity in G.M. counter (ECIL) at 1,245 MET. A reference standard was also prepared by taking 0.5 ml of diluted ^{32}P solution containing 0.000775 mg of ^{32}P which was dried on a planchet at 70° C.

CALCULATION:

Quantity of ^{32}P added to the medium 0.062 mg.

Quantity of ^{31}P contributed by the buffer = 3.654 mg.

Standard count represents 0.000775 mg of ^{32}P

Hence quantity of total phosphorus represented by standard count = 0.000775 x 59.9355 x 100 = 4.645 mg. Where 100 is the dilution of factor.

Total quantity of phosphorus in the 40 ml of unknown =

$\frac{Cu}{Cs}$ x 4.645 mg where Cu = count of unknown and Cs = count of standard.

(Count of the negative control was subtracted from the other counts)

RESULTS AND DISCUSSION

EXPERIMENT 1 INFLUENCE OF TANNINS ON N SOLUBILITY AND ON DIGESTIBILITY

The solubility of N and IVDMD decreased as the levels of tannic acid increased (table 9). N solubility showed an abrupt decrease in treatments 1 and 2 thereafter the decrease was less with each increment of tannic acid.

TABLE 9

Influence of tannins on *in vitro* N solubility and DM digestibility

Treatment No.	Tannic acid (%)	Mean N solubility (%)	Mean DM digestibility (%)
1	0	36.73 ± 0.425	43.80 ± 0.84
2	1.25	24.48 ± 0.311	37.60 ± 1.00
3	2.5	20.81 ± 0.589	30.27 ± 0.81
4	5.0	17.55 ± 0.312	21.80 ± 0.61
5	7.5	15.30 ± 0.473	13.20 ± 0.53

The DM digestibility decreased more or less proportionately amongst the different treatments. The trend of drop was from 45.80% in treatment 1 to 13.20% in treatment 5 with added 7.5% tannic acid.

The affinity of tannins to combine with proteins and precipitate them is a well known phenomenon. That tannin-protein complexes are insoluble in water is well recognized (Bogen et al. 1959). The extent of degradation of dietary protein is largely depended upon its solubility in rumen liquor and physical form (Ammimon and Lewis, 1959) and the process which reduces its solubility will result in decreased breakdown of protein. But due to tanning, proteins are retained longer because tannins precipitate highly soluble proteins easily and make them unavailable for microbial utilisation. Bhargava et al. (1975) reported that 6% level of tannic acid decreased protein solubility of groundnut cake by76%. They suggested that treatment of groundnut cake with tannic acid protected the protein from microbial degradation. McCullough and Cummins (1974) observed high tannin sorghums having low protein digestibility in bullocks.

In the present studies there was a considerable reduction in IVDMD. The results are in agreement with the observations of some other workers (Donellyand Anthony, 1970; Saba et al., 1972; Burns et al., 1972; Donelly and Anthony 1975; Schaffert et al., 1974b; Tripathi, 1974; Green, 1974; Armstrong et al., 1974a; Arora and Luthra, 1974 and Burns and Depo, 1974 and 1976). Since the DM digestibility in in vitro studies was less, the inhibiting effect of tannic acid on microbial multiplication was demonstrated. At lower concentrations of tannic acid also, the adverse effect seemed to be proportionate because of proportionate decrease in availability of substrates.

EXPERIMENT 2 INFLUENCE OF TANNINS ON SYNTHESIS OF RNA, DNA AND PROTEIN BY MICROBES

Addition of tannic acid in the *in vitro* system resulted in a decrease in concentrations of protein, RNA and DNA . Microbial RNA, DNA and protein contents were maximum in treatment 1 and decreased with every increment of added tannic acid upto 7.5%. Similarly TVFA was also me found maximum in treatment 1, but their production decreased with increase in amount of tannic acid. At 7.5%, the VFA level was more or less similar to 0 hour control indicating complete inhibition of microbial fermentation which was evident also from other parameters like RNA, DNA and protein contents. In the case of protein - N, the critical difference showed significant treatment differences between one another except treatments 4 and 5. The RNA -N and DNA -N values also followed the same trend. The VFA values showed highly significant differences among all treatments ($p < 0.001$). RNA -N : protein - N ratios varied from 0.058 to 0.072 and DNA - N : protein - N ratios varied from 0.0196 to 0.026. The wider ratios were evident in treatment 5 (tables 10 and 11).

Reports regarding the influence of tannins on *in vitro* microbial synthesis of protein, RNA and DNA and production of VFA are scanty. In the present experiments, ammonium sulphate was used as the sole N source. Hence tannins added to the system were free to exert their harmful effects on micro-organisms. In systems, where natural diets are used as substrates, the tannins may form complexes with protein source. But in experiments, where SPN is the only N source, the effect of tannins may be only on microbial activity.

The harmful effects of tannins and their mechanism of action on microbes have been reported by Honig *et al.* (1964). Tannic might effect

TABLE 10 Influence of tannins on *in vitro* synthesis of protein, RNA-N and DNA-N and VFA concentration

Treatment	Level of tannic acid (%)	Mean protein - N (mg/100 ml)	Mean RNA (mg/100 ml)	Mean RNA - N[a] (mg/100 ml)	Mean DNA (mg/100 ml)	Mean DNA - N[b] (mg/100 ml)	Mean VFA (Meq)/ 100 ml)
1	0	30.19 ± 1.274	15.45	2.156 ± 0.107	5.37	0.795 ± 0.054	15.46 ± 0.315
2	1.25	23.84 ± 1.021	11.17	1.565 ± 0.101	3.78	0.561 ± 0.025	12.34 ± 0.194
3	2.5	18.59 ± 0.582	8.47	1.185 ± 0.046	2.68	0.426 ± 0.021	9.38 ± 0.425
4	5.0	16.27 ± 1.318	7.17	1.00 ± 0.423	2.28	0.337 ± 0.013	7.29 ± 0.359
5	7.5	14.61 ± 0.271	6.17	0.865 ± 0.034	1.965	0.290 ± 0.006	5.49 ± 0.235
C.D.(5%)		2.087		0.185		0.079	0.923
0 hour control		15.27	6.47	0.905	1.995	0.295	5.49

a N in RNA - 14% b N in DNA - 14.8% (McAllan and Smith, 1969)

Analysis of variance

Sources of variations	df	MS			
		Protein - N	RNA - N	DNA - N	VFA
Between animals	3	15.105*	0.147*	0.017	0.597
Between treatments	4	321.94**	2.163**	0.0333**	126.687**
Error	32	4.198	0.033	0.006	0.821

* $p < 0.05$ ** $p < 0.01$

TABLE 11

Influence of tannins on nucleic acid – N : protein – N ratios

	Treatments (tannic acid (%))				
	1 (0)	2 (1.25)	3 (2.5)	4 (5.0)	5 (7.5)
RNA – N : protein – N	0.072 ± 0.0018	0.066 ± 0.0018	0.064 ± 0.0028	0.062 ± 0.0016	0.058 ± 0.0013
DNA – N : protein – N	0.026 ± 0.0013	0.0234 ± 0.00043	0.0229 ± 0.00084	0.0207 ± 00077	0.0196 ± 0.00055
Nucleic acid – N : protein – N	0.0979 ± 0.0015	0.0886 ± 0.0022	0.0867 ± 0.0024	0.0824 ± 0.0020	0.0778 ± 0.0016

bacteria either by absorption on their surface or by penetrating into the bacterial cell and reacting with its components. The inhibition of cell wall synthesis is one of the early effects exerted by tannins on sensitive bacteria. The integrity of osmotic barrier is also impaired. Once the cell wall permeability is irreversibly affected, viability is also lost. According to Nagari et al. (1965) inhibition of microbial proteolytic activity by tannins could result from the formation of insoluble complexes with protein substrate and thus lead to inactivation of proteolytic enzymes.

Nagari et al. (1965) and Tripathi (1974) observed that tannins from different sources differed in the severity of action on micro-organisms. Variations in the degree of severity as well as lack of adaptation of microbes to tannins might explain why in this experiment there had been more profound effect than that reported by some other workers. (Delort - Laval et al., 1972; Verma et al., 1974 and Bhargava and Ranjhan, 1974). Since the rumen liquor samples used in the present experiment were collected from animals whose diet was free from tannic acid, the influence of added tannic acid in these studies was well marked. Perhaps the situation would have been a little different, if it had been collected from animals fed tannin containing diets.

Smith and McAllan (1970c) reported NH₃ - N and DAA - N values as low as 5.2% and 2.7% of the total protein - N when microbes were separated from rumen liquor. Values higher than this have been reported (Smith et al., 1968b and Smith, 1969). There could be a great variation because of different species of microbes from different sources. (Smith and McAllan 1974). Herbert (1961) cultivated pure strains of bacteria in continuous culture, where there was restriction of nutrients. He obtained bacteria having greater total - N content and decrease in NH_3 - N : total N ratio.

Maung and Baldwin (1976) observed that ratios of NH3 - N to total microbial - N were slightly higher in urea plus amino acids medium to that of urea medium alone.

EXPERIMENT 3 INFLUENCE OF TANNINS ON ^{32}P UPTAKE BY RUMEN MICROBES

The results of the experiment are given in table 12. The phosphorus uptake in the group without tannic acid (control) was2.64 mg per 100 ml. The ^{32}P in microbial population decreased with increase in levels of tannic acid in different treatments, signifying suppressed rate of microbial fermentation. The results obtained in this experiment further confirmed the results of the previous experiment where the tannins adversely affected microbial multiplication because there was less $^{32}PO_4$ utilisation.

Taylor (1946) reported 2.72% phosphorus present in E.coli culture on DM basis. According to Hungate (1966), protein formed 65% of dry cell mass in rumen microbes. As per these proportions, nearly 97 mg of dry cell mass might beproduced to give 63.05 mg of protein in the control group.

In the present experiment the micro-organisms were separated from TRL. In the process certain micro-organisms and growth factors might have been lost. Glucose used in this study might not be as efficient as cellulose powder used in experiment 2 in supporting microbial growth for 24 hour incubation period. Hence a comparatively higher value of net protein synthesis (93.34 mg/100 ml) was obtained in experiment 2.

TABLE 12

Influence of tannins on phosphorus utilization by rumen microbes

Treatment	Tannic acid (%)	Precipitate (CPM)	Supernatant (CPM)	% recovery of isotope	^{32}P incorporated (mg/40ml)	Total P incorporated (mg/40ml)	Total P incorporated (mg/100 ml)
Negative control	0	500	2532	83.23	—	—	—
1	0	2335	1941	92.26	0.0176	1.056 ± 0.057	2.640
2	1.25	1758	2095	86.43	0.0123	0.734 ± 0.077	1.835
3	2.50	1535	2364	90.63	0.0083	0.481 ± 0.036	1.202
4	5.0	851	2405	96.07	0.0035	0.208 ± 0.032	0.52 (0.52)
5	7.50	479	2670	87.70	—	—	—

Standard count = 6199

CHAPTER V

INFLUENCE OF DIETARY TANNIC ACID ON PROTEIN AND NUCLEIC ACID METABOLISM IN THE RUMEN

MATERIAL AND METHODS

EXPERIMENT NO. 1 INFLUENCE OF DIETARY TANNIC ACID ON PROTEIN, RNA AND DNA METABOLISM AND VFA PRODUCTION IN RUMEN OF BUFFALOES

Selection of animals and experimental design

A 4 x 4 Latin square design was adopted for rumen studies in four buffaloes. The details of distribution of animals are given in table 13.

TABLE 13

Distribution of animals

Periods	Treatments			
	I	II	III	IV
I	31	61	1	4
II	61	31	4	1
III	1	4	61	31
IV	4	1	31	61

Experimental animals were subjected to four feeding treatments. The DCP and TDN contents of all rations remained the same. But the content of total tannic acid varied in different rations.

The composition of ration under different feeding treatments is given in table - 14

The ingredients were procured in one lot to avoid qualitative differences. Based on the composition given by Sen and Ray (1964),

TABLE 14

Ingredient composition of concentrate rations

Ingredients	RATIONS			
	I	II	III	IV
Oilseed meal	—	14.00	28.00	40.00
Maize	50.00	22.00	24.00	—
Ground nut cake	21.00	30.00	35.00	44.00
Wheat bran	26.00	20.50	—	—
Starch	—	6.50	5.00	7.00
Linseed oil	—	4.00	5.00	8.564
Tannic acid	—	—	—	1.436
Supermindif	2.50	2.50	2.50	2.50
Salt	0.50	0.50	0.50	0.50
Total	10 0.00	100.00	100.00	100.00
DCP	13.97	14.50	14.00	14.00
TDN	72.65	73.99	72.63	71.90
Tannic acid (%)	—	1.25	2.50	5.00

(Movimix was given @ 5 g/animal once in a week).

the rations were computed.

The foregoing tables indicate that the four treatments contained similar quantities of TDN and DCP. But the oilseed meal content was different. In treatment IV pure tannic acid was also added so that the total tannic acid content would be 5%.

Feeding and management

A weighed quantity of wheat straw was given to each animal *ad libitum*. The two animals weighing 475 and 480 kg were given 2 kg of concentrate mixture each and the other two heavier animals weighing 545 and 554 kg were given 2.4 kg each.

Observations were taken after a preliminary feeding period of 20 days. Two collections of rumen liquor samples were made at an interval of one week while the animals were kept under the same treatment. At the time of switch over from one treatment to the other, the animals were brought back to treatment I for 10 days before being subjected to another treatment to avoid carry over effect of the previous treatment.

The schedule of experiment was as follows:

8 AM	Watering *ad libitum*
10 AM	Collection of 0 hr. sample followed by concentrate feeding.
2 PM	Collection of second sample
4 PM	Collection of third sample
4.30 PM	Watering and feeding of wheat straw *ad libitum* in succession.

Sampling and processing of rumen liquor

Rumen liquor samples were drawn from the fistulated experimental animals at 0,4 and 6 hours after offering the concentrates. Samples taken just before feeding the concentrates represented 0 hour sample. About 200 ml samples were collected at each time. The samples, soon after collection were placed in ice and brought to the laboratory, strained through two layers of muslin cloth and transferred to clean and dry polethene bottles. This RRL was used for estimation of protein -N, AMA, AIA and TVFA.

For nucleic acid estimation 20 ml RRL was mixed with 20ml 10% TCA in ethanol, kept at 4°C and then proceeded as given in Chapter III. Protein - N and VFA were determined by the methods described in Chapter III. The N in the salseed meal and ration samples as was estimated by Kjeldahl method (A.O.A.C., 1970). Tannins were estimated by A.O.A.C. (1970) method.

EXPERIMENT 2 INFLUENCE OF SALSEED MEAL ON RNA - N : PROTEIN - N AND TOTAL NUCLEIC ACID - N : PROTEIN - N RATIO IN SEPARATED BACTERIA

The animals and the experimental regime were the same as described under experiment 1. But only one sample, 6 hours after feeding was collected from each animal. To separate bacteria from rumen liquor, the method described by Weaver et al. (1974) was followed. 500 ml RRL was placed in a 500 ml measuring cylinder and allowed to stand for 10 minutes. The supernatant 400 ml was removed and used for estimating bacterial protein - N, RNA and DNA as given in Chapter III. The values obtained were used to calculate RNA - N : protein - N and total nucleic acid - N : protein - N ratios.

RESULTS AND DISCUSSIONS

EXPERIMENT 1 INFLUENCE OF SALSEED MEAL ON PROTEIN - N, RNA AND DNA - N AND VFA LEVELS.

Salseed meal contained 8.91% tannins and 10.21% CP. On calculation, the CP contribution of salseed meal to treatments II, III and IV were found to be 1.43, 2.86 and 4.08% respectively. The CP content of the treatments respectively for I, II, III and IV were 18.94, 20.25, 22.0 and 22.67%.

The protein - N levels in the rumen liquor were higher when salseed meal and tannic acid were added to the ration. Protein - N contents of the treatments I, II, III and IV were ß 43.72 ± 1.613, 49.09 ± 1.9124 54.86 ± 1.850 and 61.89 ± 2.050 mg/100 ml respectively. The difference amongst treatments were highly significant ($p < 0.01$) (table 15).

TABLE 15 Influence of tannins on protein – N, RNA – N, DNA – N and Total VFA levels in the rumen liquor
Data given are averages of 24 observations (8 replicates x 3 time samples) in duplicate.

Treatment (tannins %)	I (0)	II (1.25)	III (2.5)	IV (5%)	Critical difference (5%)
Protein-N (mg/100 ml)	43.72 ± 1.615	49.09 ± 1.912	54.86 ± 1.850	61.89 ± 2.050	5.545
RNA (mg/100 ml)	27.57	26.79	25.86	23.29	
RNA-N* (mg/100 ml)	3.86 ± 0.154	3.75 ± 0.115	3.62 ± 0.089	3.26 ± 0.097	0.271
DNA (mg/100 ml)	11.01	10.74	10.14	9.26	
DNA-N° (mg/100 ml)	1.63 ± 0.53	1.59 ± 0.067	1.50 ± 0.041	1.37 ± 0.046	0.102
Total nucleic acid – N (mg/100 ml)	5.49	5.34	5.12	4.63	
VFA (meq/100 ml)	9.59 ± 0.205	9.43 ± 0.215	9.20 ± 0.188	8.48 ± 0.283	0.733
Nucleic acid – N : protein – N ratios	0.125 ± 0.0012	0.108 ± 0.0020	0.093 ± 0.0026	0.072 ± 0.0011	0.017

* N in RNA = 14.0% ° N in DNA = 14.8% (McAllan and Smith, 1969)

Analysis of variance

Source of variation	df	ms				
		Protein – N	RNA –N	DNA –N	VFA	Nucleic Acid-N : protein-N ratios
Between periods	3	27.581	0.09	0.0167	0.537	0.00004
Between treatments	3	1459.647**	1.646**	0.357**	5.829*	0.00376**
Between animals	3	379.130*	0.926*	0.248	1.089	0.0000067
Error	86	95.458	0.224	0.0316	1.634	0.000291

*$p < 0.05$ **$p < 0.01$

RNA - N and DNA - N contents and total VFA levels followed the opposite trend. In case of RNA, the significant difference was observed only between treatments I and IV ($p < 0.01$) DNA levels showed highly significant differences between treatments I and III and III and IV ($p < 0.01$). The VFA levels showed a tendency for decrease with the increase in levels of tannins. The differences were significant between treatments I and IV ($p < 0.05$).

The nucleic acid - N : protein - N ratios for treatments I, II, III and IV were : 0.125 ± 0.0012, 0.108 ± 0.0020, 0.093 ± 0.0026 and 0.072 ± 0.0011 respectively. The differences were highly significant ($p < 0.01$) amongst all treatments.

The relative proportions of nucleic acid - N, microbial protein - N and dietary protein - N present in DUL at a particular time are influenced by several factors. One is the nucleic acid - N : microbial protein - N ratio. The ratios might differ in samples from difference animals reared in dissimilar dietary and environmental conditions, though it may be relatively constant under the same environment (Smith and McAllan, 1970a). Second factor which might influence is the solubility of the dietary protein. The extent of protein degradation is largely dependent on its solubility in rumen liquor (Annison and Lewis, 1959). Some relatively insoluble proteins like zein are comparatively resistant to microbial attack (McDonald, 1954). Tannins reduce solubility, thereby making proteins unavailable to microbes in the rumen.

Ammonia is used as an intermediary in the synthesis of protein - N by micro-organisms (Bryant and Robinson, 1963). Tannic acid could decrease the deamination process (Bridges and Hatfield, 1972) and this limited availability of ammonia may become another factor contributing to the decrease in microbial growth.

In the present investigation, the protein - N content in JRL was significantly greater in the tannin fed groups. Yet the microbial activity was less with increase in tannins which was evident from the lower levels of nucleic acid - N. A significant decrease was found only in treatment IV. The results were similar, if DNA - N was also considered as an index of microbial population because DNA - N has been reported to be proportional to the number of organisms (Caldwell and Hinshelwood, 1950).

When proteins are disintegrated in the rumen, ammonia, methane, carbon-di-oxide and VFA are produced (Philipson, 1970). As a result, only a fraction of dietary protein is converted into microbial protein. Hence when natural proteins are fed, it is quite probable that a decrease in microbial activity may result in the increase in total protein - N levels in the rumen liquor. Bhargava and Ranjhan (1974) observed slight increase in protein - N concentration in rumen liquor due to tannic acid treatment. The result of the present studies indicated that the rate of microbial degradation of dietary proteins in tannin treatments was less as compared to control (treatment 1).

The microbial multiplication was adversely affected, probably due to the limitations in the effective supply of available - N. Harmful effects of tannins in salseed meal on protein utilisation have already been reported by several workers. (Anon, 1972; Shukla and Talpada, 1973 and Pal et al., 1973). This might be due to precipitation of protein by tannins from the salseed meal. Tripathi (1975) observed a highly significant increase in protein breakdown in tannin extracted salseed meal indicating thereby that tannins prevented breakdown of protein by microbes.

Schults and Schults (1970) reported that tungestic acid precipitate of rumen contained food protein contamination. Hume (1970) observed that approximately as 25% of tungestic acid precipitate of the rumen liquor was dietary protein when comparatively insoluble protein zein was included in the ration. Since the microbial protein contribution became less and dietary protein contribution increased, the ratio of total nucleic acid - N to protein - N became wider (table 15).

Naga and Harmeyer (1975) observed that the relationship between VFA production and microbial growth as rate was negative. Under in vivo conditions, the level of VFA in rumen fluid is influenced not only by rate of production, but also by its absorption from the rumen, rate of passage, dilution of rumen contents by salivary and other secretions into the stomach (Annison and Lewis, 1959). Nevertheless observations of Hatepathy (1969), Fonnesbeck et al. (1970) and Hume (1970) showed that protein availability could influence production of VFA. The lower levels of VFA obtained in treatment IV probably demonstrated its limitations in the availability of proteins to the microbes.

EXPERIMENT 2 INFLUENCE OF VARIED LEVELS OF RNA - N : PROTEIN - N AND TOTAL NUCLEIC ACID - N : PROTEIN - N RATIO IN DIETARY SUPPLEMENTS ON RUMEN LIQUOR

The results are presented in table - 16. A significant effect ($p < 0.01$) due to treatments in protein - N, RNA - N and DNA - N contents was found only between treatments I and IV. There was no significant difference in RNA - N : protein - N and total nucleic acid - N : protein - N ratios. The RNA - N : protein - N and total nucleic acid - N : protein -N ratios respectively for treatments I, II, III and IV were : 0.000 ± 0.0030, 0.150 ± 0.0027; 0.097 ± 0.0023, 0.147 ± 0.0029; 0.098 ± 0.0024, 0.147 ±

TABLE 16 Influence of tannin on portein - N, RNA - N and DNA - N in bacteria seperated from RPC

Treatments (tannin %)	I (o)	II (1.25)	III (2.5)	IV (5.0)	Critical difference (5 %)
Protein -N (mg/100 ml)	23.93 ± 0.571	23.71 ± 0.627	22.79 ± 0.590	20.91 ± 0.544	1.4151
RNA (mg/100 ml)	17.03	16.40	15.93	14.64	—
RNA -N (mg/100 ml)*	2.385 ± 0.087	2.296 ± 0.062	2.230 ± 0.044	2.05 ± 0.046	0.1647
DNA (mg/100 ml)	8.14	7.97	7.51	6.82	
DNA -N (mg/100 ml)+	1.204 ± 0.036	1.180 ± 0.019	1.111 ± 0.059	1.010 ± 0.055	0.1361
DNA -N : protein -N ration	0.0994 ± 0.0030	.0970 ± 0.0025	0.0983 ± 0.0024	0.0982 ± 0.0020	
Total nucleic acid -N : protein - N ration	0.150 ± 0.0027	0.147 ± 0.0029	0.147 ± 0.0017	0.146 ± 0.0025	

*N in RNA = 14 % +N in DNA = 14.8%

Analysis of variance

Source of variation	df	Ms				
		Protein -N	RNA -N	DNA -N	RNA -N : protein N	Nucleic acid -N: protein - N ratios
Between periods	3	4.863	0.073	0.0333	0.000067	0.0000383
Between treatments	3	15.120**	0.160**	0.0600*	0.000033	0.0000188
Between animals	3	1.952	0.075	0.0133	0.00050	0.0000169
Error	22	1.862	0.025	0.017	0.00029	0.0000296

*p < 0.05 p < 0.01

0.0017; 0.098 ± 0.0020; 0.146 ± 0.0025. Wide variations in the nucleic acid – N : protein – N and RNA – N : protein – N ratios (0.08 to0.24 and 0.052 to 0.116 respectively) have been reported (McDonald cited by Ellis and Pfander, 1949; Ellis Pfander, 1965; Smith et al., 1968b; Smith, 1969; Smith and McAllan, 1970a, b, c and 1971; Porter et al., 1972; McAllan and Smith, 1972; Smith and McAllan, 1974 and Cole et al., 1976). The values obtained, in this experiment, also tend to agree with the reported ones.

The results substantiate the findings of previous experiments, showing thereby that tannins might be adversely affecting microbial multiplication at 5% level. Hence total nucleic acid and protein – N levels were less with tannin treatments. Inspite of such differences, the ratios of RNA – N : protein – N and total nucleic acid – N : protein – N remained relatively constant (Smith and McAllan, 1970c). Smith and McAllan (1970c) also reported that the total nucleic acid – N concentrations were directly proportional to protein – N concentration in the rumen liquor.

Smith and McAllan (1970c) postulated that calculation of protein synthesis could be made based on the nucleic acid – N values of the bacteria from a particular sample. The error due to not taking protozoa into account would be negligible. Based on this assumption, the contribution of microbial protein to tungstic acid protein of 5% was calculated (table 17). The maximum microbial contribution was observed in treatment I (83.37%) and minimum in treatment IV (51.14%). The results thus indicated that in control ration most of the dietary protein was converted into microbial protein. Estimates made by Smith and McAllan (1970a) revealed that 4 – 7 hours after feeding urea and fishmeal diets to calves 90% of the total – N in the rumen fluid was microbial – N. With ground nut meal diet also microbial – N contribution was fairly high (80%). But when the ration contained 5% tannins

TABLE 17

Influence of tannins on relative level of microbial - N and tungstic acid - N in the RL

Treatments (tannins %)	I (0)	II (1.25)	III (2.5)	IV (5.0)
Total nucleic acid - N[a] (mg/100 ml)	5.49	5.34	5.12	4.63
Nucleic acid - N/protein - N[b] ratios	0.150	0.147	0.147	0.146
Microbial - N (mg/100 ml)[c]	36.67	36.33	34.80	31.65
Tungstic acid - N (TA -N)[a]	43.72	49.09	54.06	61.89
Microbial - N (TA-N)	85.87	74.01	63.58	51.14

a Taken from table 15

b Taken from table 16

c Values calculated from nucleic acid - N : protein - N ratios and total nucleic acids.

as in treatment IV, large quantities of dietary protein escaped microbial attack.

Since the digestibility of CP from salseed meal is considered negligible, it was overlooked while computing the rations when only DCP values were taken into account. Hence inspite of the DC contents of the rations being the same, the total N contents were actually higher in salseed meal groups. This might have been responsible for part of the higher protein - N contents in tungstic acid precipitates of RL of those groups. The contribution of salseed meal - N to the tungstic acid precipitate cannot be accurately calculated. Nevertheless the available information is summarized in table - 18.

From the data given in table - 18, it is clear that the relative increase in protein - N in tungstic acid precipitates of RL from experimental groups was more than the increase in CP content of the experimental rations. It is evident that the significantly higher protein - N levels observed in the tungstic acid precipitates of treatments II, III and IV were due to (1) the dietary proteins, otherwise soluble and metabolised in the rumen, being precipitated by tannins and (2) the unaccounted CP of salseed meal itself. The figures given in column 8 of table -18 indicate the probable effect of tannic acid.

Table - 18

Influence of tannins on protein - N contents in RL

1	2	3	4	5	6	7	8
Treatments	Crude Protein-N in the feed (%)	Crude protein-N in the oilseed meal (%)	Protein - N in the precipitate (mg/100 ml)	Groups compared	Difference in crude protein - N relative to control (feed)	Difference in protein-N relative to control (RL)	Effect of tannins (7 minus 6)
I	18.94	0.0	43.72	I & II	6.92	12.28	5.36
II	20.25	1.43	49.09	I & III	16.15	25.48	9.33
III	22.00	2.86	54.86	I & IV	20.74	41.55	20.81
IV	22.87	4.08	61.89				

CHAPTER VI

SUMMARY

A study was conducted to elucidate the influence of tannins on synthesis of nucleic acids and protein in liver of rats. *In vitro* and *in vivo* studies in buffaloes were also conducted to ascertain the effect of tannins on rumen metabolism.

In experiment 1, 30 weanling rats were distributed into three groups of 10 each in a randomised block design. The influence of addition of 0% (group A), 2.5% (group B) and 5% (group C) tannins in the feed on feed consumption, growth rate, nitrogen and % dry matter digestibility was investigated. Further RNA, DNA and protein in liver were estimated to assess liver function.

The feed consumed daily on DM basis (g); weight gain per three day interval (g); and gram feed per gram weight gain, respectively for groups A, B and C were : 20.05 ± 7.6, 7.87 ± 0.41, 2.73 ± 0.05; 16.66 ± 6.0, 5.69 ± 0.35, 3.14 ± 0.07 and 16.11 ± 5.4, 4.55 ± 0.21, 3.80 ± 0.11. The DM and N digestibilities (%), respectively for groups A, B and C were : 78.56 ± 0.44, 78.28 ± 0.56; 78.62 ± 0.64, 73.50 ± 0.86 and 78.82 ± 0.52, 69.97 ± 0.75. Feed consumption in group A was significantly ($p < 0.01$) higher than in groups B and C. The difference in feed consumption between groups B and C was not significant. Significant differences were found amongst all treatment groups in weight gain ($p < 0.05$) and feed : gain ratios ($p < 0.01$). DM digestibility did not reveal any significant difference between groups whereas the differences in N - digestibility were significant ($p < 0.01$). The addition of tannins in the diet significantly depressed feed consumption, weight gain, and N - digestibility which resulted in widened feed : gain ratios.

The average liver weights (g); total protein (mg); RNA (mg) and DNA (mg), respectively for groups A, B and C were : 3.61 ± 0.21, 717.3 ± 4.76, 21.42 ± 1.41, 5.36 ± 0.41; 2.85 ± 0.23, 569.0 ± 4.31, 16.40 ± 1.60, 4.29 ± 0.45 and 2.44 ± 0.01, 507.9 ± 2.55, 13.79 ± 0.53, 3.34 ± 0.16. The liver weight in group A was significantly ($p < 0.05$) higher than in group C. The total protein content in group A was significantly ($p < 0.01$) higher than in other groups B and C. But the difference between groups B and C was not significant. RNA and DNA contents differed significantly ($p < 0.01$) amongst the three groups. The average protein (mg); RNA (mg) and DNA (mg), respectively for the groups A, B and C were : 198.69 ± 3.31, 5.943 ± 0.18, 1.48 ± 0.05; 199.89 ±5.14, 5.68 ± 0.16, 1.49 ± 0.06 and 208.16 ± 2.32, 5.66 ± 0.03, 1.38 ± 0.04 per gram of tissue. There were no significant differences in the parameters studied amongst the three groups. The body weight : liver weight ratios, protein : RNA ratios and protein : DNA ration, respectively for groups A, B and C were : 29.0 ± 2.23, 33.2 ± 1.01, 134.9 ± 5.04, 30.3 ± 2.45, 35.4 ± 1.22, 136.7 ± 5.75 and 31.0 ± 2.66, 36.9 ± 1.01, 153.7 ± 4.17. There were no significant differences amongst the ratios except that protein : DNA ratio in group C was significantly ($p < 0.05$) wider than in group A and B indicating probable hypertrophy of liver cells in that group. It was apparent that tannins exerted their harmful effects by affecting protein digestibility in the gastro-intestinal tract and thereby adversely affected liver size and growth rate.

In experiment 2, *in vitro* trials were conducted by taking buffalo rumen liquor through a rumen fistula on a control ration without tannic acid. For N solubility and N digestibility studies, the substrate used was : maize, 50 parts; ground nut cake, 21 parts and wheat bran 26 parts,

parts, ground into 40 mesh size. To study the influence of tannins on protein synthesis, nucleic acid synthesis and production of VFA, the substrates used were : cellulose 0.85 g, Starch 0.25 g and ammonium sulphate 151 mg. McDougall's artificial saliva was used as buffer (pH 6.8). For ^{32}P uptake by rumen microbes, the substrate was prepared from glucose 600 mg and ammonium sulphate 85 mg. A mineral solution containing cysteine-HCl described by Bucholtz and Bergan (1973) was used as a buffer. The levels of tannic acid, respectively in groups 1, 2, 3, 4 and 5 were : 0, 1.25, 2.5, 5.0 and 7.5% in all the experiments.

The N solubility and DM digestibility (%) respectively, for treatments 1, 2, 3, 4 and 5 were : 36.72 ± 0.425, 43.80 ± 2.63; 24.48 ± 0.311, 37.60 ± 2.14; 20.81 ± 0.589, 30.27 ± 1.85; 17.55 ± 0.312, 21.80 ± 1.93 and 15.30 ± 0.473, 13.20 ± 1.15. Addition of tannins depressed N solubility and DM digestibility. The protein - N (mg); DNA - N (mg); RNA - N (mg) and TVFA (meq) (all per 100 ml) respectively for treatments 1, 2, 3, 4 and 5 were : 30.15 ± 1.274, 2.156 ± 0.107, 0.755 ± 0.054, 15.46 ± 0.315; 23.84 ± 1.021, 1.565 ± 0.101, 0.561 ± 0.025, 12.34 ± 0.194; 18.59 ±0.582, 1.185 ± 0.046, 0.426 ± 0.021, 9.38 ± 0.425; 16.27 ± 1.318, 1.00 ± 0.042; 0.337 ± 0.013, 7.29 ± 0.359 and 14.61 ± 0.271, 0.865 ± 0.034, 0.290 ± 0.006, 5.49 ± 0.235. Addition of tannins significantly ($p < 0.01$) depressed all the parameters studied and in treatment 5, the levels were more or less the same as in 0 hour control indicating complete inhibition of microbial multiplication at 7.5% tannic acid level. The RNA - N : protein - N, DNA - N : protein - N and total nucleic acid - N : protein - N ratios respectively for treatments 1, 2, 3, 4 and 5 were : 0.072 ± 0.0018, 0.026 ± 0.0013, 0.098 ± 0.0015, 0.066 ± 0.0018, 0.023 ± 0.0045, 0.089 ± 0.0022; 0.064 ± 0.0018, 0.023 ± 0.00084,

0.087 ± 0.0024; 0.062 ± 0.0016, 0.021 ± 0.00077, 0.082 ± 0.0020 and and 0.058 ± 0.0013, 0.020 ± 0.00055, 0.078 ± 0.0016. The ratios were narrower in control group when compared to tannic acid groups.

With regards to ^{32}P uptake by rumen microbes, a progressive decrease was observed with increase in tannin concentration. ^{32}P uptake (mg) per 100 ml respectively for groups 1, 2, 3, and 4 were : 2.640, 1.855, 1.202 and 0.52. In group 5 there was no ^{32}P uptake. Tannins depressed microbial multiplication indirectly by making the protein source not available due to its precipitation. Direct harmful effect was also possible on microbes, especially, at higher concentrations of tannins in the media without any protein source.

In experiment 3, four adult fistulated female buffaloes were randomly distributed in a Latin square design. The treatments I, II, III and IV respectively contained 0, 1.25, 2.5 and 5% tannins made available from 0, 14, 28 and 40% mahua seed meal in the ration. In treatment IV, 1.436% pure tannic acid was also added to get 5% total tannins. The DCP and TDN contents were approximately 14 and 70% in all the rations. The effect of tannins in feeds was determined through the levels of protein - N, NPN - N, DNA - N and TVFA.

The protein - N, NPN - N and DNA - N levels (all in mg per 100 ml of RL) and TVFA levels (meq/100 ml of RL), respectively for treatments I, II, III and IV were : 45.73 ± 1.813, 3.86 ± 0.134, 1.65 ± 0.053 9.59 ± 0.205; 49.087 ± 1.8912, 3.75 ± 0.115, 1.59 ± 0.057, 9.43 ± 0.215; 54.86 ± 1.850, 3.62 ± 0.089, 1.50 ± 0.041, 9.20 ± 0.186 and 61.89 ± 2.050, 3.26 ± 0.097, 1.37 ± 0.046, 8.48 ± 0.203. Protein - N level in treatment I was significantly ($p < 0.05$) lesser than in rations II, III and IV and there

was a progressive and significant ($p < 0.05$) increase in order of treatments. RNA – N and TVFA levels in treatment I were significantly higher ($p<0.01$) than in treatment IV. DNA levels were significantly lesser ($p<0.05$) in treatment III than in treatment I and again lower in treatment IV than in treatment III. Nucleic acid – N : protein – N ratios in RL respectively for treatments I, II, III and IV were : 0.125 ± 0.0012, 0.108 ± 0.0020, 0.093 ± 0.0026 and 0.072 ± 0.0011. The ratio in treatment I was significantly higher than in treatments II, III and IV. The differences amongst the four treatments were significant ($p<0.01$). Addition of tannins in the rations resulted in an increase in protein – N, but progressively depressed the RNA – N and DNA – N levels with less production of TVFA.

Further in experiment 3, the protein – N, RNA – N and DNA – N contents of bacteria separated from RL were also determined to ascertain the effect of tannins on RNA – N : protein – N; DNA – N : protein – N and total nucleic acid : protein – N ratios. Protein – N (mg); RNA – N (mg); and DNA – N (mg) in bacteria separated from 100 ml RL respectively for treatments I, II, III and IV were : 23.93 ± 0.571, 2.385 ± 0.87, 1.204 ± 0.056; 23.71 ± 0.627, 2.296 ± 0.062, 1.180 ± 0.019; 22.79 ± 0.590, 2.230 ± 0.044, 1.111 ± 0.059 and 20.91 ± 0.544, 205 ± 0.046, 1.010 ± 0.055. Protein – N, RNA – N and DNA – N levels decreased as levels of tannins in rations increased. But the differences were significant ($p<0.01$) only between treatment I and IV. RNA – N : protein – N, and total nucleic acid – N : protein – N ratios respectively for treatments I, II, III and IV were : 0.099 ± 0.0030, 0.150 ± 0.0027; 0.097 ± 0.0023, 0.147 ± 0.0029; 0.098 ± 0.0024, 0.147 ± 0.0017 and 0.098 ± 0.0020, 0.146 ± 0.0025. The differences in the ratios amongst the different treatments were not statistically significant. The addition of tannins at the levels tried,

had no significant influence on the nucleic acid - N : protein - N ratios in the bacteria.

From the value obtained for nucleic acid - N and nucleic acid - N; protein - N ratios in separated bacteria, the microbial contribution of protein - N to the tungstic acid precipitate of SRL was calculated. The values obtained were : 83.67, 74.01, 65.58 and 51.14% for treatments I, II, III and IV respectively. The tannins present in the feed partially protected the proteins from microbial attack and hence the contribution of dietary protein - N in the SRL increased. Simultaneously the quantity of microbial protein synthesis decreased due to the limitations imposed by tannins on microbial multiplication.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

1. Incorporation of tannic acid @ 2.5 and 5 percent in the diet was harmful to rats. Tannins depressed feed consumption, weight gain and syntheses of protein, RNA and DNA in liver tissue. The harmful effects of tannins could be due to its interference with digestion and absorption of proteins and amino acids respectively.
2. Addition of tannins in the *in vitro* media without proteins had profound depressive effects on microbial multiplication. Tannic acid in a natural diet depressed N - solubility and DM digestibility.
3. Salseed meal in the ration at 20 percent level and very little deleterious effect on microbial multiplication. Five percent tannins contributed by 40 percent salseed meal plus 1.4% percent added tannic acid significantly depressed microbial activity. The protein - N contents in rumen liquor from tannin treatments were higher when compared to control, whereas DNA contents were lesser in tannin groups.

The tannins protected the proteins from microbial attack in the rumen and hence the availability of proteins in the lower digestive tract for enzymatic digestion was higher. In the present investigations it has been found that salseed meal upt 20 percent had very little harmful effect on microbial multiplications. Hence salseed meal, a cheap agro-industrial by-product, can be incorporated upto 20% in the ration with benefit. But it needs treatment for the removal of tannins for use at higher levels.

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