MANIPULATION OF DEAMINATION REACTIONS IN THE RUMEN MICROORGANISMS

Dissertation

Submitted to the Punjab Agricultural University
in partial fulfilment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

IN

BIOCHEMISTRY

(Minor Field : Microbiology)

by
Kawal Preet Singh

(L-87-BS-43-D)

Department of Biochemistry
College of Basic Sciences & Humnities
PUNJAB AGRICULTURAL UNIVERSITY
LUDHIANA-141 004
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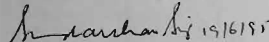
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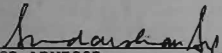PUNJAB AGRICULTURAL UNIVERSITY
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1993

CERTIFICATE I

This is to certify that this dissertation entitled, "Manipulation of deamination reactions in the rumen microorganisms" submitted to the Punjab Agricultural University, Ludhiana, in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the subject of Biochemistry (minor subject: Microbiology) is a bonafide research work carried out by Kawal Preet Singh (L-87-BS-43-D) under my supervision and that no part of this thesis has been submitted for any other degree.
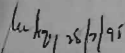
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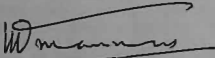

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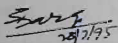Ludhiana-141004, Punjab

CERTIFICATE II

This is to certify that this dissertation entitled, "Manipulation of deamination reactions in the rumen micro-organisms" submitted by Kawal Preet Singh (L-87-BS-43-D) to the Punjab Agricultural University, Ludhiana, in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the subject of Biochemistry (minor subject: Microbiology) has been approved by the student's Advisory Committee after an oral examination on the same in collaboration with an External examiner.

MAJOR ADVISOR
(Dr. Sudarshan Singh) 28/7/95

EXTERNAL EXAMINER

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Department of Molecular
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HEAD OF THE DEPARTMENT
(Dr. K.L. Bajaj) 28/7/95

DEAN OF POSTGRADUATE STUDIES
(Dr. K.D. Mannan)

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and Vinki deserve a special mention for always boosting my morale from across the seas and strengthening my faith in my self.
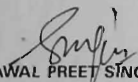
Jerry's toothless smiles, Komal's love, a long and tedious wait by Roami, Ruby and Sandy has gone a long way to help me accomplish this. I am sorry for the inconvenience caused.

Over and above, I am thankful to Baljeet, my life companion and best friend who suffered an emotional trauma during the course of this study. Her firm belief in my knowledge, academic capabilities and unwavering love were the anchors that held me tied to sanity during the annui of the research work and loneliness. Her sacrifices, I am sure, have not gone in vain. Above all, God help Ph.D.scholar's wife.

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(KAWAL PREET SINGH)

CONTENTS

Title of dissertation	:	Manipulation of deamination reactions in the rumen micro-organisms
Name of the student and Admission No.	:	Kawal Preet Singh (L-87-BS-43-D)
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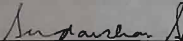
ABSTRACT
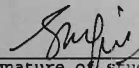
Degradation of various feed proteins and individual amino acids and biosynthesis of proteins in the presence of various deaminase and methanogenic inhibitors by rumen microorganisms obtained from fistulated buffalo (Bubalus bubalis) bulls were investigated. The deaminase activity of mixed rumen bacterial extract was also characterized. Intact, soluble and insoluble proteins of casein, soyabean seed meal (Soya), groundnut cake (GC), mustard cake (MC) and sunflower seed meal (SF) were used as protein sources. Intact, soluble and insoluble proteins of casein were degraded by rumen fluid (SRF) and its fraction viz. bacteria, protozoa and cell-free supernatant to maximum extent followed, in order, by soya, GC, MC and SF. Soluble proteins were degraded to maximum extent and the insoluble proteins were degraded to minimum extent by SRF and its fractions. All these proteins were degraded maximally by SRF, followed by cell-free supernatant, bacteria and protozoa. There was almost direct relationship between the production of ammonia and the degree of protein degradation by SRF and its fractions. Individual amino acids were degraded at different rates by both SRF and bacteria. With regard to their relative rate of degradation and deamination by SRF the amino acids were divided into 4 groups. Serine, cysteine and aspartic acid (87-92%) were attacked mostly completely, followed by arginine, phenylalanine, threonine, glutamate, glutamine, lysine, tyrosine and cystine (44-70%), tryptophan, alanine,

methionine and histidine (30-35%) and isoleucine, valine, glycine, hydroxyproline and proline (9-18%). The pattern of degradation of amino acids was the same with bacteria but the rate of degradation was lower than with SRF.

The pH and temperature optima for deaminase activity was 6.75 and 45°C and the activity was thermal stable upto 50°C. Out of large number of chemicals found to have inhibitory effect on deaminase activity, sodium azide, p-chloromercuri-benzoate, hydrazine and its derivatives, sodium arsenate, sodium arsenite, dimethyldiphenyl iodonium chloride (DDIC), phosphoramidon, monensin, chloroform and chloralhydrate were found to be strong inhibitors (50-80% inhibition). The deaminase seems to be a metalloenzyme and requires -SH groups for its catalytic activity. It was observed that 61.4% of activity was present in cell-free supernatant and 43.7% was associated with bacteria. Within the bacterial cell 65% of the activity was present in cytosol and 35% was associated with membranes.

All the above mentioned inhibitors except sodium azide and p-CMB (which were not examined) significantly increased the concentration of TCA-precipitable proteins in the incubation mixture when different intact proteins were incubated with SRF and bacteria. The order of effectiveness of these inhibitors for increasing the TCA-precipitable proteins was phenyl-hydrazine > sodium arsenate > DDIC > sodium arsenite > hydrazine > p-Nitrophenylhydrazine > hydrazine sulfate > hydroxylamine-HCl with SRF and hydrazine sulfate > hydrazine > DDIC > sodium arsenate/sodium arsenite > hydroxylamine-HCl > phenylhydrazine > p-nitrophenyl hydrazine with bacteria. In the presence of these inhibitors the per cent increase in the concentration of TCA-precipitable proteins was higher with casein (50-86% with SRF and 33-112% with bacteria) and soya (18-74% with SRF and 28-79% with bacteria) followed by MC (32-62% with SRF and 17-74% with bacteria), SF (30-58% with SRF and 12-64% with bacteria) and GC (25-52% with SRF and 19-75% with bacteria).

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Signature of Major Advisor   15/6/95

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Signature of student

CHAPTER I

INTRODUCTION

Feedstuff consumed by the ruminants are initially exposed to microbial fermentation in the reticulo-rumen prior to post-gastric enzymic digestion (Mackie and White, 1990). Dietary proteins are extensively degraded by microbial proteases to peptides and amino acids in the reticulo-rumen (Hobson and Wallace, 1982a,b). The free amino acids released can be assimilated directly by rumen microbes for protein biosynthesis but most are rapidly deaminated to yield ammonia and other intermediate products (Chalupa, 1974). The ammonia, so produced, is the main nitrogen source for protein biosynthesis by rumen microorganisms (Chalupa, 1974). However, the degradation of dietary proteins and free amino acids is often in excess and all the ammonia produced, especially from high quality dietary proteins, is not fully utilized for microbial protein synthesis. The excess ammonia is absorbed from the rumen and is excreted as urea in the urine and hence goes waste (Nolan and Leng, 1972; Chalupa, 1975). Depending upon various factors 40 to 60% of the dietary proteins are

degraded in the rumen (Leng and Nolan, 1984; Mackie and Kistner, 1985). The undegraded dietary proteins and microbial proteins are digested post-ruminally and meet the amino acids requirement of the host animal. Quantitative losses upto 55% may occur during the transformation of dietary proteins and free amino acids to microbial proteins (Smith and McAllen, 1974; Chalupa, 1975).

For efficient utilization of dietary proteins by ruminants, it is therefore, desirable to avoid the losses occurring due to the transformation of dietary proteins to microbial proteins by reducing the extent of degradation of dietary proteins in the rumen. This can be achieved by (i) protecting the dietary proteins against ruminal degradation by treating them with various chemicals like formaldehyde, tannins and zinc salts (Dreider and Hatfield, 1970; Phillips et al., 1981; Mahadevan et al., 1983; Siddons et al., 1984; Britton and Klopfenstein, 1986; Cecava et al., 1993), (ii) depressing proteolysis in the rumen (Chalupa, 1975), and (iii) using those dietary proteins which are more resistant to ruminal degradation (Ganev et al., 1979; Siddons and Parandine, 1981).

Deamination of amino acids in the rumen is also of primary significance in the utilization of dietary nitrogen by ruminants, since this is the principal step by which dietary amino acids may be irreversibly lost to the animal. Inhibition of deamination, therefore, assumes practical significance as a

means of increasing ruminal escape of dietary amino acids (Broderick and Balthorp, Jr. 1979).

Keeping in view the above observations, the present study was undertaken to optimise the use of dietary proteins in ruminants by decreasing the proteolysis of feed proteins and deamination of amino acids in the rumen. To achieve this objective it was proposed to examine:

(i) Proteolysis of different types of dietary proteins and deamination of amino acids by rumen fluid and its fractions.

(ii) Isolation and characterization of deaminases from rumen bacteria.

(iii) Effect of selected deaminase and methanogenic inhibitors on protein synthesis by rumen microorganisms.

CHAPTER I

REVIEW OF LITERATURE

Dietary proteins are extensively degraded to peptides, amino acids and finally to ammonia (Chalupa, 1974; Tamminga, 1979) in the reticulo-rumen. The ammonia, so produced, is the main nitrogen source for protein biosynthesis in rumen micro-organisms. However, all the ammonia produced, especially from high quality proteins, is not utilized for protein biosynthesis and the excess is absorbed from rumen and is lost as urea in the urine. Quantitative losses upto 55% may occur during the transformation of dietary proteins to microbial proteins. For efficient utilization of feed proteins, therefore, it is desirable that there should be maximum incorporation of intact dietary amino acids into microbial proteins or least catabolized in the rumen for maximum absorption post-ruminally. In the light of this preamble, the literature is reviewed under the following heads:

2.1   Proteolysis of dietary proteins in the reticulo-rumen

2.2   Deamination of amino acids

2.3   Protection of dietary proteins and amino acids against ruminal degradation

2.4   Chemical inhibition of amino acid deamination

2.1 Proteolysis of dietary proteins in the reticulo-rumen

Dietary proteins are extensivley degraded in the rumen by ruminal bacteria and protozoa to peptides, amino acids and ammonia (Chalupa, 1974; Tamminga, 1979; Mackie and White, 1990). The information regarding the microorganisms responsible for proteolysis of dietary proteins and the nature of microbial proteases has been reviewed by several workers (Blackburn, 1965; Hobson and Howard, 1969; Allison, 1970; Bryant, 1970; Lewis and Swan, 1971; Armstrong and Hutton, 1972 and Vaz Portugal, 1972). It has been observed that ruminal bacteria are mainly responsible for proteolysis (Broderick and Craig, 1983; Cotta and Hespell, 1986; Mahadevan et al., 1987 and Nugent and Mangan, 1981). Bacterial proteases are membrane bound and located on the surface to facilitate the access to substrate and include both exo- and endo-peptidases (Nugent and Mangan, 1981; Kopecny and Wallace, 1982 and Wallace, 1985). These enzymes are constitutive in nature and do not appear to be subjected to metabolic control(Chalupa, 1974).

Predominant proteolytic bacteria include gram negative species such as _Bacteroides ruminicola, Bacteroides amylophilus, Megasphaera elsdenii, Selenomonas ruminantium_ and _Succinovibrio_ species (Chalupa, 1974 and Cotta and Hespell, 1986). Major gram positive strains include _Butyrivibrio fibrisolvens, Streptococcus bovis, Lachnospira_ sp.,

Propionibacterium sp., and Clostridium sp. (Bryant and Robinson, 1962; Blackburn, 1968; Allison,1970; Russell et al., 1981; Brock et al., 1982; Hazlewood and Edwards, 1982 and Wallace and Brammall, 1985). Main proteolytic protozoal species include Entodinium, Isotricha, Eudiplodium and Ophryscolex (Foresberg et al., 1964; Coleman, 1967 a,b; 196 8; 1969a,b; Allison, 1970 and Bryant, 1970). Onodera and Kandatsu (1970) described the protozoal proteases to be located internally and hence of not much significance to dietary protein degradation.

Protein degradation can be envisioned in distinct steps involving (i) hydration (ii) solubilisation (iii) proteolysis (iv) Uptake (transport) and (v) either fermentation or microbial protein synthesis (Chen et al., 1987). It has been generally assumed that proteolysis may be a rate limiting step in protein degradation (Nugent and Mangan, 1978; Satter and Roffler, 1975; Tamminga, 1979).

It has been suggested that peptide uptake and not the proteolysis could be the rate limiting step in dietary protein degradation by rumen microorganisms (Bladen et al., 1961; Russell, 1983; Russell et al., 1983; Hino and Russell, 1985, 1987; Chen et al., 1987). Broderick et al.(1988) also reported that peptides would accumulate in rumen fluid during hydrolysis of rapidly degradable proteins whereas the peptide uptake would exceed their release in case of slowly degradable proteins.

2.1.1 Various factors influencing protein degradation in rumen

The various factors influencing the protein

degradation in the rumen are briefly summarized below:

2.1.1.1 Protein solubility

It is assumed that soluble proteins are rapidly and more completely degraded than insoluble ones, because of their greater accessibility to proteases in solution. Actually soluble proteins differ greatly in the rate at which they are hydrolysed. Nugent and Mangan (1978) showed that casein, leaf protein fraction I and bovine serum albumin, though soluble in buffer, were hydrolyzed at different rates viz. casein < leaf protein fraction I < bovine serum albumin. Hence it was suggested that differences in rates of microbial hydrolysis of certain proteins are caused by structural rather than solubility differences (Nugent and Mangan, 1978; Mahadevan et al., 1980). Protein solubility is, therefore, expected to predict differences in protein degradation more accurately when applied to a similar group of feeds across a diverse group of feeds differing in physical and chemical properties (Satter, 1986). The proteins may also have altered solubility at lower pH and hence may exhibit altered degradability (Ganev et al., 1979).

2.1.1.2 Protein structure

Mahadevan et al.(1980) showed that degradation of soluble and insoluble proteins by Bacterioides amylophilus protease and by rumen microorganisms proceeded at almost identical rates and suggested that structural characteristics of protein determines its degradation in the rumen. It was

further shown that treatment of a resistant protein with mercaptoethanol rendered it to be more susceptible to hydrolysis indicating that proteins having disulfide bonds are more resistant to degradation. Ovalbumin which inspite of being soluble in rumen fluid, resists proteolytic attack, probably due to the cyclic feature of this protein.

2.1.1.3 Retention time in rumen

Extent of protein degradation is influenced by resident time of proteins in the rumen. All the factors which determine the resident time of a protein in the rumen such as level of feed intake (Tamminga, 1979), dilution rate of rumen fluid (Harrison et al., 1975; Cole et al., 1976; Prigge et al., 1978; Hemsley, 1975) and environmental temperature (Kennedy et al., 1976; Keeney et al., 1980) will, therefore, influence the protein degradation in the rumen.

2.1.1.4 Feed processing and storage

Some feeds are exposed to heat during processing. By-product feeds are often dried for marketing and ensiled feeds may experience elevated temperatures for sufficient time. Feed processing methods such as pelleting, extrusion, steam, rolling or flaking may generate enough heat to alter a protein (Satter, 1986). All such treatments may protect the dietary proteins against ruminal degradation.

2.1.1.5 Ammonia concentration in the rumen

Several workers have studied the influence of ammonia concentration on protein degradation in the rumen. Orskov et

al. (1974) concluded that inclusion of urea in the diet did not have a sparing effect on degradation of dietary proteins from barley and fish meal in lambs. Wallace et al.(1979) reported that when ammonia concentrations in the rumen fluid of sheep, fed whole barley, were increased from 6.1 to 13.4 mM, there was 90% increase in degradation rate of rolled barley but only smaller increases in rates of degradation of dietary proteins and plant fibres. Nikolic and Filipovic (1981), however, showed that low ammonia concentrations did not affect the degradation rate of dietary maize proteins, atleast in short term expression.

Song and Kennelly (1991) showed that although removal of protozoa from rumen fluid decreased the extent of degradation of dietary protein, the rate and extent of degradation was not influenced by ammonia concentrations, but was highly correlated to protein solubility. it was concluded that ammonia concentration was not the primary factor regulating the proteolytic activity of microorganisms, rather their activities may depend on the solubility and other physico-chemical characteristics of dietary proteins.

2.1.2 Comparative role of bacteria and protozoa in degradation of proteins

Not much is known about the quantitative contribution of ruminal bacteria and protozoa to the degradation of dietary protein in the rumen, except that bacteria are primarily responsible for proteolysis (Nugent and Mangan, 1981; Brock et

al., 1982; Kopecny and Wallace, 1982; Foresberg et al., 1984; Wallace and Brammall, 1985). It was observed that mixed ruminal bacteria adsorb dietary proteins on the cell wall and hydrolysis occurs there (Nugent and Mangan, 1981; Kopecny and Wallace, 1982; Wallace, 1985). In this process, solubility and primary amino acid sequence of proteins are important determinants of proteolysis. Ammonia is the major end product of bacterial degradation of proteins.

Entodiniomorphs protozoa only take up particulate proteins, whereas Isotrichs (Holotrichs) utilize both soluble and particulate proteins (Abou Akkada and Howard, 1962; Onodera and Kandatsu, 1970). Weller and Pilgrim (1974) reported that the contribution of protozoa to the amount of microbial proteins entering small intestine was very low. Ushida and Jounay (1985) reported that in vitro degradability of proteins was significantly lower in defaunated sheep and the protozoal effect was greater when protein solubility was low. The protozoal species, particularly large Ophyrscoleidae, (100 μm), degraded more, relatively insoluble fractions of dietary proteins. Entodinium simplex engulfed all types of bacteria and utilized their amino acids without any change or interconversion (Coleman, 1972).

Coleman (1964) and Coleman and Hall (1969) also reported that Entodinium ecoudatum rapidly engulfed bacteria and it was likely that this organism may obtain its amino acid requirements for its protein synthesis from engulfed bacteria.

It was estimated that maximum rate of uptake by this organism was approximately 200 bacteria per minute and each organism contained upto 10^4 E. coli when completely filled.

Heald and Oxford (1953) were the first to observe the loss of soluble peptides and proteins from holotrich protozoa. Harmeyer (1971) reported that as much as 25% of the cellular nitrogen was excreted into the surrounding medium by Isotricha species in a 24 h period, one-third of which was amino acids (alanine, glutamate, aspartate, proline, ornithine) and ammonia. The excretion was not affected by the presence of exogenous amino acids and represented the degradations of ingested nitrogenous material.

Eadie and Gill (1971) observed that ruminal ammonia concentrations were about twice as high in faunated than in defaunated sheep. Stern et al. (1977) observed that ciliate protozoa were able to synthesize amino acids as well as utilize preformed amino acids released by proteolysis of ingested bacteria or plant material. It was suggested that amino acids of undigested chloroplast were incorporated into protozoal proteins.

Hino and Russell (1987) used heat killed bacteria (bacterial fraction heated at 80°C for 20 min in NaCl) as a protein source and showed that addition of amphoterin (10 μg/ml) that killed protozoa in incubation mixture, decreased ammonia production when killed bacteria were used as protein source. Defaunation, however, had no effect on deamination of

casein. Protonophore monensin (5 $\mu g/ml$), that also killed protozoa, decreased the casein deamination to a much greater extent than amphoterin. Antibacterial antibiotics such as penicillin G, polymixin B, cephalosporin and streptomycin greatly reduced ammonia formation from casein. It was further shown that isolated bacteria produced more ammonia from casein than isolated protozoa, but the difference was less with heat treated particulate proteins such as heat killed bacteria. Non-ammonia- non-protein nitrogen accumulation with protozoa was grater than with bacteria. It was suggested that (i) soluble proteins were primarily degraded by bacteria (ii) protozoa could contribute to the degradation of insoluble particulate proteins (iii) protozoa were limited in their ability to assimilate peptides (or amino acids) (iv) low molecular weight products could be fermented more rapidly by bacteria and (v) Monensin was toxic to protozoa and decreases in ammonia concentrations were primarily due to its action on bacteria.

Ushida et al.(1990) reported that elimination of protozoa (defaunation) resulted in increased duodenal flow of primarily non-ammonia nitrogen. This was primarily due to increased microbial protein flow and to a lesser extent to higher dietary protein flow. It was further shown that defaunation markedly increased the efficiency of microbial protein synthesis.

2.2 Deamination of amino acids

2.2.1 Mechanism of deamination

Available information indicates that fermentation of amino acids, which are formed as a result of proteolysis, mainly involves oxidative deamination (or transamination with a keto acid) to keto acids, followed by a decarboxylation (or transamination with another amino acid) of keto acids (Deimeijer, 1976; Prins, 1977: Van Nevel and Demeyer, 1979). This is most important pathway for amino acid degradation operating in the rumen and involves a yield of one ATP per decarboxylation.

Hungate (1966) suggested that Stickland type reaction may be important for amino acid degradation. Broderick and Balthorp Jr. (1979) reported that addition of arsenate, an inhibitor of reductive step in Stickland reaction, also inhibited the deamination of amino acids by rumen microorganisms completely. This observation confirmed the hypothesis advanced by Hungate (1966). Generalised Stickland reaction may be summarized as follows:

$$R'CHNH_2COOH + 2 R''CHNH_2COOH + 2H_2O$$
$$------> R'COOH + CO_2 + 3NH_2 + 2R''CH_2 COOH.$$
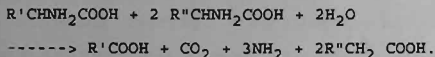
The above reaction involves coupled oxidation-reduction between suitable pairs of amino acids where one amino acid is oxidatively deaminated and decarboxylated while the other amino acid is reductively deaminated.

Stickland (1934) classified amino acids as proton

donors (e.g. alanine, valine and leucine) and proton acceptors (e.g. glycine and proline) on the basis of their ability to reduce dyes such as methylene blue. Proton acceptors were ineffective in reducing methylene blue, but rapidly oxidized the reduced form of low redox potential dyes such as benzene viologen. This observation indicated that oxidative and reductive deamination occurred at quite different redox potentials. Stickland (1935) while studying the interactions between amino acids and suitable redox dyes in presence of cell suspension of Clostridum sporogenes, presented the evidence that the reduced power generated in oxidative deamination yielding three moles of acid for every three moles of amino acid consumed produced one mole of ATP.

Cohen-Bazzire et al.(1948) showed that cell suspension of Clostridium sporogenes and related organisms converted valine to isobutyrate, leucine to isovalerate and isoleucine to an optically active valeric acid (probably 2-methyl butyrate). Britz and Wilkinson (1982) showed that leucine dissimilation by cell suspension of Clostridia and Peptostreptococcus anaerobius followed stickland reaction stoichiometry as the ratio of isovalerate to isocaproate was 1:2, amount of CO_2 produced being equal to isovalerate and ammonium ion concentration equal to total C_5 and C_6 acids formed. Presence of alanine and valine (proton donors) in incubations effectively increased the concentration of isovalerate at the expense of isocaproate, implying that leucine, in this case, primarily acted as proton

acceptor. Glycine and proline (proton acceptor) stimulated both isocaproate and isovalerate from leucine, indicating that leucine here acted as proton donor. It was concluded that leucine played dual role during amino acid deamination. It was further shown that addition of glucose stimulated the conversion of leucine to volatile fatty acids production indicating that glucose inhibited the oxidative deamination and not the reductive deamination. This effect could be due to glycosylation process utilizing cofactors such as NAD^+ which are otherwise available for amino acid deamination.

The stoichiometric relationships of amino acids degradation are not well understood. Demeijer (1976), Prins (1977) and Tamminga (1978) suggested that microbial growth efficiency during protein fermentation is inferior to carbohydrate fermentation. In vitro experiments on fermentation of casein by Demeijer (1976) indicated that 0.43 moles of amino acids in casein yielded 0.14 moles of propionate and 0.09 moles of methane. Since casein contains some 0.85 moles of amino acids per 100 g and fermentation of 100 g of casein would, therefore, yield 1.3 moles of ATP (0.85 due to decarboxylation of α-keto acids, 0.27 from the formation of propionate and 0.18 from formation of methane). This is considerably less than the generally accepted minimal yield of 4 to 5 moles of ATP per mole of hexose equivalent (162 g of polysaccharide) fermented in rumen (Prins, 1977). Van Nevel and Demeyer (1979) conducted experiments where this stoichiometry and microbial energetics

could be calculated based on following general reactions:

2H produced $(2H_p)$

(i) $R \cdot CH(NH_2) \cdot COOH + H_2O \longrightarrow RCO \cdot COOH + NH_2 + 2H$

(ii) $RCOCOOH + CH_2CH(NH_2)COOH \dashrightarrow R \cdot CH(NH_2)COOH + CH_3CO \cdot COOH$

(iii) $CH_3CO \cdot COOH + H_2O \dashrightarrow CH_3COOH + CO_2 + 2H$

2H is utilized $(2H_u)$ as follows:

(i) $CH_3CO \cdot COOH + 4H \dashrightarrow CH_3CH_2COOH + H_2O$

(ii) $2CH_3COOH + 4H \dashrightarrow CH_3(CH_2)COOH + 2H_2O$

(iii) $CO_2 + 8H \dashrightarrow CH_4 + 2H_2O$

(iv) $NH_2(CH_2)_4 \cdot COOH + 2H \dashrightarrow NH_3 + CH_3(CH_2)_3COOH$

Isovalerate (IV) and valerate (V) were assumed to be formed from deamination followed by decarboxylation of leucine (isoleucine) and lysine respectively. Regeneration of reduced cofactors involves the production of acetate(A), propionate (P), butyrate (B) and methane (M) and of valerate (V) from α-NH_2-valerate.

The molar proportions of amino acids (AA) fermented and NH_3 formed were calculated from net molar amounts of volatile fatty acids (VFA) formed as follows:

$AA = A + P + IV + V + B.2$ and

$NH_3 = A + P + IV + 2V + B.2$.

$= AA - V$

The amount of protein synthesized in relation to organic matter fermented was calculated as $AA \times 114$ where 114 was equivalent to calculated average molecular weight of a casein amino acid unit.

Hydrogen recovery (%) was calculated as follows:

$$2H_u/2H_p \times 100$$

where

$$2H_u = 2P + 2B + 4H + V$$

and

$$2H_p = 2A + P + 4B + 2IV + 2V$$

It was further shown that incubation of rumen fluid with casein resulted in net disappearance of protein-N and net production of ammonia, methane and volatile fatty acids. The concentration of free α-NH_2-nitrogen was nearly doubled indicating that rate of proteolysis exceeded the rate of amino acid fermentation. The only amine detected in traces in the incubation mixture was 2-phenyl methylamine showing that decarboxylation of amino acid was of minor importance. From above calculations, it was found that ammonia production accounts for 96% of protein-nitrogen disappearance, whereas ammonia production, calculated from volatile fatty acids and methane produced accounts for 90% of the amount formed. The data suggested that microbial growth efficiency in protein fermentation is about half the value obtained for carbohydrate fermentation.

2.2.2 Amino acid transport into the cell

Russell (1983) showed that <u>Bacteroides</u> <u>ruminicola</u> B_1^4 was unable to grow on peptides alone because it was unable to transport or ferment (or both) peptides at a fast enough rate to meet its energy requirements. It was further observed that

during the periods of rapid growth, very little peptide was deaminated and no ammonia formation was observed but as the growth ceased, there was linear increase in ammonia production. The increase in bacterial dry matter upon the addition of trypticase to glucose limited culture suggested that trypticase metabolism was improving the energetics of bacterial growth. Since the synthesis of cell material is not always proportional to the availability of energy source, protein metabolism is generally considered wasteful.

Russell et al.(1988) observed that ruminal bacterium Streptococcus bovis possessed a sodium dependent mechanism for the transport of neutral amino acids into the cells and membrane residues. Another gram positive and monensin sensitive strain was shown to grow rapidly on glutamate or glutamine as sole energy source but only in the presence of sodium (Chen and Russell, 1990). These organisms showed complete inhibition of growth in presence of ionophore, monensin. There was little or no production of ammonia since monensin exchanges Na^+ for H^+. Another antibiotic valinomycin and ATPase inhibitor dicyclohexyl-carbodiimide had very little effect on growth and ammonia production. Strain F was shown to have separate carriers for glutamate and glutamine which could be driven by a chemical gradient of sodium. The glutamate carrier had one site whereas the glutamine carrier had more than one site for transport of amino acid. Neither carrier could use lithium in place of sodium. Amino acids histidine and serine were rapidly

transported by sodium dependent systems, while serine alone did not allow growth of the organism even when sodium was present. All these observations clearly indicated that most of the ruminal bacterial strains were solely dependent on sodium dependent systems to utilize and transport amino acids and peptides.

Chen and Russell (1989 *a,b*) isolated two monensin sensitive strains of ruminal bacteria that grow poorly on carbohydrates but rapidly on amino acids. The short rods (SR strain) fermented arginine, serine, lysine, glutamine and threonine very rapidly at the rate of more than 159 nmoles/mg protein/hr and grew fast on casein digest containing short peptides than on free amino acids (0.34 vs 0.29 hr^{-1}). Gelatin hydrolysate, an amino acid source containing long peptides, was unable to support the growth or ammonia production. However, there was large increase in ammonia production when SR strain was cocultured with peptidase producing bacteria such as Bacteroides ruminicola and Streptococcus bovis. The other strain called strain F, deaminated glutamine, histidine, glutamate and serine rapidly at more than 137 n moles/mg protein/hr and grew faster on free amino acids than on short peptides (0.43 vs 0.21 hr^{-1}). It was concluded from above observations that both strains (SR and F) were unable to transport or hydrolyse peptides extra-cellularly.

Chen and Russell (1989 *b*) further described sodium dependent transport of branched chain amino acids by another

monensin sensitive ruminal <u>Peptostreptococcus</u> bacterium. This organism was shown to grow rapidly on leucine as an energy source and produced large amount of branched chain volatile fatty acids, strictly according to Stickland reaction, but only in presence of sodium. Leucine could be driven by an artificial membrane potential only when sodium was available. Since sodium was taken up along with leucine, it appeared that leucine was transported in symport with sodium. The leucine carrier could also use lithium and had a single site for sodium. Valine and isoleucine competitively inhibited leucine transport which showed that the organism used a common carrier for branched chain amino acids. Pittman <u>et</u> <u>al</u>.(1967) observed that some of the rumen microorganisms could transport peptides but were unable to take up any free amino acid present in the rumen fluid.

The presence of strains of ruminal bacteria that require sodium for transport and utilization of free amino acids present in rumen fluid has also been reported by Russell <u>et</u> <u>al</u>.(1988), Chen and Russell (1989a,b,1990). It was also observed that rumen is a sodium rich environment with concentrations of sodium upto 90 mM and most of the ruminal strains were solely dependent on sodium dependent transport systems for transport and utilization of amino acids and peptides.

2.2.3 Deaminase activity in rumen bacteria and protozoa

Deaminase activity occurs less frequently in rumen

bacteria than does the proteolytic activity. Predominant deaminating bacterial species in the rumen include Selenomonas ruminantium, Bacteroides ruminicola, Megasphaera elsdenii (formerly known as Peptostreptococcus) and some strains of Butyrivibrio fibriosolvns (Chalupa, 1974; Russell et al., 1988). Bladen et al.(1961) concluded that Bacteroides ruminicola is usually the most important ammonia producing bacterium in the rumen. Scheifinger et al.(1976) studied the degradation of amino acids by pure cultures of ruminal bacteria and identified the genera of Megasphaera, Eubacterium and Streptococcus bacteria capable of degrading all the amino acids tested. Members of Butyriovibrio totally degraded serine, aspartic acid and glutamic acid, with glycine as only amino acid not attacked. Subspecies of Selenomonas differed in their amino acid degrading patterns. Subspecies Selenomonas lactilytica degraded all the amino acids except histidine and tyrosine while subspecies Selenomonas ruminantium could not degrade glycine, leucine, isoleucine, threonine, histidine, arginine, lysine, tryptophan or tyrosine. Selenomonas, Butyriovibrio and Streptococcus bacteria, however, degraded methionine. It was, therefore, concluded that total ruminal amino acid degradation occurs as a result of intensive interactions of number of bacterial species.

Regarding the role of protozoa in the degradation of proteins and amino acids, not much is known. Amino acids either obtained from the surroundings or from catabolism of engulfed

bacteria or feed protein are used for protein synthesis or degraded in the body of the protozoa. Williams et al.(1961) and Gutierrez and Davies (1962) used rumen protozoa Ophryscolex caudatus and Epidinium ecaudatum, respectively to show that ^{14}C was incorporated from ^{14}C-DL-alanine, ^{14}C-DL-leucine and ^{14}C-DL-valine into the organism. Abou Akkada and Howard (1962) found a glutaminase like activity in the ruminal protozoan Entodinium caudatum. It was, however, shown that there was negligible amount of deamination or uptake of amino acids into the cellular material from casein or casein hydrolysate. Warner (1964) also observed this activity in other ruminal protozoa.

Bhatia et al.(1979, 1982) reported the presence of a wide range of transaminase activities in ruminal protozoal fraction and showed that this fraction could incorporate exogenous urea added to the culture medium. Onodera et al.(1983) examined the formation of urea and.or ammonia from arginine and some other amino acids and the metabolism of arginine, proline, citrulline and ornithine (urea cycle intermediates) by starved rumen ciliate protozoa and showed that not urea, but only ammonia was produced from arginine, citrulline, ornithine and also from asparagine and glutamine. Urea was not hydrolysed. Other end products, in addition to ammonia, included amino acids such as alanine, proline, glutamate, lysine, valine, small amounts of peptides and breakdown products like 2-amino butaneoic acid (Onodera and Kandatsu, 1968, 1970; 1972; 1973; Onodera et al., 1974; Onodera

and Ushijima, 1982).

Foresberg _et al_.(1984) characterised the proteolytic activities of mixed rumen protozoa. The organisms were known to possess low proteolytic activity on azocasein and low endogenous proteolytic activity at 10 to 15°C. Protozoa washed in 0.1M phosphate buffer (pH 6.8) and stored on ice, autolysed when they were warmed to 39°C. At pH optimum 5.8, they were shown to exhibit low proteolytic activity on azocasein, but very high endogenous proteolytic activity. The endogenous proteolytic activity was inhibited by cysteine proteinase inhibitors, for example, iodoacetate (63.1%) and aspartic acid proteinase inhibitor pepstatin (43.9%). The inhibitors specific for serine and metallo-proteinases were without effect. The serine and cysteine proteinase inhibitors of microbial origins such as antipain, chymostatin and leupeptin, caused up to 67% inhibition of endogenous proteolysis. Hydrolysis of casein was also inhibited by cysteine proteinase inhibitors. Some of the inhibitors decreased endogenous deamination, in particular, phosphoramidon, which had little effect on proteolysis. Deamination of amino acids produced during proteolysis, was inhibited by all chemical agents blocking proteolysis including TPCK (Tosyl-phenylalanine chloromethyl-ketone) TLCK (Tosyl-leucine-chloromethyl ketone) diacetyl-DL-norleucine methyl ester plus copper and merthiolate. Phosphoramidon at 280 and 500 μg ml^{-1} concentration inhibited the deamination by 26.5 and 82.8 per cent, respectively. It was further shown that

protozoal autolysates had 10 to 78 times higher hydrolytic activity on synthetic amino peptidase substrates such as L-leucine-p-nitroanilide and aminopeptidase activity was partially inhibited by bestatin. It was finally concluded that protozoal autolysates had high amino-peptidase activity and low deaminase activity on endogenous amino acids.

Hino and Russell (1985) and Wallace et.al. (1987) observed that deaminase activity was approximately 3 times higher in ruminal protozoa as compared to ruminal bacteria.

2.2.4 Products and rates of catabolism of amino acids in rumen

Available information regarding the intermediate products formed during the catabolism of amino acids is not very comprehensive. El-Shazly (1952) identified σ-amino valeric acid as an intermediate in catabolism of amino acid proline by rumen bacteria. Cappa (1955) reported the formation of indole and skatole from tryptophan catabolism. Dehority et al.(1958) studied the metabolism of valine, proline, leucine and isoleucine by rumen microorganisms and identified some branched chain amino acids as the end products. It was found that proline was transformed into σ-amino valeric acid in the absence of another amino acid. The fact that later amino acid was formed in the absence of supplemented amino acids does not exclude its formation through Stickland reaction.

Lewis and Emery (1962a,b) examined the intermediary products arising from the catabolism of individual amino acids by rumen microorganisms chromatographically and spectrophoto-

metrically. Cheese cloth strained rumen fluid and washed bacterial suspensions were employed for degradation of amino acids. L-arginine yielded ornithine, σ-amino valeric acid and putrescine. L-ornithine gave rise to σ-amino valeric acid and putrescine and L-lysine yielded σ-amino valeric acid and cadaverine. Tests for amine production from casein hydrolysate and five individual amino acids - arginine, lysine, histidine, tryptophan and phenylalanine at pH 4.5, 5.5 and 6.5 were negative except for cadaverine and putrescine. Ammonia production was highest at higher pH from all the five amino acids studied. Lysine decarboxylase activity occurred at more acidic pH than ornithine decarboxylase activity. DL-tryptophan yielded indole and skatole in rumen fluid but skatole appeared only in traces with washed cell suspension. Glutamic acid was not dissimilated by rumen microorganisms in vitro.

Lewis and Emergy (1962c) studied the relative rates of deamination of amino acids by mixed rumen microorganisms in vitro using strained rumen fluid and washed cell suspension. It was observed that serine, cysteine, aspartic acid, threonine and arginine were attacked most completely. This was followed by glutamic acid, phenylalanine, lysine and cystine forming an intermediate group and a third group where deamination was much less pronounced was tryptophan, σ-aminoavaleric acid, methionine, alanine, valine, isoleucine, ornithine, histidine, glycine, proline and hydroxyproline. It was further observed that dissimilation rates were more rapid in whole rumen fluid

than in washed cell suspension. A mixture of amino acids was not dissimilated at a faster rate than catabolic rate of individual amino acid. D and L form of serine and tryptophan were catabolised at same relative rate, while D-enantiomers of aspartate, lysine, threonine and phenyl alanine were not metabolized at all.

Yokoyama and Carlson (1974) studied the dissimilation of tryptophan and other aromatic compounds by ruminal microorganisms in vitro. Incubation of L-(U-benzene ring-^{14}C) tryptophan with ruminal microorganisms resulted in 39% of the added radioactivity being incorporated into skatole, 7% into indole and 4% into indole acetic acid. The D-enantiomer was not degraded. The major pathway of skatole formation from L-tryptophan appeared to be the decarboxylation of indole acetic acid, which arose as a result of deamination of L-tryptophan

Onodera and Kandatsu (1975) showed that butyric acid and acetic acid were principal metabolites of lysine degradation by rumen microorganisms. It was further shown that mixed rumen bacteria, unlike ciliate protozoa, did not produce any pipecolate from lysine. The higher concentration of acetate in the incubation mixture than expected, indicated that butyrate was being further degraded to acetate. It was also reported that rumen bacteria appeared to decompose much greater amounts of lysine than did the rumen ciliate protozoa.

Chalupa (1976) determined the degradation of amino acids both in vivo and in vitro by mixed rumen bacteria. The

amino acids were used in incubation mixture at physiological quantities. In vitro rate constants for essential amino acids indicated that arginine and threonine (0.5 to 0.9 mM/hr) were most rapidly degraded; lysine, phenylalanine, leucine and isoleucine (0.2 to 0.3 mM/hr) formed an intermediate group; whereas valine and methionine (0.1 to 0.14 mM/hr) were least rapidly degraded. In vivo rates of degradation of amino acids were around 1.5 times greater than in vitro rates of the same. It was suggested that same degradative pathways operated for in vivo and in vitro systems. Incubation of threonine, arginine, lysine, phenylalanine, leucine and isoleucine along or in combination with essential amino acids showed similar amounts of degradation, whereas methionine and valine degradation was approximately twice as great when fermented alone as compared to in conjunction with other essential amino acids. Combining a mixture of non-essential amino acids (aspartate, serine, glutamate, alanine, tyrosine and ornithine) with essential amino acids did not influence the degradation of essential amino acids. Tyrosine and ornithine were the only amino acids degraded to lesser extent (30%) when fermentation system contained both essential and non-essential amino acids. it was demonstrated that amino acids were degraded at different rates and there were interactions between certain amino acids. It was concluded that with the exception of methionine, supplements of the amino acids cannot survive ruminal degradation.

Scheifinger et al.(1976) studied the degradation of

amino acids by pure cultures of rumen bacteria. Five major genus were chosen to study the degradation of amino acids at normal physiological levels in vitro. The results indicated that (i) all amino acids were not degraded by the strains studied and (ii) degradation of amino acids occurred at different rates. It was observed that genus Megasphaera and Eubacterium degraded all the amino acids tested. Members of genus Butyriovibrio totally degraded serine, aspartate and glutamate and only glycine was the amino acid not attacked by these microorganisms. Two subspecies of Selenomonas differed in their amino acid degradation patterns. Subspecies, S. lactilytica degraded all the amino acids tested except histidine and tyrosine. The subspecies of S. ruminantium could not degrade glycine, leucine, isoleucine, throenine, histidine, arginine, lysine, tryptophane or tyrosine. Methionine appeared unique in that it was produced by members of Megasphaera, Eubacterium and isolate 19D of Streptococcus while degraded by Selenomonas, Butriovibrio and Streptococcus isolate 12D. It was proposed that total ruminal degradation of dietary amino acids occurs as a result of extensive bacterial interactions.

Cottle and Velle (1989) studied the degradation and outflow of essential amino acids, which are main limiting nutrients to wool growth in sheep. It was observed that relative rate of apparent degradation in first 4 hr was highest for lysine and lowest for methionine. Apparent degradation in 24 hr was again highest for lysine and lowest for threonine.

Conversely, fraction flowing out of rumen in intact form was lowest for lysine and highest for threonine in a 24 hr period. It was noted that threonine degradation rate appeared to be very high during first 1 to 2 h but was lowest over 24 hr period. The reason for this is not known. It was also observed that degradation of methionine appeared to be slightly more rapid and more complete when given alone rather than when given alongwith lysine and threonine.

The degradation of branched chain amino acids by starved rumen protozoa was studied by Onodera and Gotto (1990). They reported that branched chain amino acids were degraded by protozoa in a way similar to that of mixed rumen bacteria and the end products were also the same. It was observed that protozoal suspensions produced carbon dioxide and five carbon volatile fatty acids(VFA) from 1-leucine,1-isoleucine and 1-valine. The five carbon VFA was identified as isobutyrate coming from degradation of valine and isovalerate and 2-methyl butyrate produced from leucine and isoleucine. It was also reported that degradation rates with protozoal suspension were intermediate for leucine and isoleucine while that of valine was slow as compared to rates of arginine, threonine, citrulline and methionine (Onodera and Ushijima,1982; Onodera et al.,1983 and Onodera and Migita,1985).

2.3 Protection of dietary proteins and amino acids against ruminal degradation.

2.3.1 Processing of dietary proteins and amino acids.

For efficient utilization of dietary proteins by ruminants, it is desirable that extent of degradation of dietary proteins and intact amino acids in the rumen be reduced so that dietary amino acids be either directly incorporated into rumen microbial proteins or be digested and absorbed post ruminally. Various practical methods have been employed over years for decreasing the degradation of proteins and inhibiting the deamination of amino acids in the rumen.

The dietary proteins can be protected from ruminal degradation by heat treatment under ideal conditions (Potter et al., 1971; Hale, 1973; Goering and Waldo, 1974; Arielli et al., 1989; Cros et al., 1991; Gos et al., 1992a,b; Moshtaghi Nia and Ingalls, 1992; Benchaar et al., 1994) and formaldehyde treatment (Reis and Tunks, 1969; Ferguson et al., 1976; Amos et al., 1979; Phillips, 1981; Mahadevan et al., 1983). Recently the metal ions have been also used to reduce the degradation of proteins by rumen microorganisms.

Britton and Klopfenstein (1986) demonstrated that treating soyabean meal with zinc salts at 1 to 2% of feed dry matter reduced the in vitro degradation of the feed protein and improved efficiency of nitrogen utilization by calves. Other workers have observed similar improvements in calf growth (Karr et al., 1991c) or milk production (Zimmermann et al., 1992)

when diets contained zinc treated soyabean meal compared to solvent extracted soyabean meal. The mechanism by which heavy metal salts can precipitate soluble proteins is well known (Haurowitz, 1950) and recent studies substantiate increased flow of feed amino acids in lambs (Karr et al., 1991b) and steers (Froetschel et al., 1990) fed diets containing zinc treated soyabean meal. Karr et al.(1991a) suggested that zinc salts may inactivate proteolytic enzymes of selected rumen bacteria, thus reducing ruminal proteolysis of dietary proteins. Cecava et al.(1993) studied the effect of zinc treated soyabean meal on ruminal fermentation and intestinal amino acid flows in steers fed corn silage based diets. A positive quadratic response (p < 0.06) was observed for total and essential amino acids flows to small intestine because flows of total and essential amino acids from ruminally undegraded dietary proteins tended to increase when solvent extracted and zinc treated soyabean meals were fed in combination of 50:50 (crude protein ratio). Absorption of amino acids from small intestine also showed a positive quadratic response for 50:50 combination meal.

Encapsulation of amino acids (Sibbald et al., 1968; Broderick et al., 1970; Neudoerffer et al., 1971; Chandler et al., 1972; Schelling et al., 1973; Titgemeyer et al., 1983; Chalupa and Chandler, 1992) and preparation of various amino acid analogues (Reis, 1970; Salisbury et al., 1971; Wright, 1971; Belasco, 1972; Digenalis et al., 1974; Amos et al., 1974

and Chalupa, 1975) were used to protect the essential amino acids from ruminal degradation. However, these amino acids were available postruminally.

2.3.2 Use of various chemical agents

The outflow of materials from rumen which consists of microbial cells, fermentation end products and undegraded feed particles may not always provide the animal with optimum nutrients. Thus there are opportunities to improve animal performance by inducing metabolic changes in the rumen (Chalupa, 1975). Various chemical agents like Ionophores, halogenated compounds, antibiotics, herbicides and insecticides, etc. have been used to modulate the selected pathways of metabolism (volatile fatty acid production, methanogenesis, proteolysis, amino acid deamination and ureolysis) in the rumen for better animal production.

2.3.2.1 Ionophores

Ionophores are highly lipophilic substances which are toxic to many bacteria, protozoa, fungi and higher organisms, and qualify for the classic definition of antibiotics (Pressman, 1965). The exterior of the molecule is hydrophobic and interior is hydrophillic and is able to bind cations. Some ionophores bind only one cation and hence are called uniporters, while others that bind more than one cation are called antiporters. Ionophores are able to shield and delocalize the charge of ions which facilitates their movements across the cell membranes (Russell and Strobel,

1989). According to Pressman (1976) an ionophore must be in anionic form before it is capable of binding a metal ion. Painter et al.(1982) observed that diffusion across the cell membrane can only occur when ionophore exists in protonated or zwitterionic form.

Most exhaustively studied ionophore so far is monensin (formerly named rumensin) which was initially developed as a coccidostat for poultry but later found to manipulate rumen fermentation like decreased ammonia production, increasing the ratio of propionate to acetate and decreasing protein degradation (Van Nevel and Demeyer, 1971; Dinius et al., 1976; Kerr et al., 1976; Richardson et al., 1976; Prange et al., 1978; Slyter, 1979 and Thronton and Owens, 1981). The others such as valinomycin, nigericin, carbonyl cyanide, m-chlorophenyl hydrazone (COCP), lasalocid, tetronasin and lysocellin etc. are not very popular (Dinius, 1976; Richardson, 1976; Thronton et al., 1976; Van Nevel and Demeyer, 1977; Lemenger et al., 1978; Chen and Wolin, 1979; Dawson, 1979; Poos et al., 1979; Van Nevel and Demeyer, 1979: Isichei and Bergen, 1980; Sauer et al., 1980; Dennis et al., 1981 a,b; Henderson et al., 1981; Nagraja et al., 1981, 1982; Rogers, 1982; Sandeaux et al., 1982; Bergen and Bates, 1984; Russell and Strobel, 1989; Chen and Russell, 1990; Sticker et al., 1991; Yang and Russell, 1991).

Richardson et al.(1976) estimated theoretically, an energy saving of 5.6% to the host animal, assuming there was 10

moles/100 moles of total volatile fatty acids increase in propionate production when monensin was fed.

Van Nevel and Demeyer (1977) indicated that monensin at 5, 25 and 100 μg/ml of strained rumen fluid decreased in vitro methane production from formate by 18, 15 and 26%, respectivley. Similar amounts of monensin showed greater decrease in methane production when washed cell suspension was incubated with formate as a substrate. The decrease was of the order of 7, 23 and 62% respectively. This decrease in methane production was accompanied by an increase in molar proportion of propionate (Thronton et.al, 1976; Chalupa, 1980) and this may lead to lower heat increment as propionate is more efficiently utilized than acetate (Blaxter and Waiman, 1964; Smith, 1979). Van Nevel and Demeyer (1977) further observed that 5 μg monensin per ml incubation mixture decreased the ammonia-nitrogen formation from 8.8 mg to 3.8 mg. At same concentration of monensin, protein degradation decreased from 15.9 to 10.8 mg of protein nitrogen. However, at 25 μg monensin level, decrease in ammonia and protein nitrogen was approximately the same as that at lower concentration.

Slyter (1979) showed that monensin at zero or 33 μg/g of diet decreased methane production from 6.41 m mole/day to 3.63 m mole/day, hydrogen production decreased from 0.92 to 0.36 m mole/day, carbon dioxide from 64.3 to 54.3 m mole/day, total volatile fatty acids from 67.3 to 63.5 m mole/day and acetate to propionate ratio from 2.6 to 1.8.

Barlety et al.(1979) reported that monensin at 22, 44, 88 and 176 ppm levels decreased the acetate to propionate ratio from 2.78 (at zero monensin level) to 2.27, 2.11, 2.02 and 1.8, respectively, in the rumen. Acetic acid production was decreased from 52.6 to 45.7 moles % while propionate production increased from 18.8% to 25.6% males at 176 ppm monensin concentration. Monensin also reduced feed intake and improved feed efficiency without affecting the weight gain.

Chalupa et al.(1980) observed that monensin at 1.0 ppm level increased the propionate production from 0.58 to 0.71 m moles, decreased acetate production from 1.42 to 1.24 m moles, respectively. Fermentation efficiency increased from 76.4% at zero level of monensin to 79.2% at 1.0 ppm level, when the diet contained 50% concentrates. It was also effective in decreasing the utilization of exogenous amino acids.

Dennis et al.(1981) studied the effect of lasalocid or monensin on lactate using or producing rumen bacteria and observed that both chemical agents inhibited most of the lactate producing bacteria such as Butyrivibrio fibrisolvens, Eubacterium cellulosolvens, Lactobacillus ruminins, Ruminococcus albus. Streptococcus bovis. Minimum inhibitory concentration ranged from 0.3 to 3.0 μg/ml. Among the lactate producers, those that produce succinate as major end product (Bacteroides, Selenomonas, Succinivibrio) were not inhibited by either monensin or lasalocid. Major lactate fermentors

(Anaerovibrio, Megasphaera, Selenomonas) were also not inhibited. Veillonella alcalescens was inhibited by 24 μg/ml of lasalocid and not by monensin. It was concluded that the reported increase in propionate production in lasalocid or monensin fed cattle (Van Nevel and Demeyer, 1977: Slyter, 1979; Bartely et al., 1979; Chalupa et al., 1980) may result from the selection for succinate and lactate producers as reported by Chen and Wolin (1979). It was further observed that inhibition of major lactate producing bacteria (Streptococcus and Lactobacillus) by monensin or lasalocid may be useful in treatment of lactic acidosis in ruminants.

Thronton and Owens (1981) studied the effect of monensin on in vivo methane production. Daily intake of monensin totaled 200 mg for every animal. Methane production in animals given low and high roughage diets decreased by 16 and 24% respectively. Total heat production at low roughage diet decreased from 6.02 Kcal/hr/g to 5.94 Kcal/hr/kg (high roughage diet). CO_2 production also decreased in monensin fed animals. Similarly energy loss due to methane production decreased from 8.0 to 7.3% of gross energy intake and total metabolizable energy increased from 67.0 to 69.9% for monensin fed animals. Dry matter and nitrogen digestibility increased from 79 to 81.4% and 69.5 to 72.3% respectively, for low roughage diet animals when given monensin. Nitrogen retention increased from 3.9 to 7.8 g/day for low roughage diet animals given monensin. These effects were almost negligible in high roughage receiving

animals.

Russell et al.(1981) examined the rates of bacterial growth, protein degradation, ammonium formation and lactic acid production by mixed rumen bacteria grown on mixed carbohydrates, casein and ammonia. Microscopic examination of fast growth incubation revealed small ovoid cells similar to Streptococcus bovis and it was suggested that proliferation of this organism might be responsible for rapid proteolysis. Inclusion of monensin and thiopeptin at 5 ppm level decreased protein degradation over control by 13% and 50% respectively. The ratio of protein degraded to bacterial protein synthesized was found to be 0.659, 0.362 and 0.628 for control, thiopeptin and monensin, respectively. Above data suggested that monensin and thiopeptin were able to decrease protein degradation by Streptococcus bovis quite effectively.

Muntifering et al.(1981) studied the effect of monensin on site and extent of digestion of whole corn and bacterial protein synthesis in beef steers. Monensin was fed at zero and 33 ppm concentration along with corn based diet. Monensin decreased ruminal true digestion of organic matter. Apparent ruminal digestion of starch was decreased by 19%. It also decreased the contribution of bacterial nitrogen to abomasal nitrogen (52 vs 58%) and increased the ruminally undegraded feed nitrogen (46 vs 40%). It also decreased the fraction of bacterial nitrogen to total nitrogen digested post ruminally (42 vs 50%) and increased the contribution of

ruminally undegraded feed nitrogen digested post ruminally (58 vs 50%). Monensin supplement caused a greater proportion of feed nitrogen and starch to be digested in intestines than in rumen (with possibly greater resultant metabolic efficiency), and this may account for some of the benefits of feeding this ionophore with high grain diets.

Rowe et al.(1981) concluded that approximately 20% more metabolizable energy was available to host when feed was supplemented with monensin. Wadegaertner and Johnson (1983) studied the digestibility, methanogenesis and heat increment of a cracked corn silage diet with or without monensin fed to steers. Monensin was given at level of 3 mg/kg body weight of animals. Monensin improved the apparent digestibility of energy from 71.8 to 74.8% and of crude protein from 61.6 to 65.8%. Methane production was reduced by 26% and metabolizable energy increased from 63.3 to 66.8% of gross energy intake. It was concluded that at equalised gross energy intakes, about one third of the improved energy utilization could be explained by reduced methanogenesis caused by monensin and two third by reduced faecal losses.

Van Nevel and Demeyer (1977) showed that monensin (5 μg/ml of incubation mixture) decreased the casein degradation in vitro from 15.9 to 10.8 mg nitrogen and ammonia production from 8.86 to 3.83 mg ammonia nitrogen. Schelling et al.(1977) indicated that monensin decreased the rate of amino acids degradation in rumen fluid. Dinius et al.(1976) observed that

decreases in rumen ammonia on monensin supplement were consistent with depression of deamination or proteolysis or both.

Short et al. (1978) and Poos et al. (1979) observed that inclusion of monensin resulted in decreased bacterial nitrogen and increased dietary proteins reaching abomasum of steers. Owens et al. (1978) indicated that monensin increased total amino nitrogen reaching abomasum. Beede et al. (1980a) and Hanson and Klopfenstein (1979) demonstrated greater nitrogen retention and animal performance in steers receiving protein deficient diet supplemented with monensin. The improved nitrogen flow, due to monensin, has been attributed to decreased peptide breakdown (Newbold et al., 1990; Wallace et al., 1990; Chen and Russell, 1991) and amino acid deamination (van Nevel and Demeyer, 1977; Horton, 1979; Chalupa, 1980; Russell and Martin, 1984; Hino and Russell, 1985; Russell and Strobel, 1988,1989; Yang and Russell, 1993) in the rumen.

Peptide breakdown was shown to be inhibited in vitro by monensin, tetronasin and protonophores like tetrachloro-salicylanilide (TCS), valinomycin and sodium arsenate (Newbold et al., 1990; Wallace et al., 1990). It was observed that monensin and tetronasin at 2.5 $\mu g/ml$ of incubation mixture decreased the breakdown rate of a series of alanine peptides from Ala_2 to Ala_5. Ala_2 breakdown rate decreased from 0.71 m mole/mg protein per min to 0.56 and 0.58 for monensin and tetronasin, respectively. Ala_5 breakdown rate, at ionophore

level of 5 μg/ml, was decreased from 2.15 m mole/mg protein/ min. to 1.64 m mole/mg protein/min for both monensin and tetronasin. Monensin and tetronasin showed 12 to 31% inhibitory effects. Toluene was shown to increase peptide breakdown by rumen microorganisms consistent with a permeability barrier being removed. Thus, at least some peptide metabolism may depend on their transport into bacteria. It was further shown that rumen fluid from sheep receiving monensin (33 mg/kg body weight) or tetronasin (10 mg/kg body weight) hydrolysed Ala_3 and Ala_4 at approximately same rates as controls, but peak concentrations of the peptides in rumen fluid after feeding was more than double in ionophore fed sheep and peptides persisted for longer periods than in control animals.

Newbold et al.(1990) demonstrated that ionophore tetronasin at 6 and 10 ppm level in barley diet lowered the proteolytic activity in rumen fluid by 76% and deaminative activity by 58% as compared to the control after 42 days of feeding. When tetronesin was added in vitro to rumen fluid of sheep not receiving ionophore, proteolytic activity remained unaffected, but the rate of ammonia production from amino acids was decreased by 87%. Oligopeptide breakdown was inhibited by 21%.

Sticker et al.(1991) observed that inclusion of lysocellin in the diet lowered proteolytic activity, improved animal performance and produced alterations in volatile fatty acids that were similar to effect to monensin. Hilliaire et al.

(1989) and Newbold et al.(1990) reported that both lysocellin and tetronasin reduced proteolysis and deamination of proteins by rumen microorganisms.

Yang and Russell (1993) demonstrated that number of ruminal bacteria utilizing peptides, carbohydrates and amino acids were decreased nearly ten fold on the addition of monensin (350 mg/day) to the timothy hay and soyabean meal diet of cows in vivo. This, in turn, decreased ruminal ammonia concentration by about 30% of the control value. It was suggested that monensin inhibited highly active amino acid fermenting bacteria, which in turn decreased ammonia production. Monensin did not increase soluble proteins, peptides or amino acids in ruminal fluid which indicated that decrease in ammonia concentration could not increase the flow of dietary amino nitrogen to lower gut. However, monensin increased the concentration of bacterial protein in rumen fluid, which could provide additional amino nitrogen for the host animal.

It can be inferred that the ionophores reduce the degradation of proteins and deamination of amino acids probably due to reduction in the number of proteolytic and deaminating bacteria directly affecting the proteolytic and deaminative activity. This results in increase in the quantity of dietary proteins escaping ruminal degradation and making it more available for post ruminal digestion and absorption. The ionophores also have depressing effects on methanogenesis.

2.3.2.2 Halogenated compounds

Van Nevel et al. (1969) showed that methane production from pyruvate by mixed rumen bacteria in vitro was nearly totally inhibited by chloral hydrate at 0.1 μ mole/ml of incubation fluid. The effect was accompanied by accumulation of gaseous hydrogen and an increase in propionic acid production from 23.6% of total volatile fatty acids (control) to 38.9%. There was a decrease in acetic acid from 63.2% (control) to 44.5% of total volatile fatty acids. However, there was a slight decrease in total amount of volatile fatty acids produced (from 9.5 meq/100ml for control to 9.0 meq/100 ml). Further it was shown that chloral hydrate at 100 mg/100 ml of rumen fluid decreased proteolysis of casein in vitro by approximately 25% whereas it increased protein synthesis by about 40 to 50%. In comparison linseed oil hydrolysate had completely opposite effects.

Amicholral (HC , a hemiacetal of chloral and starch, has been studied in detail for its effects on ruminal fermentation (Singh et al.,1971; Trei and Scot, 1971: Trei et al., 1972; Singh and Trei, 1972; Trei et al., 1973; Marty and Demeyer, 1973; Cole and McCroskey, 1975; Czerkawski and Breckenridge, 1975b; Chalupa et al., 1975 ; Chalupa, 1977; Clapperton, 1977; Horton, 1980; Chalupa et al., 1980). These studies indicated that HCS inhibited methanogenesis with concomitant increase in gaseous hydrogen. Substantial amounts

of this hydrogen, instead of being disposed of as methane normally, were utilized for propionate production. The underlying mechanism suggested was irreversible reaction of halogenated analogues with reduced vitamin B_{12} to inhibit cobamide dependent methanogenesis (Wolfe, 1982; Czerkawski and Brekenridge, 1975a; Demeyer and van Nevel, 1985). Several studies have indicated increased nitrogen retention when amichloral was supplemented in diets of the ruminants (Johnson, 1972; 1974; Trei et al., 1973; Leibholz, 1975; Horton, 1980). Ruminal ammonia concentrations were lowered by about 60% in steers fed diets containing 1500 μg amichloral/kg diet indicating that there was a decrease in protein deamination in the rumen (Horton, 1980).

Russell and Martin (1984) studied the effect of methane production inhibitors on fermentation of amino acids by mixed rumen microorganisms in vitro. Chloroform was used at concentration of 4 x 10^{-5}M in incubation mixture. It was observed that concentration of propionate was increased from 10.6 mM for control to 13.9 mM, acetate was decreased from 40.5 mM to 32.3 mM and there was overall decrease in total volatile fatty acids production. Deamination of amino acids was considerably reduced. Chloroform completely inhibited methane production, ammonia production was reduced by about 10% but some accumulation of hydrogen occurred. Demeyer and Van Nevel (1985) indicated a positive response of feeding halogenated compounds such as decreased feed intake, significant increase

in daily weight gain, and higher feed conversion ratio on a high roughage diet.

2.3.2.3 Antibiotics

Various antibiotics, other than ionophores, such as Avoparcin, Siomycin, thiopeptin, capreomycin, bacitracin, novobiocin, virginiamycin, tetracyclines such as aureomycin, terramycin and sarsaponin and salinomycin have shown favourable effects on the performance of cattle and sheep (Beede and Farlin, 1977a,b; Delay et al., 1978; Johnson et al., 1979; Hedde et al., 1980; Russell et al., 1981; Froetschel et al., 1983; Goerich et al., 1984; MacGregor and Armstrong, 1984; Spears and Harvey, 1984 and Van Nevel et al., 1984; Ellenberger et al., 1985: Gibson et al., 1985: Goetsch and Owens, 1985; Merchen and Berger, 1985; Jounay and Thivend, 1986; Olumeyen et al., 1986; Van Nevel and Demeyer, 1987, 1990).

Chalupa et al. (1981) reported that avoparcin supplementation could be associated with a decrease in ammonia and increase in α-amino nitrogen concentrations in rumen. Froetschel et al. (1983) showed that diets with low fibre and 50 ppm avoparcin fed to sheep increased propionate production in the rumen. Avoparcin decreased ammonia concentration from 50 to 45 mg/10 ml of rumen fluid and increased α-amino nitrogen from 8.6 to 14.3 mM with low fibre diets. In vitro studies demonstrated a decreased degradation of crystalline amino acids by mixed rumen microorganisms from low fibre receiving sheep with 50 ppm avoparcin. It was concluded that avoparcin appears

to modify rumen fermentation by increasing propionate production and inhibiting proteins and amino acids degradation.

MacGregor and Armstrong (1984) observed that inclusion of avoparcin at level of 225 mg/litre of normal saline infused into rumen, duodenum and proximal ileum of sheep, resulted in 7.6, 10.8 and 9.7% enhancement in the net absorption of total, essential and non-essential amino acids, respectively. When avoparcin was infused into the rumen at the same rate the uptake of total, essential and non-essential amino acids from small intestine was improved by 9.1, 10.5 and 11.90% respectively.

Jounay and Thivend (1986) showed that avoparcin (1 mg per litre) caused a decrease in degradability of different protein sources in fermentor studies with rumen fluid. The decrease was largest for highly soluble proteins. Avoparcin was found to reduce the amount of nitrogen incorporated into bacteria. There was decreased production of volatile fatty acids by 20% mainly of acetate and butyrate with no change in propionate production. Gas production was reduced by 10 to 20% and a large decrease in methane production when ammonium sulfate was used as only nitrogen source in presence of avoparcin. These results could be explained if avoparcin uncouples fermentation as shown for monensin. It was concluded that although avoparcin had no effect on ion transport, it produced same changes in fermentation pattern and protein degradation as the ionophores.

Another antibiotic virginamycin, produced by _Streptomyces_ _virginiae_ has shown positive effects on the performance of growing steers (Parigi-Bini,1979). Van Nevel _et al_.(1984) demonstrated a decrease in methane production, increase in propionate production, decrease in the amounts of hexose fermented and increase in fermentation efficiency, when virginamycin at 0 to 200 ppm concentration was added to incubation mixture _in_ _vitro_. It was concluded that concentration of 1 ppm altered the fermentation pattern more effectively. Further increase in the concentration of the antibiotic showed a constant fermentation pattern. Feeding the antibiotic at 33 mg/kg body weight to steers had no effect on the concentrations of volatile fatty acids, ammonia and urea in the rumen. However, it lowered the concentration of lactic acid in the rumen fluid.

Another polyether antibiotic salinomycin, produced by _Streptomyces_ _albus_, was found to be highly effective against gram positive bacteria (Olumeyan _et_ _al_., 1986). Merchen and Berger (1985) reported enhanced feed efficiency and daily weight gains in steers and sheep given this antibiotic. Its potency was three times higher than that of monensin and lasalocid. It was shown to have effect on total volatile fatty acid concentration when given to steers at 0.22 mg/kg body weight per day, but acetate to propionate ratio was significantly lower in salinomycin fed animals. The molar

proportion of butyrate increased and the proportion of amylolytic bacteria was increased with the supplementation. Total number of rumen protozoa also decreased initially and this reduction was due to reduced number of Entodinium species.

Van Nevel an Demeyer (1987) investigated the effect of various antibiotics on casein or casein hydrolysate degradation in vitro and observed that addition of various antibiotics such as Terramycin, aureomycin, avoparcin, bacitracin, salinomycin, thiopeptin, virginamycin etc. and ionophores such as monensin and lasalocid decreased the degradation of casein and casein hydrolysate by rumen microorganisms indicating inhibition of both proteolytic and deaminase/transaminase activity. Volatile fatty acid production was inhibited more than ammonia production and casein disappearance. Stoichiometric calculations suggested the accumulation of peptide nitrogen in inhibited incubations as well as ammonia production from non-protein cell components (e.g. nucleic acids) made available for fermentation by cell lysis. Sarsaponin, a steroidal glycoside, was shown to decrease feed protein breakdown in rumen under certain conditions (Goetsch and Owens, 1985; Gibson et al., 1985; Ellenberger et al., 1985).

Van Nevel and Demeyer (1990) observed that potent feed additives (antibiotics) were not very selective in their influence on different nitrogen transaction in rumen in vitro. It was shown that all antibiotics, except siomycin, novobiocin, capreomycin, flavomycin and sarsaponin (at concentration of 5

and 20 ppm) lowered casein and casein hydrolysate degradation in vitro. Presence of carbohydrates in the incubation mixture did not affect protein breakdown. Free amino acids were degraded at a faster rate than casein suggesting that proteolysis was rate limiting step in protein breakdown. Similarly, fermentation pattern of the amino acids was found to be different from corresponding proteins.

2.4 Chemical inhibition of amino acid deamination by rumen microorganisms

Amino acid deamination is of primary significance in metabolism of nitrogen in ruminant, since this is the principal step by which dietary amino acids may be irreversibly lost to the animal. Inhibition of deamination, therefore, assumes practical significance as a means of increasing ruminal escape of dietary amino acids (Broderick and Baltharp, Jr., 1979).

Schelling et al.(1973) observed enhancement in ruminal escape of orally fed methionine and lysine by feeding the sheep 1 g of oxytetracycline per day. Tolbert et al.(1977) and Schelling et al.(1977) suggested that monensin may have a possible nitrogen sparing effect mediated, in part, through inhibition of ruminal amino acid deamination.

Smith et al.(1967) reported the inhibition of a microbial enzyme histidine deaminase (histidine ammonia-lyase, EC 4.3.1.3) by various carbonyl reagents such as sodium borohydride, phenyl hydrazine, sodium sulfite, hydroxylamine

and cyanide. Arsenite inhibits the deaminase activity by inhibiting reduction step of Stickland type deamination reactions (Stickland, 1934; 1935; Nisman, 1954; Britz and Wilkinson, 1981). Chalupa et al. (1975) developed a new family of deamination inhibitors, with diaryliodonium salts as parent compounds. These compounds were initially developed as inhibitors of gluconeogenesis, secondary to inhibition of NADH oxidation in rat liver mitochondria (Holland et al., 1973; Gately et al., 1975).

Broderick et al. (1979) observed highest recoveries of total amino acids in the presence of hydrazine (97%) and thymol (109%) in a batch type in vitro ruminal system. Amongst the other inhibitors (though none reduced the deamination more than hydrazine) such as phenyl hydrazine, p-nitrophenyl hydrazine, methyl hydrazine and sodium arsenite provided adequate protection against amino acids degradation. It was further observed that p-hydrazinobenzoic acid, potassium cyanide and amino oxyacetic acid inhibited amino acid deamination at intermediate level between hydrazine and controls. Diphenyliodonium compounds at 0.05 mM concentration showed equal recoveries of amino acids as 5.0 mM hydrazine. Individual amino acid showed a trend similar to that of total amino acids, though aspartate and glutamate were only 40 to 60% recovered at highest concentration of hydrazine used. Arginine was not at all recovered at all hydrazine and diphenyliodonium salts concentrations. Hydrazine and diphenyliodonium salts in

particular, inhibited _in vitro_ casein degradation by rumen microorganisms.

Horton (1979, 1980) observed in an _in vivo_ study that feeding 4,4'dimethyliodonium chloride to steers at level of 25 mg/kg of diet increased organic matter digestibility. Molar proportion of butyric acid was increased but showed no effect on the molar proportions of acetate and propionate in rumen fluid. Ammonia concentration was lowered from 16.3 mg/100ml rumen fluid to 7.2 mg/100ml rumen fluid. Feed intake was lowered by 19% and feed utilization efficiency was increased by 12% when iodonium salt was supplemented with amichloral.

Chalupa _et al_.(1983a) observed that during _in vitro_ fermentation of individual amino acids with rumen fluid in the presence of 0 to 10 ppm of ten different diaryliodonium analogues, protection against degradation was greatest for valine, methionine, isoleucine, leucine and phenylalanine. Chalupa _et al_.(1983a,b) observed that animals fed 4,4'-dimethylphenyl iodonium chloride for 112 days at 0, 25, 50 and 100 ppm level had higher concentration of amino acids and lower concentrations of ammonia in the rumen fluid. There was decreased production of acetate in conjunction with increased propionate production along with improved fermentation efficiency. It was further observed that these compounds improved nitrogen utilization by adjusting ruminal degradation of dietary proteins to yield more amino nitrogen and less ammonia nitrogen.

Van Nevel and Demeyer (1990) showed in an *in vitro* study with diphenyl dimethyliodonium chloride and other antibiotics that ammonia production was decreased more than casein degradation indicating that rumen microbial deaminase activity was more inhibited than proteolysis. It was also observed that there was complete inhibition of methane production with deaminase inhibitor and this clearly affected the molar proportion of volatile fatty acids with increased proportion of propionate and decreased proportion of acetate. The amount of total volatile fatty acids formed was also much lower in the presence of inhibitor owing to more lactate formation.

Studies with methanogenic inhibitors such as monensin, chlorinated hydrocarbons, sulfite and polyunsaturated oils have demonstrated a decrease in amino acid fermentation by rumen microorganisms (Van Nevel et al., 1970a; Chalupa, 1977; Van Nevel and Demeyer, 1977; Slyter, 1979; Wallace et al., 1987; Russell and Martin, 1984). Chalupa (1977) noted a decrease in amino acid degradation when amichloral was added to the *in vitro* incubation mixture.

Russell and Martin (1984) studied the effect of methanogenic inhibitors such as carbon monoxide, which is a selective inhibitor of bacterial hydrogenases (Gray and Gest, 1965; Thauer et al., 1974). Chloroform, monensin and lasalocid on amino acid fermentation by mixed rumen microorganisms *in*

vitro. It was noted that carbon monoxide (0.5 atm),chloroform (4 x10^{-5}M), monensin and lasalocid (1 ppm each) inhibited methane production from excess of trypticase (15 gm/litre). All the chemicals reduced amino acid deamination,but degree of methane inhibition was not related to ammonia accumulation. The ammonia production was decreased by 10, 50 and 20% in the presence of chloroform, ionophores and carbon monoxide, respectively. The mechanisms for decrease in amino acid fermentation were different for each chemical used. With chloroform, some hydrogen accumulated and ratio of straight chain to branched chain fatty acids was not different from controls. Carbon monoxide caused a large decrease in straight chain to branched chain fatty acids indicating selective inhibition of branched chain amino acid fermentation.The ionophores decreased the concentration of isovalerate and 2-methyl butyrate as well as the ratio of straight chain to branched chain fatty acid.

Similarly, Russell and Jeraci (1984) showed that when one atm (101.3 KPa) of carbon monoxide was added to mixed rumen bacteria, containing timothy hay, methane production was inhibited by 88%, molar ratio of propionate to acetate increased from 0.83 to 1.53 and extracellular ammonia concentration decreased from 5.2 to 2.4 mM. Cellulose and hemicellulose digestion was inhibited by 40 and 27%, respectively. Ammonia production from amino acid source trypticase declined by 20% where carbon monoxide pressure was

increased to 1.0 atm and 93% of this decrease was explained by selective inhibition of branched chain amino acid fermentation.

Hino and Russell (1985) studied the effect of carbon monoxide and monensin on amino acid fermentation by mixed rumen microorganisms and their cell extracts. Both inhibitors decreased methane production and caused significant increase in ratio of intracellular NADH to NAD^+. The study with cell extracts of mixed microorganisms (bacteria and protozoa) indicated that ratio of NADH to NAD^+ had marked effect on deamination of reduced amino acids, particularly branched chain amino acids. Deamination was inhibited by NADH and stimulated by methylene blue, an agent that oxidised NADH. Neutral and oxidised amino acids remained unaffected. Addition of small amounts of 2-oxoglutarate greatly enhanced deamination of branched chain amino acids and indicated that transamination via glutamate dehydrogenase was important. Formation of ammonia from glutamate was similarly inhibited by NADH. It was concluded that reducing equivalents disposal and intracellular NADH to NAD^+ ratio were important effecters of branched chain amino acids fermentation.

CHAPTER III

MATERIALS AND METHODS

The present study was divided into four parts and hence materials and methods used are described under the following heads:

3.1   Experimental animals, their feeding and sampling of rumen fluid.

3.2   *In vitro* degradation of intact feed proteins and individual amino acids by rumen fluid and its fractions in the presence and absence of various inhibitors.

3.3   Characterization of deaminase activity from mixed rumen bacteria

3.4   Distribution of deaminase activity in rumen fluid and its fractions.

3.5   Localisation of deaminase activity in rumen bacterial cell

3.5   Analytical methods

3.1 Experimental animals and diet

Two rumen fistulated buffalo bulls (<u>Bos</u> <u>bubalis</u>) of 150 kg average body weight were fed a daily ration consisting of wheat straw, berseem (<u>Trifolium</u> <u>alexandrium</u>), mustard cake and crushed maize grains (Table 1). The ration was divided into two parts and fed twice a day i.e. at 09.00 and 17.00 h. The

Table 1. Details of Ration[*][+]

Ration ingredients	Daily intake (kg)	Daily organic matter (kg)	Daily DCP intake (kg)	Daily TDN intake (kg)
Wheat straw	2.500	2.250	-	1.081
Berseem (<u>Trifolium alexandrium</u>)	1.250	0.250	0.035	0.161
Crushed maize grains	0.300	0.250	0.022	0.254
Mustard cake	0.300	0.250	0.083	0.222
Total	4.350	3.000	0.140	2.718

[*]Calculated according to feeding standards laid down by Sen and Ray(1971) for adult animals

[+]Ration contained 20 gms mineral mixture

animals consumed the whole ratio within 1 h of its being offered. Water was offered _ad lib_ at 10.00 and 18.00 h. The pre-experimental period lasted three months.

3.1.1 Sampling of rumen fluid

Approximately one litre of rumen fluid was obtained by suction through a perforated plastic tube from all parts of rumen of each buffalo bull before feeding in the morning. The rumen fluid from both the animals was pooled together on equal volume basis. The rumen fluid was separated from large feed particles by straining through two layers of cheese cloth and immediately put in a prewarmed thermos for brief storage.

3.1.2 Fractionation of strained rumen fluid into its fractions

The strained rumen fluid (SRF) was fractionated into bacterial, protozoal and cell free fractions. The fractionation was carried out at 37°C except where mentioned otherwise.

One litre of SRF was centrifuged at 2000 x g for 15 min and supernatant was carefully decanted and checked microscopically to ensure that protozoa had been completely removed. The protozoal pellet which also contained fine feed particles was washed twice with McDougall buffer[*]. The final pellet obtained was made to 1 l, the original volume of the SRF with McDougall buffer which also contained 0.5% each of

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[*]Composition and preparation of McDougall buffer:

0.5g K_2HPO_4, 1.0 g KH_2PO_4, 3.0 g NaCl, 0.1 g $MgSO_4.7H_2O$, 8 mg $CaCl_2$, 1.08 g $CH_3CHCOONa$ were dissolved in 1 l distilled water. Nitrogen gas was bubbled through the solution for 10 min to remove any dissolved oxygen and pH of the buffer was made to 6.8 by flushing it with carbon dioxde gas. 1. 0 g each of $NaHCO_3$ and anhydrous Na_2S were added just before use.

penicillin G and streptomycin sulfate. The antibiotics were added to check the bacterial contamination. This suspension was termed as **washed protozoal suspension.**

The combined supernatant obtained above was centrifuged at 26,000 x g for 10 min and the supernatant was removed and kept in a separate container for further use. The pellet obtained was washed twice with McDougall buffer and supernatant obtained was added to previously stored supernatant. The pellet was suspended in McDougall buffer and made to 1 l (the original volume of SRF). This suspension was termed as **washed mixed bacterial suspension.**

The combined supernatant collected above was also made to the original volume of SRF i.e. 1 l and termed as **cell-free supernatant.**

3.2 In vitro degradation of intact feed proteins and individual amino acids by rumen fluid and its fractions in the presence and absence of various inhibitors

3.2.1 Intact feed protein sources

Casein, soyabean seeds (Soya), sunflower seeds (SF), groundnut cake powder (GC) and mustard cake powder (MC) were used as protein sources in this study. Sunflower seeds were first dehulled and dried overnight at 40°C in an oven and these along with soyabean seeds, groundnut cake and mustard cake were finely ground to powder feed. Total nitrogen and crude protein contents of these feeds were estimated by microkjeldahl method.

3.2.2 Preparation of soluble and insoluble protein fractions from intact feed proteins

Soluble and insoluble protein fractions from intact feed proteins were prepared according to the method of Mahadevan et al.(1980).

50 g of each of finely ground intact feed protein sources were separately mixed with 200 ml of 0.1 M potassium phosphate buffer of pH 7.6 in 500 ml conical flask and the contents were stirred at room temperature by placing the flasks on a shaker with circular movements for 2 h. The soluble and insoluble fractions were separated by centrifugation at 30,000 x g for 20 min in a refrigerated centrifuge. The sediment was washed thrice with 0.1M potassium phosphate buffer of pH 7.6 and was finally resuspended in 100 ml of the same buffer and termed as **insoluble protein fraction**.

The combined supernatant obtained above was concentrated by precipitation with ammonium sulphate (80% saturation). The precipitated proteins were collected by centrifugation at 26,000 x g for 10 min and dissolved in 50 ml of 0.1M potassium phosphate buffer of pH 7.6 and was dialysed against the same buffer for 72 h with three changes of the buffer at 4°C to remove small molecular weight ninhydrin positive material.The final volume was made to 100 ml with 0.1M potassium phosphate buffer of pH 7.6 and termed as **soluble fraction**. The final pH of soluble and insoluble fractions was adjusted to 6.8. Total nitrogen and crude protein contents of

soluble and insoluble fractions of intact feed proteins were also estimated by microkjeldahl method.

3.2.3 Incubation procedure for in vitro degradation studies

Substrates were dispersed in 10 ml of McDougall buffer of pH 6.8, saturated with nitrogen gas, in a conical flask fitted with Bunsen valve (Johnson, 1966) and incubated at 39°C for 20 min in the atmosphere of carbon dioxide gas. SRF or its fractions viz. mixed bacterial suspension, washed protozoal suspension or cell free supernatant (10 ml saturated with carbon dioxide gas) were added to the flasks, contents were gased again with carbon dioxide, stoppered and incubated at 39°C for different periods in a temperature controlled water bath fitted with shaking platform. All incubations were done in duplicate. The zero hour controls and incubated controls (substrates dispersed in autoclaved SRF or its fractions and incubated) were run simultaneously.

After appropriate time of incubation, 5 ml of incubation mixture were mixed with appropriate volume of 50% trichloroacetic acid (TCA) so that final concentration of TCA in incubation mixture was 10%. These samples were placed overnight at 4°C for precipitation of proteins. To the rest of incubation mixture, 0.25 ml (4-5 drops) of saturated mercuric chloride solution were added to stop the fermentation and the samples were stored in a refrigerator for estimation of ammonia, total volatile fatty acids and total amino acids.

3.2.3.1 In vitro degradation of intact feed proteins and their soluble and insoluble fractions

Intact feed proteins were dispersed in McDougall buffer of pH 6.8, at concentration of 40 mg protein/20 ml incubation mixture which contained 10 ml of McDougall buffer and 10 ml of SRF or its fractions. The soluble and insoluble fractions of intact feed proteins were dispersed in 0.1M McDougall buffer of pH 6.8 in such a way that each ml contained 20mg crude protein and 2 ml of each suspension were added to 8 ml of McDougall buffer of pH 7.8. Rest of the incubation mixture contained 10 ml of SRF or its fraction (final volume of incubation mixture was 20 ml). Incubations were done for 2,4,6, 8 and 12 h.

3.2.3.2 In vitro degradation of casein hydrolysate

50 mg casein hydrolysate dispersed in 10 ml McDougall buffer of pH 6.8 and 10 ml SRF or mixed rumen bacterial fraction were incubated as under section 3.2.3 for 4 and 8 h.

3.2.2.3 In vitro degradation of a mixture of amino acids

The mixture of amino acids (1 m mole/ml of SRF or mixed bacterial suspension) was prepared as follows:

Amino acid	mg
Glutamic acid	14.7
Tyrosine	18.1
Tryptophan	20.4
Histidine	20.5
Aspartic acid	13.3
Cysteine	12.1
Alanine	8.9
Threonine	11.9
Phenylalanine	16.5

Lysine	14.6
Glutamine	14.6
Isoleucine	11.5
Arginine	17.4
Valine	11.7
Serine	10.5
Glycine	7.5
Methionine	14.9
Cystine	24.0
Proline	11.5
Hydroxyproline	13.1

The above amino acids were mixed and dissolved in McDougall buffer (section 3.2.3) and made to 25 ml with same buffer. Amino acids, tyrosine, phenylalanine and tryptophan were dissolved in 2 to 3 ml of 0.1N HCl before mixing with other amino acids in the buffer. 1 ml of above mixture was added to incubation flask containing 10 ml SRF or mixed rumen bacterial suspension, 9 ml McDougall buffer and incubations were carried out for 4 and 8 h as described under section 3.2.3.

3.2.3.4 In vitro degradation of individual amino acids

10 m moles of each amino acid listed under section 3.2.3.3 were dispersed in 10 ml of McDougall buffer of pH 6.8 (section 3.2.3) and incubations were carried out by adding 10 ml of strained rumen fluid or mixed rumen bacterial suspension for 8 h as described under section 3.2.3.

3.2.4 In vitro degradation studies in presence of deaminase or methanogenic inhibitors

3.2.4.1 Deaminase inhibitors

Hydrazine, hydrazine sulfate, phenylhydrazine, p-

nitrophenylhydrazine, hydroxylamineHCL, sodium arsenate, sodium arsenite and dimethyldiphenyliodonium chloride (DDIC) were used as inhibitors of deamination. All these inhibitors were used at concentration of 0.1 and 1.0 mmoles/10 ml SRF or mixed bacterial suspension except DDIC which was used at 25 and 50 ppm concentration. Each inhibitor was suspended/dissolved in McDougall buffer of pH 6.8 (section 3.2.3) so that 1 ml of the solution contained 1.0 mmole (50 ppm in case of DDIC) of inhibitor. 0.1 and 1.0 ml (0.5 and 1.0 ml in case of DDIC) of these solution were put in each incubation flask containing the substrate and volume was made to 10 ml with McDougall buffer of pH 6.8.10 ml of SRF or washed mixed bacterial suspension were added to each flask and incubations were carried out for 4 and 8 h as described under section 3.2.3 to study the degradation of (i) intact feed proteins, (ii) soluble and insoluble proteins (iii) casein hydrolysate and (iv) mixture of amino acids as described under sections 3.2.3.1, 3.2.3.2 and 3.2.3.3.

3.2.4.2 Methanogenic inhibitors

Sodium sulfite, chloroform, chloral hydrate and monensin were used as methanogenic inhibitors. All these inhibitors were used at concentration of 0.1 and 1.0 m moles/10 ml SRF or washed mixed bacterial suspension except monensin which was used at concentration of 25 and 50 ppm. The inhibitors were dissolved/suspended in McDougall buffer of pH 6.8 as described under section 3.2.4.1 and incubations were carried out as described under sections 3.2.3, 3.2.3.1, 3.2.3.2

and 3.2.3.3.

3.2 Characterization of deaminase activity of mixed rumen bacteria

3.2.1 Preparation of mixed rumen bacterial extract (MRBE)

The mixed rumen bacterial extract was prepared according to the method as described by Baldwin and Palmquist (1965).

Approximately 1 l of rumen fluid was removed from the rumen of two buffalo bulls 4 h after the morning feeding. Rumen fluid was strained through double layer of cheese cloth to remove large feed particles and was immediately cooled by placing in ice bath. To this SRF 400 ml of 25 mM ice cold phosphate buffer (pH 7.5) were added. One litre of this mixture was centrifuged twice at 2000 x g for 15 min to remove feed particles and protozoa. The supernatant, carefully decanted, was subjected to centrifugation at 26,000 x g for 10 min in a refrigerated centrifuge to obtain the bacterial pellet. This pellet was washed twice with 25 mM ice cold phosphate buffer of pH 7.5 to remove any soluble contaminants. The washed pellet was resuspended in 10 ml of 25 mM cold phosphate buffer of pH 7.5 and subjected to repeated sonication while kept in ice bath(sonication burst for 1 min followed by rest for 1 min, the whole process repeated five times). The undegraded cells and debris were removed by centrifugation at 26,000 x g for 30 min in a refrigerated centrifuge. The clear supernatant which contained 12 to 15 mg protein per ml of the extract was termed

as mixed rumen bacterial extract (MRBE). All operations were carried out in cold at 4°C. The enzyme extracts were stored in small vials at 0°C, each vial containing exactly 0.5 ml of the extract. At 0°C enzyme extract was found to be stable for several days.

3.3.2 Deamination assay procedure

Deaminase assay procedure was the same as described by Wallace et al.(1987).

Assay mixed contained 0.5 ml of 25 mM phosphate buffer (pH 7.0, boiled before use and saturated with CO_2), 1 ml of 0.5% casein hydrolysate solution prepared in 25 mM phosphate buffer and 0.5 ml of enzyme extract containing 6 to 8 mg protein. This mixture was incubated at 39°C for 2 h. The reaction was stopped by adding 0.25 ml of trichloroacetic acid (50%). The mixture was centrifuged and supernatant was used for estimation of ammonia nitrogen by phenol-hypochlorite method (Weatherburn, 1967). The protein content in the enzyme extract was estimated according to the method as described by Lowry et al.(1951). Enzyme activity was expressed as μM of ammonia produced per mg protein per hr.

3.3.3 Characterisation of deaminase activity of mixed rumen bacterial extract

3.3.3.1 Effect of pH

The reaction mixtures for deaminase assay were prepared in buffers of varying pH values ranging from 5.0 to

9.0 with an increment of 0.5 and deaminase activities were estimated at the end of incubation period. Different buffers used were 25 mM citrate buffer for pH 3.0 to 4.0, 25 mM phosphate buffer for pH range 4.0 to 7.5 and 26 mM Tris HCl buffer for pH range 7.5 to 9.0.

3.3.3.2 Heat stability of enzyme

The MRBE was pre-incubated at temperature ranging from 30°C to 90°C for 10min and immediately cooled by immersing in ice cold bath and the residual deaminase activity was assayed.

3.3.3.3 Effect of incubation temperature

The assay mixtures containing MRBE were incubated at varying temperatures ranging from 25°C to 80°C, instead of 39°C and deaminase activity was measured.

3.3.3.4 Effect of various metal ions

The MRBE was pre-incubated with 0.5 Ml of various metal ions at concentrations ranging from 1 to 10 mmoles (in 25 mM phosphate buffer of pH 7.0) for 10 min. The metal ions were used as chloride salts to eliminate the effects of anionic ions. At the end of preincubation period, substrate was added into each test tube and deaminase activity assayed.

3.3.3.5 Effect of various proteinase inhibitors

The MRBE was preincubated with 0.5 ml of various proteinase inhibitors of concentration ranging from 1 to 10 m moles (except for p-chloromercuribenzoate and sodium azide which were used at 0.1 and 1.0 mmole concentrations) for 10 min at 4°C. The inhibitors were dissolved or suspended in 25 mM

phosphate buffer (pH 7.0). The substrate was added after preincubation period and deaminase activity assayed.

3.3.3.6 Effect of various deaminase inhibitors

0.5 ml of various deaminase inhibitors in 25 mM phosphate buffer in concentration range of 0.1 and 1.0 mmole (except DDIC which was used at concentration range of 25 and 50 ppm) was added to MRBE and were incubated at 4°C for 10 min. At the end of this incubation period, substrate was added and deaminase activity determined.

3.3.3.7 Effect of various methanogenic inhibitors

The MRBE(0.5 ml) was preincubated with various methanogenic inhibitors (0.5 ml) in concentration range of 0.1 and 1.0 mmole (except Monensin which was used at 25 and 50 ppm concentration) for 10 min at 4°C. The inhibitors were dissolved or suspended in 25 mM phosphate buffer (pH 7.0). The substrate was then added and deaminase activity assayed.

3.3.3.8 Effect of various electrolytes

0.5 ml MRBE was preincubated with 0.5 ml of various electrolytes (in 25 mM phosphate buffer, pH 7.0) in concentration range of 1 to 10 mmole for 10 min at 4°C. After the preincubation period, standard assay procedure was carried out to determine the effect of various electrolytes (anions) on deaminase activity. The electrolytes were used as their sodium salts to eliminate the effect of cations.

3.3.3.9 Effect of concentration of amino acid mixture

Amino acid mixture was prepared as described in

section 3.2.3.3 except that it was dissolved in 0.05M phosphate buffer of pH 7.0 (boiled and saturated with CO_2 gas). 1 ml of this solution (1 to 10 mmole amino acids mixture) was put in the test tubes instead of casein hydrolysate solution and deaminase assay was carried out with MRBE.

3.2.3.10 Extent of deamination of individual amino acids

1.0 ml each of individual amino acids, containing 10 mmoles in 25 mM phosphate buffer of pH 7.0 (boiled and saturated with CO_2 gas), was used as substrate instead of casein hydrolysate solution for assay with MRBE, and deaminase activity determined.

3.3.3.11 Extent of deamination of various intact feed proteins and their soluble and insoluble fractions

Various intact feed proteins and their soluble and insoluble fractions were used as substrates instead of casein hydrolysate for deaminase assay with MRBE. 1.0 ml each of intact feed proteins, their soluble and insoluble fractions in 25 mM phosphate buffer of pH 7.0 (boiled and saturated with CO_2 gas) and containing 5 mg crude protein were incubated with 0.5 ml of MRBE and deaminase assay was carried out.

3.4 Distribution of deaminase activity in rumen fluid and its fraction

Approximately 500 ml of SRF were collected from two buffalo bulls on equal volume basis 4 hr after morning feeding. This SRF was fractionated according to the method described in

section 3.1 with a few exceptions.

The protozoal and bacterial pellets were suspended in 10 ml of 25 mM phosphate buffer of pH 7.0 (boiled and saturated with CO_2 gas). Penicillin G and streptomycin sulfate were not added to the protozoal suspension.

1.0 ml SRF, 0.2 ml each of washed bacterial or washed protozoal suspension or 0.5 ml cell-free supernatant were used in the assay mixture. Deaminase activity was determined by the procedure described under section 3.3.2. Each assay was done in triplicate. Protein contents of SRF and its fractions was determined by method of Lowry et al. (1951). The enzyme activity was expressed as μM of ammonia produced per mg protein per hr as well as % deaminase activity as compared to that of SRF taken as 100%.

3.5 Localization of deaminase activity in mixed rumen bacteria cell

Localization of deaminase activity in mixed rumen bacteria cells was done according to the method as used by Kopecny and Wallace (1981).

Approximately 1 l of rumen fluid was collected from the experimental animals on equal volume basis 4 h after morning feeding and was strained through double layer of cheese cloth to remove large feed particles. The SRF was centrifuged twice at 2000 x g for 15 min to remove protozoa and fine feed particles. The supernatant was carefully decanted and checked microscopically to ensure that the protozoa had been removed.

It was centrifuged again at 26,000 x g for 10 min to obtain the bacterial pellet, which was washed twice with 50 mM phosphate buffer of pH 7.5. The pellet was suspended in one fifth the original volume of SRF (approx. 200 ml) in 50 mM potassium phosphate buffer of pH 7.5 and containing 4 mmoles of dithiothreitol (DDT).

20 ml of this bacterial pellet suspension was shaken at 2 cycles per second in a shaker water bath at room temperature for 10 min. The mixture was centrifuged again at 26,000 x g for 10 min, supernatant stored for further use and the pellet obtained was resuspended in 25 ml of 100 mM potassium phosphate buffer, pH 7.5, which contained 4 mmoles of DTT and 10 mmoles $MgCl_2$ per litre of the buffer. This preparation was placed in ice bath and repeatedly sonicated (1 min sonication burst followed by 1 min rest, repeated five times) for 10 min. The unbroken cells and debris were removed by centrifugation at 26,000 x g for 10 min and the pellet was stored for further use. The supernatant containing cell membrane was subjected to centrifugation at 200,000 x g for 30 min and the pellet was further used for extraction of membrane proteins. The supernatant was also preserved for further use.

The membrane proteins were extracted by the method as described by Schnaitman (1974). The cell membrane pellet obtained above, was resuspended in 25 ml of 10 mM HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid) of pH 7.4 which also contained 2% triton X-100 and incubated at 39°C

in a water bath for 10 min, followed by centrifugation at 200,000 x g for 30 min. The supernatant contained inner membrane proteins. The pellet containing outer membrane proteins were solubilized by resuspending the pellet in 25 ml of 10 mM HEPES-Triton X-100 buffer of pH 7.4 and containing 10 mmoles of EDTA. This mixture was incubated at room temperature for 10 min and centrifuged again at 200,000 x g for 30 min to obtain outer membrane proteins in supernatant. The pellet obtained above was used to extract the peptidoglycan proteins according to the method of Lugtenberg et al.(1977). The pellet was resuspended in 25 ml of 25 mM potassium phosphate buffer of pH 7.5 and containing 2% sodium dodecyl sulfate and incubated at 60°C for 20 min. Proteins were harvested by centrifugation at 200,000xg for 30 min.

At each step of extraction, the deaminase activity of pellet and supernatant was determined according to the assay procedure described in section 3.3.3. The protein contents of the same were also determined at each step by dissolving the pellet in 0.1N NaOH by method of Lowry et al.(1951). Deaminase activity was expressed as μM ammonia produced per mg protein from each fraction per hr. Phosphate buffer used for entire operations was boiled and saturated with CO_2 gas.

Effect of various detergents on the release of deaminase activity from mixed rumen bacteria

The mixed rumen bacterial pellet prepared as described

in section 3.5 was suspended in 200 ml of 50 mM potassium phosphate buffer of pH 7.5 (boiled and saturated with CO_2 gas). Triton X-100, Tween-20, Tween-40, Tween-60, Tween-80, Centrimide and sodium dodecyl sulfate were used as detergents for the release of deaminase activity. The detergents were dissolved in 50 mM potassium phosphate buffer of pH 7.0 at 0.05 and 0.1% concentrations. 10 ml of bacterial suspension was mixed with 2 ml of buffer detergent mixture containing 0.05 and 0.1% detergent and shaken on a shaker water bath at 2 cycles per second for 10 min. The mixture was immediately centrifuged at 26,000 x g for 10 min and deaminase activity associated with pellet and released into supernatant was assayed (section 3.3.2). The pellet was dissolved in 0.1N NaOH and protein contents of both pellet and supernatant were also determined (Lowry et al., 1951) and deaminase activity was expressed as μM ammonia produced per mg protein per hr as well as % activity associated with pellet or released in to supernatant as compared to the activity of each before treatment with the detergent.

3.6 Analytical methods

3.6.1 Estimation of total volatile fatty acids

Total volatile fatty acids were estimated by the procedure as described by Barnett and Reid (1957).

Reagents

i)   5% oxalic acid

ii)   10% potassium oxalate

iii)   0.05N NaOH

Procedure

The boiling flask of Markham distillation apparatus was filled with distilled water and a few glass beads were placed at the bottom to prevent the bumping of distilled water during boiling. The Markham distillation apparatus was steamed for 10 min and the condensate collected in steam distillation compartment was emptied by pinching the plastic tube connecting the boiling flask to the distillation apparatus. The water drain tap was opened to drain off the condensed water that collected in the outer jacket of the apparatus. 1.0 ml of the sample and 2 ml of 5% oxalic acid and 10% potassium oxalate mixture (1:1) were added into the steam distillation compartment through the inlet without touching the sides of the inlet with pipette tip. The receiving flask was placed at the distillate receiving end and distillate was collected and titrated against 0.05N NaOH using phenolphthalein as an indicator. Before putting in the next sample, condensate was removed from steam jacket and apparatus was washed with distilled water. For calculation of total volatile fatty acid concentration, the following equivalent was used:

1 ml of .05N NaOH $\equiv$ 0.05m moles of acid

3.6.2 Estimation of proteins

The estimation of proteins was done according to the method of Lowry et al.(1951).

Reagents

Reagent A: 2% Na_2CO_3 in 0.1N NaOH

Reagent B: 0.5% $CuSO_4.5H_2O$ in 1% sodium citrate $(Na_3C_6H_5O_7.2H_2O)$ solution.

Reagent C: 50 parts of reagent A were mixed with 1 part of reagent B just before use.

Folin-phenol reagent: Stock solution (SRL, India) was diluted with 2 volumes of distilled water.

Procedure

Proteins precipitated with trichloroacetic acid from incubation mixture were centrifuged at 4000 rpm for 30 min. The supernatants were stored for estimation of ammonia and total amino acids. The precipitates obtained were mixed with 5 ml of 0.1N NaOH and test tubes were placed in boiling water bath till the entire precipitates were dissolved.

0.1 ml of the above solution was taken in a test tube and made to 1.0 ml with distilled water. 5.0 ml of reagent C were added to each test tube and contents were mixed well and allowed to stand at room temperature for 10 min. 0.5 ml of diluted folin-phenol reagent was added to each test tube and contents were vigorously shaken immediately. The test tubes were allowed to stand at 37°C for 30 min and blue colour developed was read at 525 nm against a reagent blank in spectronic-20 spectrophotometer (Bausch and Lomb). The protein contents were calculated from a standard curve prepared by using bovine serum albumin (BSA) in concentration range of 20

to 100 µg in 0.1N NaOH.

3.6.3 Estimation of ammonia nitrogen (Weatherburn, 1967)

Reagents:

Reagents A: 5 g distilled phenol and 25 mg sodium nitroprusside ($Na_2Fe(CH_5NO_2)$ were dissolved in glass distilled water. The reagent was stored in amber coloured bottle in a refrigerator.

Reagent B (Alkaline hypochlorite solution): 2.5 g NaOH and 4.2 ml sodium hypochlorite (NaOCl) were mixed and made to 500 ml with glass distilled water. The reagent was put in amber coloured bottle and kept in a refrigerator.

Standard solution: A stock solution was prepared by dissolving 330 mg ammonium sulfate in 100 ml of 0.1N HCl (0.35 mg ammonia nitrogen/ml). The working standards were prepared by appropriate dilution of stock solution with 0.1N HCl to get a concentration range of 0.7 to 3.5 µg of above solution.

Procedure

20 ml of sample or standard (containing 0.7 to 3.5 µg ammonia nitrogen) were placed in test tubes and 5 ml of reagent A were added to each test tube. The contents were mixed well and 5 ml of reagent B were then added to each test tube. The colour was developed by placing the test tubes in water bath at 37°C for exactly 15 min or at room temperature for 30 min. The absorbance was read at 535 nm using spectronic-20 spectrophotometer, against a reagent blank and concentration of ammonia nitrogen was calculated from a standard curve prepared simultaneously.

3.6.4 Estimation of total amino acids

The supernatant obtained after precipitation of proteins by trichloroacetic acid was used for the estimation of total amino acids by the method as described by Lee and Takahashi (1966).

Reagents

i) **0.5M citrate buffer**: 52.5 g of citric acid monohydrate ($C_6H_8O_7.H_2O$) and 73.5 g trisodium citrate dihdyrate ($C_6H_8O_7.Na_3.2H_2O$) were each dissolved in 1 l of distilled water. These solutions were mixed in appropriate ratio to set the pH of buffer at 5.5 and final volume was made to 1 l with distilled water.

ii)   1% ninhdyrin in 0.5M citrate buffer

iii)   Glycerol

Procedure

20 μl of supernatant or standard in 10% trichloroacetic acid were put in test tubes. 3.8 ml of a mixture of 1% ninhydrin in 0.5M citrate buffer, pH 5.5, glycerol and citrate buffer were added to the test tubes and contents were mixed well on a vortex mixer. Above mixture contained 1 ml of 1% ninhydrin in 0.5M citrate buffer of pH 5.5, 2.4 ml of 0.5M citrate buffer of pH 5.5 and 0.4 ml of glycerol and large quantities of this mixture were prepared before use. The test tubes, after having vortexed were placed in a boiling water bath for 12 min and then cooled under tap

water to the room temperature. The mixture was shaken again on a vortex mixer and absorbance was read against a reagent blank at 570 nm in spectronic-20 spectrophotometer. A standard curve was prepared using tryptophane in concentration range of 2 to 20 μg and amino acid concentration was calculated.

3.6.5 Estimation of total nitrogen and crude protein contents

Total nitrogen was estimated by microkjeldahl method (McKenzie and Wallace, 1954)

Reagents

Digestion mixture: Copper sulfate powder and potassium sulfate powder were mixed well n ratio of 1:10 (w/w)

4% boric acid solution: 40 g of anhydrous boric acid were dissolved in 1 l of distilled water.

Mixed indicator: 55 mg of bromocresol green and 33 mg of methyl red were dissolved in 100 ml of absolute alcohol.

Boric acid mixed indicator mixture: 2 ml of mixed indicator solution were added to 1 l of 4% boric acid solution and kept in amber coloured bottle. This mixture was freshly prepared.

40% NaOH

0.05 N HCl

Procedure

Digestion of the samples: 100 mg finely ground feed protein source, 10 ml of soluble proteins fraction or 10 ml of insoluble proteins fraction were placed in 100 ml long necked digestion flasks along with 0.5 g of digestion mixture and 5 ml of concentrated sulphuric acid. Digestion mixture was added to

prevent bumping as well as to indicate the completion of digestion by clear yellowish blue solution in the flask. The digestion flasks were heated over a digestion heater till the solutions were clear of all the fumes. The flasks were removed, cooled to room temperature and volume of solution made to 25 ml with distilled water by repeatedly washing the digestion flask. **Distillation of the digested solution**: Boiling flask of the markham steam distillation apparatus was filled with distilled water and apparatus steamed for 10 min. 2.0 ml of digested sample and 5 ml of 40% NaOH were introduced into steam distillation chamber of the apparatus. Simultaneously a conical flask containing 5 ml of boric acid-mixed indicator mixture were placed at the distillation receiving end and 25 ml of distillate was collected. The distillate was titrated against 0.05 N HCl. Appropriate digestion blanks were run along with protein samples. Total nitrogen in the sample was calculated as follows:

% Nitrogen =

$$\frac{0.00014 \times \text{vol.of } 0.05N \text{ HCl used} \times \text{Total vol.of distillate}}{\text{Volume of sample distilled} \times \text{Weight of sample digested}} \times 100$$

Crude protein contents were calculated by multiplying % nitrogen by a factor of 6.25.

CHAPTER IV

RESULTS

4.1 **In vitro** degradation of intact feed proteins and its soluble and insoluble fractions with strained rumen fluid and its fractions

4.1.1 **In vitro** degradation of intact feed proteins by rumen fluid and its fractions

4.1.1.1 Strained rumen fluid

The results of the degradation of intact feed proteins when incubated for different periods with strained rumen fluid (SRF) are given in Table 3 (Fig.1).

The degradation of all the intact feed proteins examined was found to increase with increase in the incubation period from 2 to 12 h. At 6 h incubation period, the intact protein of casein, soyabean seeds (Soya), ground nut cake (GC), mustard cake (MC) and sunflower seeds (SF) were degraded to the extent of 62.0, 57.3, 46.8, 38.3 and 38.3%, respectively, and corresponding values for 12 h incubation period were 75.8, 74.8, 64.5, 60.3 and 53.8%.

At 6 h of incubation, extent of degradation of casein and Soya was significantly ($P < 0.01$) higher than that of GC,

Table 2. Crude protein content s (g/100 g feed dry matter) and solubility (%) of various intact feed proteins and their fractions

Feed protein source	Crude protein content of different protein fractions			Solubility (%)
	Intact	Soluble	Insoluble	
Groundnut Cake (GC)	46.37	16.88	16.68	35.9
Mustard Cake (MC)	30.68	10.62	20.87	32.0
Sunflower Seeds (SF)	20.5	10.44	10.06	51.0
Soyabean seeds (soya)	37.25	15.31	22.31	40.9
Casein	23.62	19.56	4.06	82.8

Figures represent mean of four observations.

The means superscribed by letters a, b, c, d (at $p < 0.01$) and e, f, g , h (at $p < 0.05$) in the proceeding tables (unless mentioned otherwise) mean the following are **significant**:

a,e   between casein and soya on one hand GC, MC and SF on the other hand

b,f   between casein and that of soya

c,g   between GC and that of MC and SF

d,h   between MC and that of SF

Table 3. Degradation of intact feed proteins when incubated for different periods with strained rumen fluid.

Incubation period (h)	TCA-precipitable proteins left after incubation (mg)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	40.0	40.0	40.0	40.0	40.0
2	$29.3^{a}\pm0.96$ (26.8)	$31.9^{a,g}\pm0.72$ (20.3)	$32.9^{a,g}0.67$ (17.8)	$28.2^{f}\pm0.64$ (29.5)	25.9 ± 0.98 (35.3
4	$24.0^{a}\pm0.19$ (40.0)	$27.9^{a,c,g}\pm0.94$ (30.3)	$28.8^{a}\pm0.49$ (28.0)	$21.8^{f}\pm0.28$ (45.5)	20.6 ± 0.65 (48.5)
6	$21.3^{a}\pm1.18$ (46.8)	$24.7^{a}\pm0.92$ (38.3)	$24.7^{a}\pm0.73$ (38.3)	$17.1^{b}\pm0.63$ (57.3)	15.2 ± 0.18 (62.0)
8	$17.5^{a}\pm0.46$ (56.3)	$20.2^{a,c}\pm0.12$ (49.3)	$22.6^{a,c,d}\pm1.14$ (43.7)	$13.4^{f}\pm0.71$ (66.5)	11.9 ± 0.18 (70.3)
12	$14.2^{a}\pm0.81$ (64.5)	$15.9^{a}\pm0.91$ (60.3)	$18.5^{a,h}\pm0.83$ (53.0)	10.1 ± 0.85 (74.8)	9.7 ± 0.98 (75.8)

Figures represent mean of four observations ± standard deviation
Figures in parenthesis represent per cent degradation over control
Conditions of incubation : 200 mg soluble starch, 40 mg intact protein on crude protein basis, 10 ml strained rumen fluid, 10 ml buffer were incubated for different periods.

FIG.1 DEGRADATION OF INTACT PROTEINS & THEIR SOLUBLE & INSOLUBLE FRACTIONS WHEN INCUBATED WITH STRAINED RUMEN FLUID FOR 12 h.
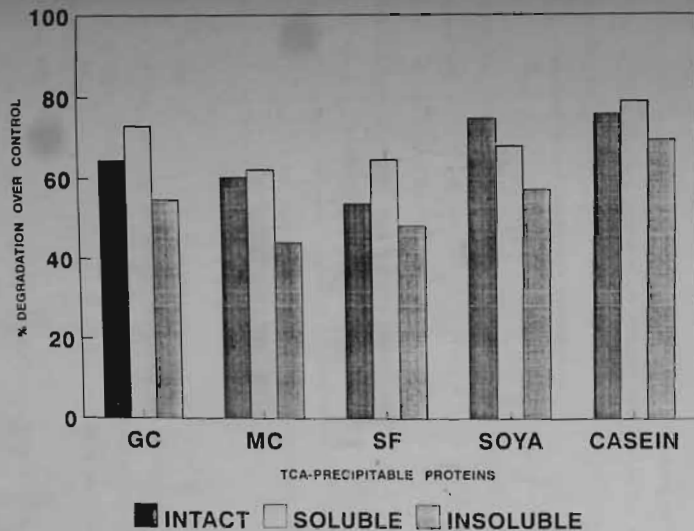
MC and SF protein, while that of GC was significantly (P < 0.01) higher than that of MC and SF. Between Soya and casein the difference was significant (P < 0.01) at 6 h of incubation and between MC and SF significant (P < 0.05) at 12 h of incubation.

The degradation values for all incubation periods indicated that intact protein of casein was maximally degraded followed, in order, by soya, GC, MC and SF.

4.1.1.2 Washed mixed bacterial suspension

The data on degradation of intact feed proteins when incubated for different periods with washed bacterial suspension are given in Table 4 (Fig.2). As with SRF, the extent of degradation of intact proteins of all the feeds examined was increased with increasing incubation periods upto 12 h. At 6 h incubation period, the intact proteins of casein, Soya, GC, MC and SF were degraded to the extent of 20.5, 19.2, 15.5, 12.7 and 11.7% respectively and the corresponding values for 12 h incubation period were 36.2, 34.2, 27.2, 24.5 and 22.2%.

At 6 and 12 h incubation periods, the difference in extent of degradation of casein and soya was significantly (p < 0.01) higher than that of GC, MC and SF intact proteins. No significant difference was observed between soya and casein, GC and MC and SF and GC.

At all the incubation periods, there was maximum degradation of casein followed, in order, by Soya, GC, MC and

Table 4. Degradation of intact feed proteins when incubated for different periods with washed bacterial suspension

Incubation period (h)	TCA-precipitable proteins left after incubation (mg)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	40.0	40.0	40.0	40.0	40.0
2	$38.1^a \pm 0.32$ (4.75)	$38.3^a \pm 0.81$ (4.2)	$38.5^a 0.38$ (3.7)	36.8 ± 0.64 (8.0)	36.6 ± 0.86 (8.5)
4	$36.2^a \pm 0.49$ (9.5)	$36.1^a \pm 0.72$ (9.7)	$36.9^a \pm 0.52$ (7.7)	34.28 ± 0.91 (14.5)	34.1 ± 0.92 (14.7)
6	$33.8^a \pm 0.63$ (15.5)	34.9 ± 0.93 (12.7)	$35.3^a \pm 0.76$ (11.7)	32.3 ± 0.84 (19.2)	31.8 ± 0.98 (20.5)
8	$31.7^a \pm 0.72$ (20.7)	$32.5^a \pm 0.92$ (18.7)	$33.6^a \pm 0.41$ (16.0)	29.2 ± 0.32 (27.0)	28.8 ± 0.74 (30.0)
12	$29.1^a \pm 0.61$ (27.2)	$30.2^a \pm 0.49$ (24.5)	$31.1^a \pm 0.77$ (22.2)	26.3 ± 0.99 (34.2)	25.5 ± 0.36 (36.2)

Figures represent mean of four observations ± standard deviation
Figures in parenthesis represent per cent degradation over control
Conditions of incubation : Same as in table 3 except that 10 ml of washed mixed bacterial suspension were added in place of strained rumen fluid.

FIG.2 DEGRADATION OF INTACT PROTEINS & THEIR SOLUBLE & INSOLUBLE FRACTIONS WHEN INCUBATED WITH WASHED BACTERIAL SUSPENSION FOR 12 h.
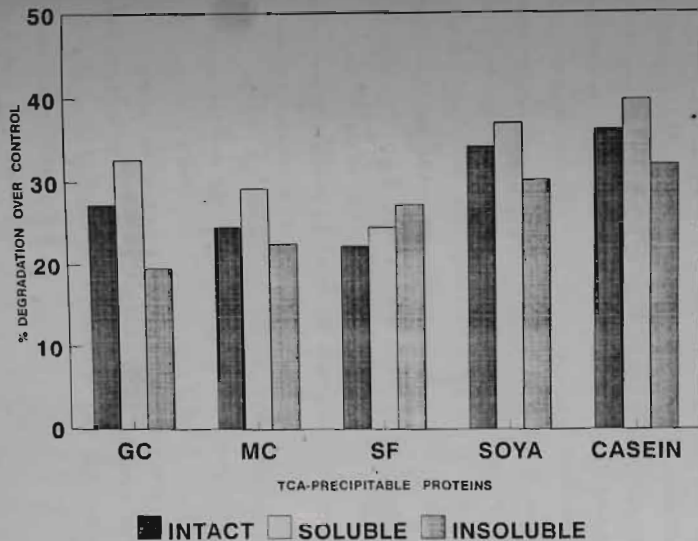
SF.

4.1.1.3 Washed protozoal suspension

The data on the degradation of intact proteins of all feeds when incubated with washed protozoal suspension for different periods are given in Table 5 (Fig.3). As with SRF and washed bacterial suspension, the extent of degradation of intact proteins of all the feeds studied increased with increase in the incubation period upto 12 h. At 6h incubation period, the extent of degradation of casein, soya, GC, MC and SF intact proteins was 11.5, 7.7, 4.5, 3.2 and 2.7% respectfully, and the corresponding values for 12 h incubation period were 14.7, 14.0, 9.2, 6.2 and 5.7%.

At 6 h ($p < 0.05$) and 12 h ($p < 0.01$) incubation periods, the difference in extent of degradation of casein and soya was significantly higher than that of GC, MC and SF. However, at 6 h incubation period, the difference in degradation between Soya and Casein intact proteins was also significant ($p < 0.05$).

Extent of degradation of intact proteins remained maximum in case of casein and was followed in order by Soya, GC, MC and SF as in case of degradation with SRF and washed bacterial suspension.

4.1.1.4 Cell free supernatant

Table 6 (Fig.4) represents the data on the degradation of intact feed proteins when incubated for different periods with cell free supernatant of SRF. As with SRF and its

Table 5. Degradation of intact feed proteins when incubated for different periods with washed protozoal suspension

Incubation period (h)	TCA-precipitable proteins left after incubation (mg)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	40.0	40.0	40.0	40.0	40.0
2	$39.6^e \pm 0.41$ (1.05)	$39.8^e \pm 0.18$ (0.5)	$39.9^e 0.0.44$ (0.25	38.2 ± 0.84 (4.5)	38.1 ± 0.66 (4.7)
4	$38.9^a \pm 0.22$ (2.7)	$39.2^a \pm 0.39$ (2.0)	$39.5^a \pm 0.36$ (1.2)	37.58 ± 0.49 (6.2)	36.9 ± 0.41 (7.7)
6	$38.2^e \pm 0.39$ (4.5)	$38.7^e \pm 0.81$ (3.2)	$38.9^e \pm 0.77$ (2.7)	$36.9^f \pm 0.64$ (7.7)	35.4 ± 0.83 (11.5)
8	$37.6^a \pm 0.58$ (6.0)	$38.4^a \pm 0.72$ (4.0)	$38.4^{a, d} \pm 0.34$ (4.0)	$35.8^f \pm 0.12$ (10.5)	34.9 ± 0.59 (13.0)
12	$36.3^a \pm 0.61$ (9.2)	$37.5^a \pm 0.22$ (6.2)	$37.7^a \pm 0.59$ (5.7)	34.4 ± 0.51 (14.0)	34.1 ± 0.38 (14.7)

Figures represent mean of four observations ± standard deviation
Figures in parenthesis represent per cent degradation over control
Conditions of incubation : Same as in table 3 except that 10 ml of washed protozoal
                           suspension were added in place of strained rumen fluid.

Table 6.   Degradation of intact feed proteins when incubated for different periods with cell free supernatant

Incubation period (h)	TCA-precipitable proteins left after incubation (mg)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	40.0	40.0	40.0	40.0	40.0
2	36.5±0.42 (8.7)	36.8±0.56 (8.0)	36.8±0.72 (8.0)	35.7±0.51 (10.7)	35.3±0.38 (11.8)
4	32.8^a±0.91 (18.0)	33.4^a±0.63 (8.0)	34.2^a±0.41 (8.0)	30.1±0.88 (10.7)	28.7±0.34 (28.2)
6	28.6^a±0.46 (28.5)	30.2^{a,g}±0.72 (24.5)	30.8^{a,g}±0.37 (23.0)	27.6^f±0.71 (31.0)	25.7±0.49 (35.7)
8	23.5^a±0.57 (41.3)	25.2^{a,g}±0.77 (37.0)	26.3^{a,g,h}±0.48 (34.2)	22.1^b±0.59 (44.7)	20.1±0.56 (49.8)
12	21.4^a±0.42 (46.5)	23.0^{a,g}±0.91 (42.5)	24.2^{a,g,h}±0.56 (39.5)	19.5±0.55 (51.2)	18.3±0.57 (54.3)

Figures represent mean of four observations ± standard deviation
Figures in parenthesis represent per cent degradation over control
Conditions of incubation : Same as in table 3 except that 10 ml of cell free supernatant were added in place of strained rumen fluid.

FIG.3 DEGRADATION OF INTACT PROTEINS & THEIR SOLUBLE & INSOLUBLE FRACTIONS WHEN INCUBATED WITH WASHED PROTOZOAL SUSPENSION FOR 12 h.
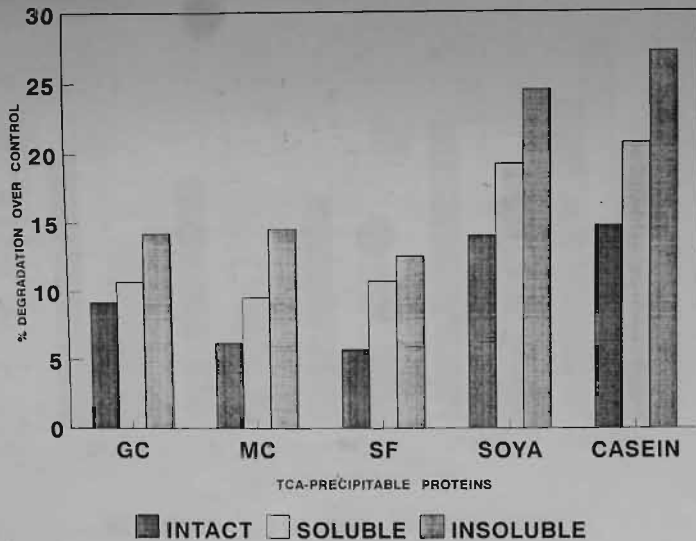
FIG.4 DEGRADATION OF INTACT FEED PROTEINS AND THEIR SOLUBLE & INSOLUBLE FRACTIONS WHEN INCUBATED WITH CELL FREE SUPERNATANT FOR 12 h.
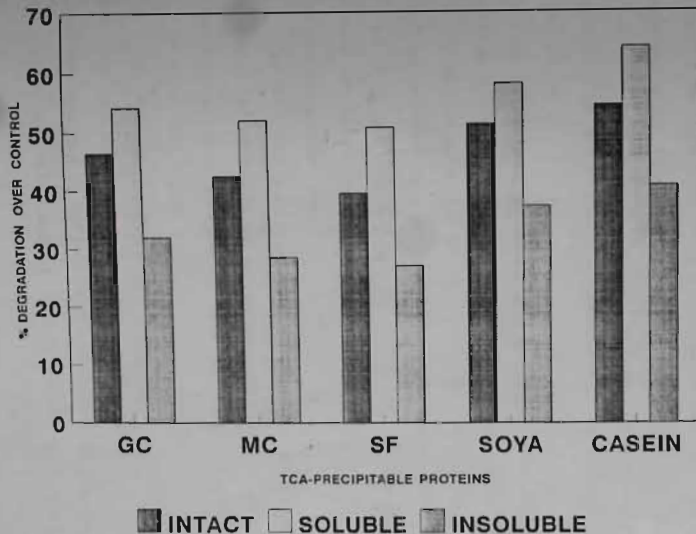
fractions studied earlier, the extent of degradation of intact proteins of all the feeds examined increased with increase in incubation time upto 12 h. At 6 h incubation period, the extent of degradation of intact proteins was 35.7, 31.0, 28.5, 24.5 and 23.0% for casein, Soya, GC, MC and SF, respectively, and corresponding values for 12 h incubation were 54.3, 51.2, 46.5, 42.5 and 39.5%.

At 6 and 12 h incubation periods, the extent of degradation between casein and soya protein was significantly (p < 0.01) higher than that of GC, MC and SF. The difference in degradation between Soya and casein was also significant (p < 0.05) at 6 h of incubation. At 12 h of incubation, the difference in extent of degradation between GC and SC and MC and SF was also significant (p < 0.05).

As with SRF, bacterial cell suspension, protozoal cell suspension, the degradation of intact protein by cell free supernatant was also maximum in case of casein followed, in order, by Soya, GC, MC and SF.

The data given in Tables 3, 4, 5 and 6 (Fig.1,2,3,4) suggest that the degradation of intact proteins of all the feed examined was maximum by SRF, followed by cell free supernatant, washed bacterial cell suspension and washed protozoal cell suspension.

4.1.2 In vitro degradation of soluble fraction of intact feed proteins by rumen fluid and its fractions

The soluble fraction of intact feed proteins was

incubated with SRF and its fractions for 0, 2, 4, 6, 8 and 12 h and their extent of degradation is presented as follows:

4.1.2.1 Strained rumen fluid

The data on extent of degradation of soluble fraction of intact feed proteins of different feeds by SRF are given in Table 7 (Fig.1). The extent of degradation of soluble proteins of all the feeds, increased with increase in incubation time from 2 to 12 h. At 6 h incubation period, the soluble fraction of casein, Soya, GC, MC and SF intact proteins was degraded to the extent of 59.5, 54.0, 50.3, 50.5 and 38.0% respectively, and the corresponding values for 12 h incubation period were 79.0, 68.0, 73.0, 62.2 and 64.7%.

At 6 h incubation period, extent of degradation of soluble proteins of casein and Soya was significantly ($p < 0.01$) higher than that of GC, MC and SF. The extent of degradation in case of MC and GC was significantly ($p < 0.01$) higher than that of SF and that of casein was significantly ($p < 0.01$) higher than that of Soya. At 12 h incubation period, the difference in extent of degradation between Soya and casein was significantly ($p < 0.01$) higher than that of GC and MC; that of MC was higher ($p < 0.01$) than that of SF.

Above data indicated that at 6 h incubation period casein was degraded to maximum extent followed, in order by Soya, GC, MC and SF, though GC and SC were degraded to almost same degree. AT 12 h incubation period, the degradation of

Table 7. Degradation of soluble fractions of intact feed proteins when incubated for different periods with SRF

Incubation period (h)	TCA-precipitable proteins left after incubation (mg)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	40.0	40.0	40.0	40.0	40.0
2	$26.1^e \pm 0.44$ (34.7)	$27.2^{e,g} \pm 0.41$ (32.0)	$28.9^{e,g,h} \pm 0.88$ (27.7)	26.2 ± 1.16 (34.0)	26.0 ± 0.51 (34.7)
4	$23.2^a \pm 0.54$ (42.0)	$23.4^a \pm 0.85$ (41.5)	$26.5^{a,d} \pm 0.78$ (33.7)	21.1 ± 1.22 (47.2)	21.0 ± 0.11 (11.(47.5)
6	$19.9^a \pm 0.91$ (50.3)	$19.8^a \pm 0.85$ (50.5)	$24.8^{a,d} \pm 0.74$ (38.0)	$18.4^b \pm 0.76$ (54.0)	16.2 ± 0.12 (59.5)
8	$16.1^a \pm 0.72$ (59.7)	$15.6^a \pm 0.64$ (61.0)	$19.2^{g,h} \pm 0.18$ (52.0)	$13.1^f \pm 0.38$ (67.2)	11.2 ± 0.82 (72.0)
12	10.8 ± 0.48 (73.0)	$12.3^g \pm 0.91$ (62.2)	$14.1^{g,h} \pm 0.33$ (64.7)	$12.8^b \pm 0.91$ (68.0)	8.4 ± 0.42 (79.0)

Figures represent mean of four observations ± standard deviation
Figures in parenthesis represent per cent degradation over control
Conditions of incubation : 200 mg soluble starch,· 40 mg soluble protein in 2 ml buffer, 10 ml strained rumen fluid and 8 ml buffer incubated at 39°C for different periods.

soluble proteins of casein was maximum followed by GC, Soya, SF and MC.

4.1.2.2 Washed mixed bacterial suspension

The data on degradation of soluble fraction of intact feed proteins when incubated for different periods with washed bacterial suspension are presented in Table 8 (Fig.2). As in all previous studies, the extent of degradation of soluble proteins for all the feeds examined, increased with increase in incubation period upto 12 h. At 6 h incubation period, the soluble proteins of casein, Soya, GC, MC and SF were degraded to the extent of 23.2, 22.0, 21.0, 16.2 and 13.0% respectively, and corresponding values for 12 h incubation period were 39.7, 37.0, 32.7, 29.0 and 24.5%, .

At 6 and 12 h of incubation, the extent of degradation of soluble proteins of casein and soya was significantly higher (p < 0.01) than that of GC, MC and SF. However, at 12 h of incubation the difference between GC and SF, SF and GC and MC and SF were also significant (p < 0.01). At 12 h of incubation, extent of degradation of casein was also significantly (p < 0.05) higher than that of Soya.

Data showed that the soluble fraction of casein intact protein was degraded to maximum extent by washed bacterial suspension followed, in order, by Soya, GC, MC and SF.

4.1.2.3 Washed protozoal suspension

The data on the extent of degradation of soluble fraction of intact feed proteins when incubated with washed

Table 8. Degradation of soluble fraction of intact feed proteins when incubated for different periods with washed bacterial suspension

Incubation period (h)	TCA-precipitable proteins left after incubation (mg)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	40.0	40.0	40.0	40.0	40.0
2	$37.7^e \pm 0.44$ (5.7)	$38.3^e \pm 0.45$ (4.2)	$38.7^e \pm 0.81$ (3.2)	36.9 ± 0.94 (7.7)	36.8 ± 0.72 (8.0)
4	$34.8^a \pm 0.59$ (13.0)	$36.2^a \pm 0.82$ (9.5)	$36.2^a \pm 0.36$ (9.5)	34.5 ± 0.45 (13.7)	33.6 ± 0.81 (16.0)
6	$31.6^a \pm 0.82$ (21.0)	$33.5^{a,c} \pm 0.91$ (16.2)	$34.8^a \pm 0.52$ (13.0)	31.2 ± 0.19 (22.0)	30.7 ± 0.77 (23.2)
8	$28.3^a \pm 0.42$ (29.2)	$30.5^{a,c} \pm 0.92$ (24.5)	$32.1^{a,c,h} \pm 0.61$ (23.0)	$28.3^f \pm 0.26$ (31.0)	26.4 ± 0.92 (35.7)
12	$26.9^a \pm 0.36$ (32.7)	$28.4^{a,c} \pm 0.38$ (29.0)	$32.1^{a,c,d} \pm 0.67$ (24.5)	$25.2^b \pm 0.14$ (37.0)	24.1 ± 0.49 (39.7)

Figures represent mean of four observations ± standard deviation
Figures in parenthesis represent per cent degradation over control
Conditions of incubation : Same as in table 7 except that 10 ml of washed mixed bacterial suspension were added in place of strained rumen fluid.

Table 9. Degradation of soluble fraction of intact feed proteins when incubated for different periods in washed protozoal suspension

Incubation period (h)	TCA-precipitable proteins left after incubation (mg)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	40.0	40.0	40.0	40.0	40.0
2	$39.4^a\pm0.61$ (1.5)	$39.5^a\pm0.33$ (1.2)	$39.6^a\pm0.21$ (1.0)	38.1 ± 0.42 (4.7)	37.8 ± 0.44 (5.5)
4	$38.6^e\pm0.48$ (3.5)	$38.7^e\pm0.42$ (3.2)	$38.4^e\pm0.18$ (4.0)	36.8 ± 0.73 (8.0)	36.1 ± 0.16 (9.7)
6	$37.8^e\pm0.82$ (5.5)	$38.2^e\pm0.55$ (4.5)	$37.6^e\pm0.76$ (6.0)	35.2 ± 0.92 (12.0)	34.8 ± 0.41 (13.0)
8	$37.2^a\pm0.39$ (7.0)	$37.8^a\pm0.46$ (5.5)	$36.8^{a,h}\pm0.41$ (8.0)	33.7 ± 0.81 (15.7)	33.3 ± 0.28 (16.7)
12	$35.7^a\pm0.21$ (10.7)	$36.2^a\pm0.41$ (9.5)	$35.7^a\pm0.38$ (10.7)	32.3 ± 0.77 (19.2)	31.7 ± 0.36 (20.7)

Figures represent mean of four observations ± standard deviation
Figures in parenthesis represent per cent degradation over control
Conditions of incubation : Same as in table 7 except that 10 ml of washed protozoal suspension were added in place of strained rumen fluid.

protozoal suspension for different period are given in Table 9 (Fig.3). The extent of degradation, as usual, increased with increase in incubation time upto 12 h. AT 6 h after incubation, soluble proteins of casein, Soya, GC, MC and SF were degraded to the extent of 13.0, 12.0, 5.5, 4.5 and 6.0% respectively, while the corresponding values for 12 h incubation period were 20.7, 19.2, 10.7, 9.5 and 10.7%.

At 6 ($p < 0.05$) and 12 h ($p < 0.01$) incubation period, extent of degradation of casein and soya soluble proteins were significantly higher than that of GC, MC and SF.

The soluble protein of casein was degraded to maximum extent followed, in order, by Soya, GC and SF being equally degraded, and MC.

4.1.2.4 Cell-free supernatant

The data on the degradation of soluble fraction of intact feed proteins when incubated with cell-free supernatant for different periods are presented in Table 10 (Fig.4). The extent of degradation of this fraction of proteins increased with increase in incubation period upto 12 h. At 6 h incubation period, it was 34.0, 30.5, 37.2, 35.2 and 36.7% for casein, Soya, GC, MC and SF, respectively, and corresponding values for 12 h incubation period were 64.0, 58.0, 54.2, 52.0 and 50.7%.

At 6 h incubation period, extent of degradation of casein and soya soluble proteins were significantly ($p < 0.01$) lower than that of GC, MC and SF, while at 12 h incubation period the same was significantly ($p < 0.01$) higher. However,

Table 10. Degradation of soluble fraction of intact feed proteins when incubated for different periods with cell free supernatant

Incubation period (h)	TCA-precipitable proteins left after incubation (mg)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	40.0	40.0	40.0	40.0	40.0
2	$35.2^e \pm 0.66$ (12.0)	$35.8^e \pm 0.31$ (10.5)	$36.0^e \pm 0.42$ (10.0)	34.1 ± 0.91 (14.7)	33.8 ± 0.42 (15.5)
4	$31.5^a \pm 0.72$ (21.2)	$32.3^a \pm 0.92$ (19.2)	$32.5^a \pm 0.38$ (18.7)	30.6 ± 0.81 (23.5)	31.2 ± 0.11 (22.0)
6	$25.1^a \pm 0.88$ (37.2)	$25.9^a \pm 0.85$ (35.2)	$25.3^a \pm 0.41'$ (36.7)	27.8 ± 0.78 (30.5)	26.4 ± 0.65 (34.0)
8	$22.5^a \pm 0.61$ (43.7)	$22.4^a \pm 0.76$ (44.0)	$23.1^a \pm 0.22$ (42.2)	$21.8^f \pm 0.41$ (45.5)	20.4 ± 0.77 (49.0)
12	$18.3^a \pm 0.24$ (54.2)	$19.2^{a,g,d} \pm 0.19$ (52.0)	$19.7^{a,g,d} \pm 0.24$ (50.7)	$16.8^b \pm 0.65$ (58.0)	14.4 ± 0.89 (64.0)

Figures represent mean of four observations ± standard deviation
Figures in parenthesis represent per cent degradation over control
Conditions of incubation : Same as in table 7 except that 10 ml of cell free supernatant were added instead of strained rumen fluid.

at 12 h incubation period, the extent of degradation of soluble protein of GC was significantly (p < 0.01) higher than that of SF and that of MC significantly higher (p < 0.01) than that of SF. Similarly, the difference in the degradation between casein and soya was also highly significant (p < 0.01) at 12 h of incubation.

Data indicated that at 6 h of incubation, soluble proteins of GC were maximally degraded followed, in order, by SF, MC, casein and Soya, whereas at 12 h of incubation casein soluble proteins were degraded to maximum extent followed, in order, by Soya, GC, MC and SF. The data given in tables 7, 8, 9 and 10 (Fig.1,2,3, and 4) indicated that in general the pattern of extent of degradation of soluble proteins of different feeds was almost the same as with intact proteins. The degradation of soluble proteins of casein was maximum followed by Soya, GC and MC and SF.

These results also indicated that soluble fractions of intact proteins was maximally degraded by SRF followed, in order, by cell-free supernatant, washed bacterial fraction and washed protozoal fraction.

4.1.3 In vitro degradation of insoluble fraction of intact feed proteins by rumen fluid and its fractions

4.1.3.1 Strained rumen fluid

The data given in table 11 (Fig.1) show the extent of degradation of insoluble fraction of intact feed proteins when

Table 11. Degradation of insoluble fraction of intact feed proteins when incubated for different periods with strained rumen fluid

Incubation period (h)	TCA-precipitable proteins left after incubation (mg)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	40.0	40.0	40.0	40.0	40.0
2	$34.0^{e}\pm0.16$ (15.0)	$35.9^{e,c}\pm0.76$ (10.2)	$37.9^{e,c,d}\pm0.31$ (7.2)	$36.9^{b}\pm0.42$ (7.7)	34.7 ± 0.83 (13.2)
4	$30.1^{a}\pm0.89$ (24.7)	$32.2^{a,c}\pm0.25$ (19.5)	$34.3^{a,c,d}\pm0.47$ (14.2)	$32.8^{b}\pm0.47$ (18.0)	28.3 ± 0.64 (29.2)
6	$28.2^{e}\pm0.75$ (29.5)	$29.5^{e,g}\pm0.83$ (26.2)	$30.3^{e,g}\pm0.99$ (24.2)	$27.6^{b}\pm0.88$ (31.0)	23.2 ± 0.51 (42.0)
8	$24.4^{a}\pm0.70$ (39.0)	$27.3^{a,c}\pm0.84$ (31.7)	$25.5^{a,c,d}\pm0.18$ (36.2)	$20.5^{b}\pm0.54$ (48.7)	15.3 ± 0.58 (61.7)
12	$18.1^{a}\pm0.86$ (54.7)	$22.4^{a,c}\pm0.59$ (44.0)	$20.7^{a,c,h}\pm0.78$ (48.2)	$17.1^{b}\pm0.91$ (57.2)	12.2 ± 0.33 (69.5)

Figures represent mean of four observations ± standard deviation
Figures in parenthesis represent per cent degradation over control
Conditions of incubation : 200 mg starch, 40 mg insoluble proteins in 2 ml buffer,
10 ml strain rumen fluid and 8 ml buffer incubated at 39°C
for different periods.

incubated for different periods with SRF. As with intact proteins and their soluble fractions, the insoluble fractions showed increased degradation with increase in incubation time with SRF. At 6 h after incubation, the extent of degradation for insoluble proteins of casein, Soya, GC, MC and SF was 42.0, 31.0, 29.5, 26.2 and 24.2%, respectively, and corresponding values for 12 h incubation period were 69.5, 57.2, 54.7, 44.0 and 48.2%.

At 6 h incubation period, the difference in degradation of insoluble proteins between casein and Soya, GC, MC and SF was significant at $p < 0.01$, between Soya and GC, MC and SF at $p < 0.05$, between GC and MC and SF at $p < 0.05$, and between MC and SF at $p < 0.05$ and between casein and Soya at $p < 0.01$. At 12 h of incubation, extent of degradation for casein and soya was significantly ($p < 0.01$) higher than that of GC, MC and SF, while that of GC was significantly higher ($p < 0.01$) than that of MC and SF. The extent of degradation of casein insoluble proteins at 12 h incubation was also significantly ($p < 0.01$) higher than that of Soya.

The above data indicated that insoluble fraction of casein intact proteins was maximally degraded by SRF followed, in order, by Soya, GC, MC and SF.

4.1.3.2 Washed mixed bacterial suspension

The data presented in Table 12 (Fig.2) show the extent of degradation of insoluble fraction of intact seed proteins

Table 12. Degradation of insoluble fraction of intact feed proteins when incubated for different periods with washed mixed bacterial suspension

Incubation period (h)	TCA-precipitable proteins left after incubation (mg)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	40.0	40.0	40.0	40.0	40.0
2	38.7±0.41 (3.2)	38.7±0.43 (3.2)	38.9±0.82 (2.7)	37.9±0.23 (5.2)	37.8±0.81 (5.5)
4	38.1^a±0.38 (4.7)	37.2^a±0.81 (7.0)	37.8^a±0.16 (5.5)	34.7±0.51 (13.2)	34.9±0.31 (12.7)
6	37.1^a±0.92 (7.2)	35.8^a±0.26 (10.5)	36.3^{a,d}±0.24 (9.2)	32.6±0.46 (18.5)	32.3±0.72 (19.2)
8	34.8^a±0.23 (13.0)	33.4^a±0.41 (16.5)	34.9^{a,d}±0.56 (12.7)	30.2±0.32 (24.5)	29.7±0.86 (25.7)
12	32.2^a±0.61 (19.5)	31.0^a±0.77 (22.5)	33.1^{a,d}±0.29 (17.2)	27.9±0.76 (30.2)	27.2±0.39 (32.0)

Figures represent mean of four observations ± standard deviation
Figures in parenthesis represent per cent degradation over control
Conditions of incubation : Same as in table 11 except 10 ml washed mixed bacterial suspension were added in place of strained rumen fluid.

when incubated for different periods with washed mixed bacterial suspension. Extent of degradation was found to increase with increase in incubation time upto 12 h. At 6 h incubation period, per cent degradation of casein , Soya, GC, MC and SF was 19.2, 18.5, 7.2, 10.5 and 9.2%, respectively, and corresponding values for 12 h incubation period were 32.0, 30.2, 19.5, 22.5 and 17.2%.

At 6 and 12 h incubation periods, extent of degradation of casein and Soya insoluble proteins was significantly ($p < 0.01$) higher than that of GC, MC and SF, while that of MC was significantly higher ($p < 0.01$) than that of SF and that of SF was significantly ($p < 0.05$) higher than that of GC. At 12 h incubation period, extent of degradation of MC was significantly ($p < 0.01$) higher than that of SF.

Though casein insoluble proteins were maximally degraded followed by Soya, MC, SF and GC in that order at 6 hr of incubation but at 12 h of incubation casein and Soya degradation was followed by that of MC, GC and SF.

4.1.3.3 Washed protozoal suspension

Data on extent of degradation of insoluble fraction of intact proteins when incubated for different periods with washed protozoal suspension are given in table 13 (Fig.3). Per cent degradation was shown to increase with increase in incubation time upto 12 h. At 6 h incubation period, the extent of degradation for casein, Soya, SC, MC and SF was 15.7, 15.0, 9.7, 6.5 and 5.2% respectively and corresponding values for 12

Table 13. Degradation of insoluble fraction of intact feed proteins when incubated for different periods with washed protozoal suspension

Incubation period (h)	TCA-precipitable proteins left after incubation (mg)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	40.0	40.0	40.0	40.0	40.0
2	$39.1^a \pm 0.71$ (2.2)	$39.3^a \pm 0.72$ (1.7)	$39.3^a \pm 0.44$ (1.7)	37.4 ± 0.38 (6.5)	37.2 ± 0.51 (7.0)
4	$37.8^a \pm 0.66$ (5.5)	$38.1^a \pm 0.81$ (4.7)	$38.6^a \pm 0.56$ (3.5)	35.5 ± 0.51 (11.2)	35.0 ± 0.46 (12.5)
6	$36.1^a \pm 0.42$ (9.7)	$37.4^{a,c} \pm 0.39$ (6.5)	$37.9^{a,c} \pm 0.89$ (5.2)	34.0 ± 0.56 (15.0)	33.7 ± 0.38 (15.7)
8	$35.1^a \pm 0.51$ (12.2)	$35.6^a \pm 0.36$ (11.0)	$36.2^a \pm 0.91$ (9.5)	$32.8^b \pm 0.61$ (18.0)	31.1 ± 0.44 (22.2)
12	$34.3^a \pm 0.58$ (14.2)	$34.2^a \pm 0.42$ (14.5)	$35.0^a \pm 0.77$ (12.5)	30.2 ± 0.76 (24.5)	29.1 ± 0.85 (27.2)

Figures represent mean of four observations ± standard deviation
Figures in parenthesis represent per cent degradation over control
Conditions of incubation : Same as in table 11 except 10 ml washed protozoal suspension were added instead of strained rumen fluid.

h incubation were 27.2, 24.5, 14.2, 14.5 and 12.5%.

At 6 and 12 h after incubation the extent of degradation of casein and soya insoluble proteins was significantly (p < 0.01) higher than that of GC, MC and SF. Further, at 6 h of incubation per cent degradation of GC was significantly higher (p < 0.01) than that of MC and SF. Casein insoluble protein fraction was degraded to maximum followed, in order, by soya, MC, GC and SF. At 6 h of incubation,. per cent degradation of GC was higher than that of MC, whereas at 12 h incubation it was almost equal in extent.

4.1.3.4 Cell-free supernatant

The results on the per cent degradation of insoluble fraction of intact feed proteins when incubated with cell-free supernatant for different periods are given in Table 14 (Fig.4). As with SRF and its bacterial and protozoal fractions, the extent of degradation was found to increase with increase in incubation time. AT 6 h of incubation,. insoluble fraction of casein Soya, GC, MC and SF intact proteins was degraded by 24.5, 23.0, 19.5, 18.2 and 15.5% respectively, and corresponding values for 12 h of incubation were 40.7, 37.2, 32.0, 28.5 and 27.0%.

At 6 and 12 h incubation period, the extent of degradation of casein and Soya was significantly (p < 0.01) higher than that of GC, MC and SF. However, at 6 h of incubation, per cent degradation of MC was significantly (p·< 0.05) higher than that of SF. Casein insoluble proteins were

Table 14. Degradation of insoluble fraction of intact feed proteins when incubated for different periods with cell-free supernatant

Incubation period (h)	TCA-precipitable proteins left after incubation (mg)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	40.0	40.0	40.0	40.0	40.0
2	$38.5^{a}\pm0.23$ (3.7)	$38.7^{a}0.17$ (3.2)	$39.2^{a,h}\pm0.22$ (2.0)	$37.5\pm0.0.38$ (6.2)	36.8 ± 0.65 (8.0)
4	$35.3^{a}\pm0.48$ (11.7)	$35.9^{a}\pm0.24$ (10.2)	$37.0^{a,h}\pm0.48$ (7.5)	34.2 ± 0.42 (14.5)	32.3 ± 0.57 (19.2)
6	$32.2^{a}\pm0.56$ (19.5)	$32.7^{a}\pm0.31$ (18.2)	$33.8^{a,h}\pm0.86$ (15.5)	30.8 ± 0.81 (23.0)	30.2 ± 0.52 (24.5)
8	$30.8^{a}\pm0.62$ (23.0)	$31.3^{a}\pm0.72$ (21.7)	$32.6^{a,h}\pm0.49$ (18.5)	$28.4^{f}\pm0.93$ (29.0)	26.5 ± 0.79 (33.7)
12	$27.2^{a}\pm0.71$ (32.0)	$28.6^{a}\pm0.94$ (28.5)	$29.2^{a}\pm0.75$ (27.0)	$25.1^{f}\pm0.81$ (37.2)	23.9 ± 0.98 (40.7)

Figures represent mean of four observations ± standard deviation
Figures in parenthesis represent per cent degradation over control
Conditions of incubation : Same as in table 11 except 10 ml of cell-free supernatant were added instead of strained rumen fluid.

degraded to maximum extent followed, in order, by GC, MC and SF.

The results of this experiment indicated that intact proteins and its fractions viz. soluble and insoluble of casein were maximally degraded by SRF and its fractions followed, in order, by Soya, GC, and MC/SF. SRF degraded these proteins to maximum extent followed, in order, by cell-free supernatant, washed mixed bacterial suspension and washed protozoal suspension. Amongst the intact proteins and its fractions, the soluble fractions were degraded to maximum extent while insoluble fractions degraded the least. Insoluble fractions of all the intact proteins were degraded to maximum extent by washed protozoal suspension.

4.2 In vitro degradation of individual amino acids when incubated with strained rumen fluid and washed mixed bacterial suspension for 8 h

Different amino acids were degraded at different rates by SRF (Tables 15, 16). Serine was maximally degraded while proline was degraded to minimum extent. The amino acids are grouped according to degradation rates. Serine, cysteine and aspartate were degraded maximally followed by arginine, lysine, tyrosine and cystine group; tryptophane, alanine, methionine and histidine group; isoleucine and valine group; glycine, hydroxyproline and proline group. The pattern of rates of degradation of different amino acids was same with washed bacterial cell suspension, but the extent of degradation was

Table 15. Degradation of individual amino acids when incubated with strained rumen fluid for 8 h

Amino acid	Amount added (mg)	Amount undegraded (mg)	Degradation (%)	Ammonia (mg/100 ml RF)
Serine	10.5	0.8±0.91	92.4	29.6±0.63
Cysteine	12.1	1.5±0.38	87.6	20.0±0.31
Aspartate	13.3	1.7±0.16	87.2	28.7±0.46
Arginine	17.4	5.3±0.23	69.5	24.0±0.56
Phenylalaine	16.5	5.1±0.18	69.1	22.7±0.26
Threonine	11.9	3.7±0.21	68.9	25.1±0.18
Glutamate	14.7	5.8±0.36	60.5	19.3±0.52
Glutamine	14.7	6.6±0.32	54.8	18.4±0.38
Lysine	14.6	7.1±0.41	51.4	17.2±0.41
Tyrosine	18.1	9.1±0.21	49.7	15.7±0.43
Cystine	24.0	13.5±0.24	43.8	14.2±0.22
Tryptophan	20.4	13.2±0.46	35.3	11.2±0.21
Alanine	8.9	5.8±0.36	34.8	8.5±0.12
Methionine	14.9	9.8±0.14	34.2	9.7±0.24
Histidine	20.4	14.1±0.29	30.8	9.9±0.22
Isoleucine	11.5	9.4±0.56	18.3	6.7±0.52
Valine	11.7	9.6±0.31	17.9	6.3±0.51
Glycine	7.5	6.5±0.06	13.3	3.0±0.12
Hydroxyproline	13.1	11.4±0.31	13.0	2.7±0.91
Proline	11.5	10.5±0.02	8.7	2.4±0.38

Figures represent mean of 4 observations ± SD

Conditions of incubation: 10m moles of each amino acid suspended in 10 ml McDougall buffer (saturated with CO_2) and 10 ml SRF were incubated at 39°C for 8 h.

Table 16. Degradation of individual amino acids when incubated with washed/mixed bacterial suspension for 8 h

Amino acid	Amount added (mg)	Amount undegraded (mg)	Degradation (%)	Ammonia (mg/100 ml RF)
Serine	10.5	2.34±0.34	77.7	11.6±0.47
Cysteine	12.1	3.2±0.24	73.5	10.5±0.72
Aspartate	13.3	3.82±0.09	71.4	10.3±0.31
Threonine	11.9	4.0±0.31	66.4	8.3±0.56
Glutamate	14.7	6.6±0.48	55.1	6.9±0.25
Arginine	17.4	8.1±0.38	53.4	6.9±0.46
Glutamine	14.6	7.4±0.52	49.3	9.2±0.42
Lysine	14.6	9.5±0.77	34.9	4.5±0.98
Tyrosine	18.1	15.2±0.23	16.0	2.0±0.41
Cystine	24.0	16.2±0.72	32.5	4.1±0.21
Phenylalaine	16.5	13.5±0.34	18.2	2.6±0.51
Tryptophan	20.4	17.5±0.51	14.2	1.8±0.63
Methionine	14.9	12.5±0.41	16.1	2.2±0.63
Alanine	8.9	7.8±0.46	12.4	1.8±0.81
Histidine	20.4	17.8±0.64	12.7	1.7±0.25
Valine	11.7	10.7±0.42	8.5	1.3±0.21
Isoleucine	11.5	10.5±0.21	8.0	1.2±0.38
Glycine	7.5	7.45±0.46	2.0	0.24±0.59
Hydroxyproline	13.1	12.8±0.28	1.98	0.24±0.78
Proline	11.5	11.3±0.16	1.04	0.14±0.38

Figures represent mean of 4 observations ± SD
Conditions of incubation: Same as described in Table 27 except
10 ml of washed mixed bacterial suspension we re~~A~~in place of added
strained rumen fluid.

lower than that with SRF.

4.3 Ammonia production with intact feed proteins and their fractions were incubated with strained rumen fluid and its fractions

Intact proteins and their soluble and insoluble fractions were incubated for different periods with SRF and its fractions and ammonia produced was measured as an index of deamination activity.

4.3.1 In vitro production of ammonia from intact feed proteins by rumen fluid and its fractions

4.3.1.1 Strained rumen fluid

The data on the production of ammonia when intact feed proteins were incubated for different periods are presented in table 17 (Fig.5). Ammonia production was found to increase to a maximum level up to 4 h and then gradually decreased with further increase in the incubation period up to 12 h. At 4 h of incubation, ammonia produced from intact proteins of casein, Soya, GC, MC and SF was 39.6, 30.1, 28.3, 26.9 and 27.2 mg/100 ml RF, respectively and the corresponding values for 12 h incubation period were 24.3, 18.8, 14.1, 13.8 and 14.5 mg/100 ml RF.

At 4 h of incubation the extent of ammonia produced from casein and soya was significantly higher ($p < 0.01$) than that of GC, MC and SF and that from GC was significantly ($p < 0.05$) higher than that of MC and SF. The degradation of casein intact proteins was significantly ($p < 0.01$) higher than that of soya.

Table 17. Ammonia production from intact feed proteins when incubated for different periods with strained rumen fluid

Incubation period (h)	Ammonia production (mg/100 ml RF)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	6.4 ± 0.26	6.4 ± 0.26	6.4 ± 0.26	6.4 ± 0.26	6.4 ± 0.26
2	$17.3^a\pm0.71$	$19.8^{a,c}0.31$	$18.1^{a,c,d}0.46$	22.8 ± 0.24	24.0 ± 0.91
4	$28.3^a\pm0.84$	$26.9^{a,g}\pm0.44$	$27.2^{a,g}\pm0.38$	$30.1^b0.77$	39.6 ± 0.18
6	$24.9^a\pm0.53$	$25.7^a\pm0.86$	$25.8^a\pm0.26$	29.7 ± 0.63	$30.4\pm0.0.54$
8	$18.7^a\pm0.91$	$15.1^{a,c}\pm0.26$	$19.9^{a,c,d}\pm0.41$	$24.3^b\pm0.82$	28.3 ± 0.44
12	$14.1^a\pm0.38$	$13.8^a\pm0.93$	$14.5^a\pm0.23$	$18.8^b\pm0.49$	24.3 ± 0.81

Figures represent mean of four observations ± standard deviation

Conditions of incubation : Same as in table 3.

FIG.5 AMMONIA PRODUCED FROM INTACT PROTEINS & THEIR SOLUBLE & INSOLUBLE FRACTIONS WITH STRAINED RUMEN FLUID FOR 4 h.
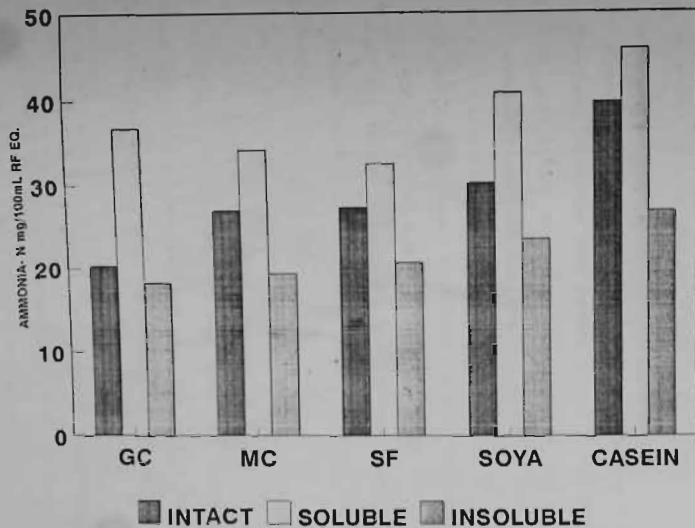
The above data indicated that at all the incubation periods, the ammonia production was maximum for casein followed, in order, by Soya, GC, SF/MC intact proteins.

4.3.1.2 Washed mixed bacterial suspension

The data on the extent of ammonia produced when intact feed proteins when incubated with washed bacterial suspension for different periods are presented in Table 18 (Fig.6). As with SRF, ammonia production was maximum at 4 h and then decreased with increasing incubation time upto 12 h. At 4 h of incubation, ammonia produced from the intact proteins of casein, Soya, GC, SF and MC was 19.9, 18.7 15.9, 16.3 and 15.8 mg/100 mg RF equivalent respectively and corresponding values for 12 h incubation were 11.2, 10.3. 9.6, 11.1 and 12.5 mg/100 ml RF equivalent.

At 4 h incubation period ammonia produced from casein and soya intact proteins was significantly ($p < 0.01$) higher than that of GC, SF and MC, whereas that of casein was significantly ($p < 0.05$) higher than that of Soya. However, the differences in ammonia produced between GC, MC and SF was not significant.

Data showed that there was maximum production of ammonia from intact proteins of casein followed, in order, by Soya, SF, MC and GC upto 6 h of incubation period.

4.3.1.3 Washed protozoal suspension

Data on the ammonia production when intact proteins of

Table 18. Ammonia production from intact feed proteins when incubated for different periods with washed bacterial suspension

Incubation period (h)	Ammonia production (mg/100 ml RF equivalent)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	8.9 ± 0.41	$8.05 \pm .41$	$8.05 \pm .41$	$8.05 \pm .41$	$8.05 \pm .41$
2	10.3 ± 0.39	$10.5^{a,c} 0.31$	$11.4^{a,c,d} 0.81$	12.4 ± 0.24	13.2 ± 0.91
4	$15.9^{a} \pm 0.82$	$15.8^{a} \pm 0.77$	$16.3^{a} 0.52$	$18.7^{f} 0.23$	$19.9 \pm 0.0.81$
6	$12.9^{e} \pm 0.44$	$14.3^{e,g} \pm 0.84$	$15.8^{e,g} \pm 0.59$	16.9 ± 0.49	17.7 ± 0.34
8	11.5 ± 0.72	$13.8^{c} \pm 0.32$	$15.0^{c} \pm 0.73$	15.9 ± 0.86	15.6 ± 0.16
12	9.6 ± 0.35	$12.5^{c} \pm 0.41$	$11.1^{c,h} \pm 0.54$	$10.3^{f} \pm 0.39$	11.2 ± 0.44

Figures represent mean of four observations ± standard deviation

Conditions of incubation : Same as in table 4.

Table 19. Ammonia production from intact feed proteins when incubated for different periods with washed protozoal suspension

Incubation period (h)	Ammonia produced (mg/100 ml RF equivalent)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	3.4 ± 0.18	3.4 ± 0.18	3.4 ± 0.18	3.4 ± 0.18	3.4 ± 0.18
2	$5.5^a\pm0.12$	$5.1^{a,g}\pm0.24$	$5.3^{a,g}\pm0.23$	$8.9^b\pm0.41$	9.1 ± 0.81
4	$8.6^a\pm0.61$	$7.9^a\pm0.61$	$8.2^a\pm0.29$	12.6 ± 0.87	13.8 ± 0.52
6	$12.7^a\pm0.42$	$10.9^{a,c}\pm0.66$	$10.9^{a,c}\pm0.21$	16.6 ± 0.98	17.0 ± 0.56
8	$19.2^a\pm0.33$	$17.8^{a,g}\pm0.72$	$16.6^{a,g,h}\pm0.18$	$20.3^b\pm0.42$	22.4 ± 0.56
12	$17.5^a\pm0.91$	$15.8^a\pm0.88$	$15.2^a\pm0.44$	$18.5^b\pm0.38$	21.7 ± 0.24

Figures represent mean of four observations ± standard deviation

Conditions of incubation : Same as in table 5.

FIG.6 AMMONIA PRODUCED FROM INTACT PROTEINS & THEIR SOLUBLE & INSOLUBLE FRACTIONS WITH WASHED BACTERIAL SUSPENSION FOR 4 h.
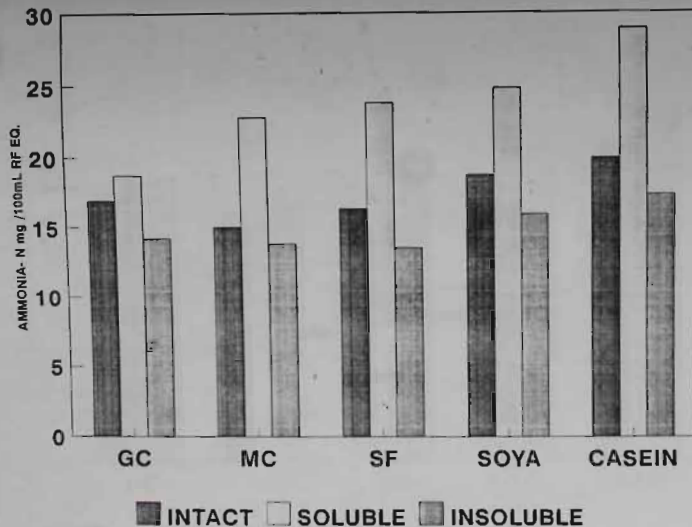
FIG.1 AMMONIA PRODUCED FROM INTACT PROTEINS & THEIR SOLUBLE & INSOLUBLE FRACTIONS WHEN INCUBATED WITH WASHED PROTOZOAL SUSPENSION FOR 8 h.
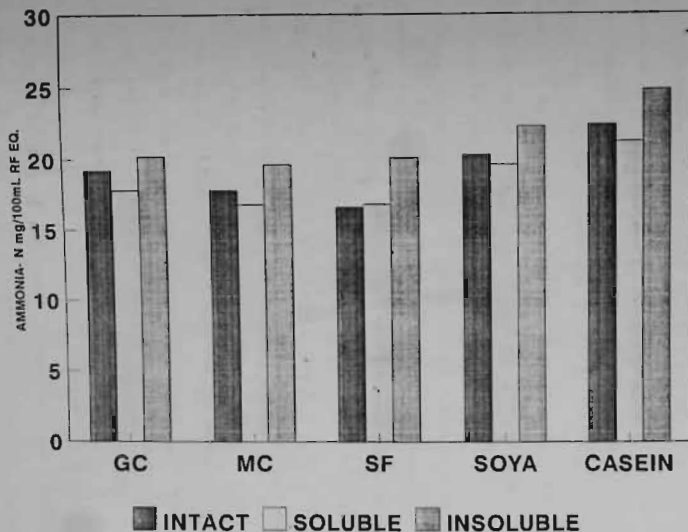
various feeds were incubated for different periods with washed protozoal suspension are given in Table 19 (Fig.7). It was observed that in contrast to SRF and washed bacterial suspension, ammonia produced from all the intact feed proteins was maximum at 8 h and then decreased upto 12 h of incubation. At 8 h incubation period, ammonia produced from intact proteins of casein, Soya, GC, MC and SF was 22.4, 20.3, 19.2, 17.8 and 16.6 mg/100 ml RF equivalent, respectively and the corresponding values for 12 h incubation period were 21.7, 18.5, 17.5, 15.8 and 15.2 mg/100 ml RF equivalent.

At 8 h of incubation, the extent of ammonia produced from casein and soya intact proteins was significantly ($p <$ 0.01) higher than of GC, MC and SF. The ammonia produced from GC was significantly ($p < 0.05$) higher than that of MC and that from MC was significantly ($p < 0.05$) higher than that of SF. The ammonia produced from casein intact proteins was significantly ($p < 0.01$) higher than that of intact Soya proteins.

The data indicated that there was maximum production of ammonia from casein intact proteins followed, in order, by soya, GC, MC and SF at all incubation periods.

4.3.1.4 Cell-free supernatant

The data on the extent of ammonia produced when various intact feed proteins were incubated for different periods with cell free supernatant are given in table 20 (Fig.8). The ammonia production was maximum at 4 h of incubation and then decreased thereafter upto 12 h of

Table 20. Ammonia production from intact feed proteins when incubated for different periods with cell-free supernatant

Incubation period (h)	Ammonia production (mg/100 ml RF equivalent)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	7.2 ± 0.24	7.2 ± 0.24	7.2 ± 0.24	7.2 ± 0.24	7.2 ± 0.24
2	$13.6^a \pm 0.18$	$13.9^a 0.23$	$13.7^a 0.26$	$20.2^b \pm 0.18$	21.4 ± 0.92
4	$22.4^a \pm 0.56$	$22.2^a \pm 0.31$	$22.3^a \pm 0.31$	$27.5^b 0.15$	31.5 ± 0.38
6	$20.8^a \pm 0.24$	$19.5^{a,c} \pm 0.44$	$20.6^{a,c,h} \pm 0.32$	$24.3^b \pm 0.24$	27.1 ± 0.41
8	$16.5^a \pm 0.41$	$15.2^{a,g} \pm 0.46$	$16.7^{a,g,h} \pm 0.61$	$21.2^b \pm 0.38$	25.2 ± 0.22
12	$11.2^a \pm 0.65$	$10.8^a \pm 0.56$	$11.5^a \pm 0.75$	$14.5^b \pm 0.91$	19.8 ± 0.18

Figures represent mean of four observations ± standard deviation

Conditions of incubation : Same as in table 6.

FIG.8 AMMONIA PRODUCED FROM INTACT PROTEINS & THEIR SOLUBLE & INSOLUBLE FRACTIONS WHEN INCUBATED WITH CELL FREE SUPERNATANT FOR 4 h.
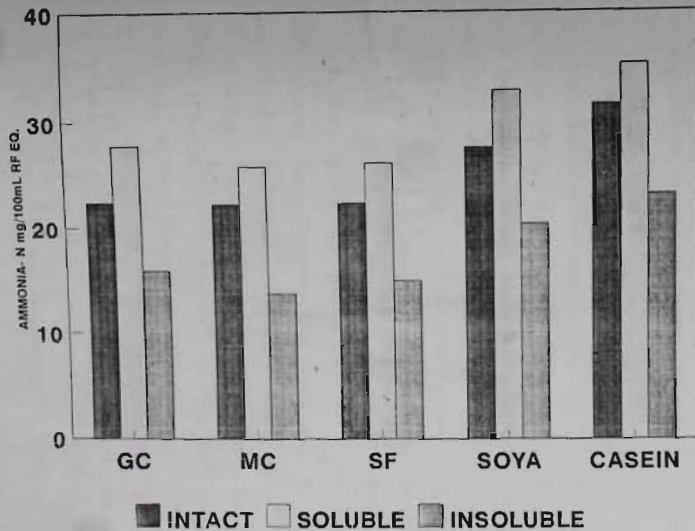
incubation. At 4 h incubation period, ammonia produced from intact proteins of casein, Soya, GC, MC and SF was 31.5, 27.5, 22.4, 22.2 and 22.3 mg/100 ml RF equivalent respectively and corresponding values for 12 h incubation period were 19.8, 14.5, 11.2, 10.8 and 11.5 mg/100 ml RF equivalent.

At 4 h incubation period, ammonia produced from casein and Soya intact proteins was significantly ($p < 0.01$) higher than that of GC, MC and SF. The differences in the production of ammonia between casein and soya intact proteins was also significant ($p < 0.05$) at 4 and 12 h of incubation.

Data indicated that maximum ammonia was produced from casein intact proteins followed, in order, by Soya, GC, MC and SF. Ammonia produced from GC, MC and SF was almost equal.

The data given in Tables 17, 18, 19 and 20 (Fig.5,6,7,8) also suggested that with SRF and its fraction there was maximum production of ammonia from casein intact proteins followed by Soya, GC, MC/SF. At all the incubation periods, SRF produced more ammonia followed by cell free supernatant and bacterial/protozoal suspension with all the intact proteins. At initial incubation periods upto 4 h bacterial suspension was more active than protozoal suspension while at prolonged incubations i.e. at 6 and 8 h protozoal suspension was more active than the bacterial suspension for the production of ammonia.

4.3.2 **In vitro** production of ammonia from soluble fraction of intact feed proteins by strained rumen fluid and its fractions

4.3.2.1 Strained rumen fluid

Table 21 (Fig.5) represents the data on extent of ammonia produced when soluble fraction of various intact feed proteins when incubated with SRF for different periods. As in case of intact proteins, ammonia production reached its maximum value at 4 h of incubation and thereafter gradually decreased upto 12 h of incubation. At 4 h incubation period, the ammonia produced from soluble fraction of intact proteins of casein, Soya, GC, MC and SF was 45.8, 40.8, 36.8, 34.2 and 32.5 mg/100 ml RF respectively, and corresponding values for 12 h incubation period were 27.4, 27.4, 18.6, 18.6 and 23.2 mg/100 ml RF.

At 4 h incubation period, ammonia produced from casein and soya soluble proteins was significantly ($p < 0.01$) higher than that of GC, MC and SF, while that of casein was significantly ($p < 0.01$) higher than that of Soya. At this incubation period ammonia produced from GC soluble proteins was significantly higher ($p < 0.01$) than that of MC.

The above data showed that maximum amount of ammonia was produced for casein soluble proteins followed, in order, by Soya, GC, MC and SF upto 8 h of incubation.

4.3.2.2 Washed mixed bacterial suspension

The data on the extent of ammonia production when soluble fraction of intact feed proteins were incubated for

Table 21. Ammonia production from soluble fractions of intact feed proteins when incubated for different periods with strained rumen fluid

| Incubation period (h) | Ammonia produced (mg/100 ml RF) | | | | |
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	7.4±0.39	7.4±0.39	7.4±0.39	7.4±0.39	7.4±0.39
2	28.9^e±0.43	28.6^{e}0.81	29.3^{e}0.44	30.4^b±0.43	34.1±0.61
4	36.8^a±0.31	34.2^{a,c}±0.72	32.5^{a,c}±0.86	40.8^b±0.91	45.8±0.38
6	30.0^a±0.91	29.2^a±0.94	28.3^a±0.92	33.5^b±0.68	40.5±0.49
8	29.2^a±0.66	24.1^{a,c}±0.23	24.6^{a,c,h}±0.24	30.2^b±0.62	33.7±0.28
12	18.6^a±0.42	18.6^a±0.14	23.2^{a,d}±0.35	27.4^b±0.74	27.4±0.17

Figures represent mean of four observations ± standard deviation

Conditions of incubation : Same as in table 7.

different periods with washed bacterial suspension are given in table 22(Fig.6). As usual, it was observed that ammonia production reached a peak value at 4 h and then gradually decreased upto 12 h of incubation. At 4 h incubation period, ammonia produced from soluble proteins of casein, Soya, GC, MC and SF was 28.9, 24.8, 18.7, 22.8 and 23.8 mg/100 ml RF equivalent, respectively, and corresponding values for 12 h incubation period were 12.8, 12.6, 10.3, 9.5 and 10.2 mg/100 ml RF equivalent.

At 4 h of incubation, ammonia produced from soluble fraction of casein intact protein was significantly ($p < 0.01$) higher than that from Soya, GC, MC and SF, while that from MC was significantly ($p < 0.01$) higher than that of GC and casein ($p < 0.01$) than that from soya. At 12 h incubation period, ammonia production from casein and soya soluble proteins was significantly ($p < 0.01$) higher than that of GC, MC and SF and there was no difference between casein and soya and between GC, MC and SF.

Data showed that maximum ammonia was produced from casein soluble proteins followed, in order, by Soya, SF, MC and GC upto 8hr of incubation.

4.3.2.3 Washed protozoal suspension

The data on the ammonia production from soluble fraction of various intact proteins when incubated for different periods with washed protozoal suspension are given in table 23 (Fig.7). As in case of intact proteins, the ammonia

Table 22. Ammonia production from soluble fractions of intact feed proteins when incubated for different periods with washed bacterial suspension

Incubation period (h)	Ammonia produced (mg/100 ml RF equivalent)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	7.2 ± 0.21	7.2 ± 0.21	7.2 ± 0.21	7.2 ± 0.21	7.2 ± 0.21
2	$14.4^e \pm 0.62$	$16.2^{e,c} \pm 0.52$	$18.4^{e,c} \pm 0.83$	$20.2^b \pm 0.92$	24.2 ± 0.31
4	18.7 ± 0.51	$22.8^c \pm 0.71$	$23.8^c \pm 0.76$	$24.8^b \pm 0.63$	28.9 ± 0.18
6	12.9 ± 0.56	$14.0^g \pm 0.22$	$17.0^g \pm 0.51$	$18.2^b \pm 0.56$	24.3 ± 0.72
8	$11.7^a \pm 0.92$	$11.5^a \pm 0.38$	$14.1^{a,d} \pm 0.16$	$16.7^b \pm 0.15$	20.8 ± 0.49
12	$10.3^a \pm 0.76$	$9.48^a \pm 0.41$	$10.2^a \pm 0.48$	$12.6^b \pm 0.83$	12.8 ± 0.61

Figures represent mean of four observations ± standard deviation

Conditions of incubation : Same as in table 8.

Table 23. Ammonia production from soluble fractions of intact feed proteins when incubated for different periods with washed protozoal suspension

Incubation period (h)	Ammonia produced (mg/100 ml RF equivalent)				
	Groundnut cake	Mustard cake'	Sunflower seed meal	Soyabean seed meal	Casein
0	4.2±0.58	4.2±0.58	4.2±0.58	4.2±0.58	4.2±0.58
2	7.2±0.31	7.1±0.42	6.8±0.81	7.6±0.38	8.1±0.44
4	10.3±0.44	10.1±0.39	10.0±0.33	10.9±0.91	12.4±0.32
6	15.2^a±0.46	14.6^a±0.81	14.4^a±0.42	16.9^f±0.42	18.4±0.98
8	17.8^a±0.62	16.8^a±0.72	16.8^a±0.36	19.6^b±0.31	21.2±0.81
12	16.4^e±0.69	16.1^a±0.77	15.8^e±0.55	17.7^f±0.52	19.6±0.77

Figures represent mean of four observations ± standard deviation

Conditions of incubation : Same as in table 9.

production from soluble fraction of intact proteins reached a peak at 8 h and then decreased thereafter upto 12 h of incubation. At 8 h incubation period, ammonia produced from soluble proteins of casein, Soya, GC, MC and SF was 21.2, 19.6, 17.8, 16.8 and 16.8 mg/100 ml RF equivalent, respectively, and corresponding values at 12 h incubation period were 19.6, 17.7, 16.4, 16.1 and 15.8 mg/100 ml RF equivalent.

At 8 h of incubation, ammonia produced from casein and soya soluble proteins was significantly ($p < 0.01$) higher than that of GC, MC and SF, whereas that of casein significantly higher ($p < 0.01$) than that of Soya. There was almost no difference between GC, MC and SF.

The data indicated that, as usual, maximum ammonia was produced from soluble proteins of casein followed, in order, by Soya, GC, MC and SF.

4.3.2.4 Cell free supernatant

Table 24 (Fig.8) presents data on ammonia production when soluble fraction of various feed proteins was incubated for different periods with cell-free supernatant. Ammonia production reached a peak at 4 h and then gradually decreased thereafter upto 12 h of incubation. AT 4 h of incubation, ammonia production from soluble proteins of casein, Soya, GC, MC and SF was 35.2, 32.8, 27.8, 25.8 and 26.1 mg/100 ml RF equivalent, respectively and corresponding values for 12 h incubation were 23.8, 19.1, 14.5, 13.6 and 14.3 mg/100 ml RF equivalent.

Table 24. Ammonia production from soluble fractions of intact feed proteins when incubated for different periods with cell free supernatant

Incubation period (h)	Ammonia produced (mg/100 ml RF equivalent)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	9.8 ± 0.19	9.8 ± 0.19	9.8 ± 0.19	9.8 ± 0.19	9.8 ± 0.19
2	$16.3^a \pm 0.18$	$15.7^{a,g} \pm 0.24$	$15.8^{a,g} \pm 0.38$	$25.9^b \pm 0.21$	$29.30.98$
4	$27.8^a \pm 0.38$	$25.8^a \pm 0.81$	$26.1^a \pm 0.36$	$32.8^b \pm 0.77$	35.2 ± 0.24
6	$22.3^a \pm 0.36$	$21.3^a \pm 0.81$	$21.7^a \pm 0.41$	$27.3^b \pm 0.32$	31.4 ± 0.31
8	$20.1^a \pm 0.24$	$18.7^{a,g} \pm 0.72$	$19.1^{a,g} \pm 0.16$	$24.7^b \pm 0.85$	27.3 ± 0.36
12	$14.5^a \pm 0.46$	$13.6^a \pm 0.81$	$14.3^a \pm 0.32$	$19.1^b \pm 0.41$	23.8 ± 0.44

Figures represent mean of four observations ± standard deviation

Conditions of incubation : Same as in table 10.

At 4 h and 12 h of incubation, ammonia production from casein and soya soluble proteins were significantly (p < 0.01) higher than that of GC, MC and SF and ammonia production from casein soluble proteins was significantly (p < 0.01) higher than that of Soya.

The data given in Tables 21, 22, 23 and 24 (Fig. 5,6,7 and 8) showed that SRF, cell-free supernatant and protozoal suspension produced maximum ammonia from soluble proteins of casein followed, in order, by Soya, GC and SF/MC. The bacterial suspension produced maximum ammonia from casein followed, in order, by Soya, SF, MC and GC at 4 h incubation period and the order for ammonia production at 12 h incubation period was casein < soya < SF/MC/GC. It is also observed that the production of ammonia from the soluble fraction of all the intact protein used was maximum by SRF followed, in order, by cell-free supernatant and bacterial/protozoal suspension. Bacterial suspension produced more ammonia than protozoal suspension upto incubation period of 6 h while protozoal produced more ammonia than bacterial suspension at 8 and 12 h of incubation.

4.3.3 In vitro production of ammonia from insoluble fraction of intact feed proteins by strained rumen fluid and its fractions

4.2.3.1 Strained rumen fluid

Data on ammonia production from insoluble fraction of intact feed proteins when incubated for different periods with

Table 25. Ammonia production from insoluble fractions of intact feed proteins when incubated for different periods with strained rumen fluid

Incubation period (h)	Ammonia produced (gm/100 ml RF)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	6.5±0.23	6.5±0.23	6.5±0.23	6.5±0.23	6.5±0.23
2	12.8[e]±0.46	12.1[e]±0.77	14.7[e]±0.18	16.3[b]±0.79	18.9±0.21
4	18.3[a]±0.81	19.3[a]±0.84	20.6[a]±0.39	23.4[b]±0.35	26.7±0.48
6	14.7[a]±0.97	13.7[a]±0.39	13.8[a]±0.46	20.0[b]±0.28	24.2±0.79
8	10.4[a]±0.48	10.6[a]±0.44	11.5[a,h]±0.31	13.7[b]±0.41	20.5±0.84
12	9.8±0.61	9.3±0.42	9.6±0.72	10.3[b]±0.18	16.7±0.22

Figures represent mean of four observations ± standard deviation

Conditions of incubation : Same as in table 11.

SRF are presented in table 25 (Fig.5). It was observed that ammonia production reached to maximum values at 4 h and then decreased gradually with increasing incubation time upto 12 h. At 4 h of incubation, extent of ammonia produced from insoluble fraction of intact proteins of casein, soya, GC, MC and SF was 26.7, 23.4, 18.3, 19.3 and 20.6 mg/100 ml RF, respectively, and corresponding values for 12 h incubation period were 16.7, 10.3, 9.8, 9.3 and 9.6 mg/100 ml RF.

At 4 h of incubation, ammonia production from casein and soya insoluble proteins were significantly ($p < 0.01$) higher than that of GC, MC and SF, while in case of casein it was ($p < 0.01$) higher than that of Soya.

Data indicated that highest amount of ammonia was produced from casein soluble proteins followed, in order, by Soya, SF, MC and GC at all the incubation times.

4.3.3.2 Washed mixed bacterial suspension

Table 26 (Fig.6) represents data on ammonia production when insoluble fraction of intact feed proteins was incubated for different periods with washed bacterial suspension. As with SRF, the ammonia production reached to maximum value at 4 h and decreased thereafter upto 12 h of incubation. At 4 h incubation period, ammonia produced from insoluble proteins of casein, soya, GC, MC and SF was 17.3, 15.9, 14.2, 13.8 and 13.5 mg/100 ml RF equivalent, respectively, and corresponding values for 12 h incubation were 10.2, 10.9, 9.9, 10.2 and 9.4 mg/100 ml RF equivalent.

Table 26. Ammonia production from insoluble fractions of intact feed proteins when incubated for different periods with washed bacterial suspension

| Incubation period (h) | Ammonia produced (mg/100 ml RF equivalent) | | | | |
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	6.4 ± 0.32	6.4 ± 0.32	6.4 ± 0.32	6.4 ± 0.32	6.4 ± 0.32
2	9.5 ± 0.59	9.7 ± 0.43	9.4 ± 0.54	$10.2^b\pm0.38$	12.9 ± 0.66
4	$14.2^a\pm0.62$	$13.8^a\pm0.87$	$13.5^a\pm0.72$	$15.9^f\pm0.54$	17.3 ± 0.42
6	$14.0^e\pm0.68$	$12.7^e\pm0.91$	$12.3^e\pm0.18$	$13.6^b\pm0.67$	15.5 ± 0.31
8	$12.4^e\pm0.18$	$11.5^e\pm0.62$	$11.5^e\pm0.22$	12.4 ± 0.39	12.1 ± 0.18
12	$9.9^a\pm0.24$	$10.2^a\pm0.77$	$9.4^a\pm0.41$	10.9 ± 0.52	10.2 ± 0.47

Figures represent mean of four observations ± standard deviation

Conditions of incubation : Same as in table 12.

At 4 and 12 h incubation, the extent of ammonia produced from casein and soya insoluble proteins was significantly (p < 0.01) higher than that Of GC,MC and SF, while that from casein was significantly (p < 0.05) higher than that of soya.

Data showed that maximum ammonia was produced from casein followed, in order, by Soya, GC, MC and SF. The amount of ammonia produced from MC and SF insoluble proteins was almost equal.

4.3.3.3 Washed protozoal suspension

Data on the production of ammonia when insoluble fraction of various intact feed proteins was incubated with washed protozoal suspension are given in Table 27 (Fig.7). The ammonia production reached to maximum value of 8 h and decreased thereafter upto 12 h of incubation. AT 8 h of incubation, ammonia produced from insoluble proteins of casein, soya, GC,MC and SF was 24.8, 22.3, 20.2, 19.6 and 20.1 mg/100 ml RF equivalent, respectively, and corresponding values for 12 h of incubation were 24.1, 21.6, 19.3, 18.8 and 18.8mg/100 ml RF equivalent.

At 8 h of incubation, ammonia production from casein insoluble proteins was significantly (p < 0.01) higher than that of soya, GC, MC and SF, while that of Soya insoluble protein was significantly (p < 0.01) higher than that of GC, MC and SF and that of GC was significantly (p < 0.05) higher than

Table 27. Ammonia production from insoluble fractions of intact feed proteins when incubated for different periods with washed protozoal suspension

Incubation period (h)	Ammonia produced (mg/100 ml RF equivalent)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	3.7±0.72	3.7±0.72	3.7±0.72	3.7±0.72	3.7±0.72
2	9.5±0.77	9.2±0.76	9.3±0.22	10.5^b±0.42	11.8±0.18
4	12.6^a±0.65	11.7^a±0.62	11.6^a±0.18	14.8^f±0.56	16.4±0.91
6	16.8^a±0.61	15.7^a±0.48	15.8^a±0.31	17.9^b±0.51	20.6±0.82
8	20.2^e±0.24	19.6^e±0.24	20.1^e±0.92	22.3^b±0.69	24.8±0.41
12	19.3^a±0.33	18.8^a±0.81	18.8^a±0.88	21.6^b±0.38	24.1±0.44

Figures represent mean of four observations ± standard deviation

Conditions of incubation : Same as in table 13.

that of MC and SF.

Data indicated that degradation of casein insoluble proteins led to maximum amount of ammonia production followed, in order, by Soya, GC, SF and MC, whereas amount of ammonia production from GC, SF and MC insoluble proteins were almost equal in amount.

4.3.3.4 Cell-free supernatant

Data on production of ammonia when insoluble fraction of intact feed proteins was incubated with cell-free supernatant for different periods are given in Table 28 (Fig.8). Ammonia production was highest at 4 h and then decreased with increasing incubation time upto 12 h. At 4 h of incubation, ammonia produced from insoluble proteins of casein, Soya, GC, MC and SF was 23.1, 20.3, 15.8, 13.7 and 14.9 mg/100 ml RF equivalent, respectively, and corresponding values for 12 h of incubation were 13.1, 9.2, 8.3, 7.6and 8.4 mg/100 ml RF equivalent.

At 4 h Incubation period, ammonia produced from casein and soya insoluble proteins was significantly ($p < 0.01$) higher than that of GC, MC and SF, that of GC was significantly ($p < 0.01$) higher than that of MC and that of SF was significantly ($p < 0.01$) higher than that of MC. AT 4 h of Incubation, ammonia production from casein insoluble proteins was significantly ($p < 0.01$) higher than that of Soya, GC, MC and SF insoluble proteins.

The data presented in Table 25, 26, 27 and 28 (Fig.6,

Table 28. Ammonia production from insoluble fractions of intact feed proteins when incubated for different periods with cell free supernatant

Incubation period (h)	Ammonia produced (mg/100 ml RF equivalent)				
	Groundnut cake	Mustard cake	Sunflower seed meal	Soyabean seed meal	Casein
0	8.4 ± 0.36	8.4 ± 0.36	8.4 ± 0.36	8.4 ± 0.36	8.4 ± 0.36
2	$10.2^a0.31$	$9.8^a\pm0.61$	$10.1^a0.59$	$13.8^b\pm0.32$	16.8 ± 0.44
4	$15.8^a\pm0.22$	$13.7^{a,c}\pm0.56$	$14.9^{a,c,h}\pm0.28$	$20.3^b\pm0.31$	23.1 ± 0.56
6	$14.7^a\pm0.84$	$11.8^{a,c}\pm0.49$	$14.2^{ac,d}\pm0.31$	$18.1^b\pm0.92$	21.4 ± 0.66
8	$11.4^a\pm0.76$	$9.3^{a,c}\pm0.44$	$11.1^{a,c,d}\pm0.24$	$12.1^b\pm0.99$	17.7 ± 0.31
12	$8.3^a\pm0.32$	$7.6^{a,g}\pm0.36$	$8.4^{a,g}\pm0.72$	$9.2^b\pm0.81$	13.1 ± 0.42

Figures represent mean of four observations ± standard deviation

Conditions of incubation : Same as in table 14.

7 and 8) indicated that SRF and its fractions produced maximum ammonia from casein insoluble proteins followed, in order, by Soya, GC/MC/SF at all the incubation periods. It is also observed that SRF was more active in the production of ammonia from insoluble proteins followed by cell-free supernatant and bacterial/protozoal suspension. Upto 4 h of incubation bacterial suspension produced more ammonia than protozoal suspension and the reverse was true after 6 h of incubation. It means that ammonia production by protozoa was more at prolonged incubation periods (6 to 8 h).

The experiment on the production of ammonia from intact proteins and its fraction of casein, Soya, GC, MC and SF by rumen fluid and its fraction indicated that SRF, bacteria and cell-free supernatant produced ammonia maximally from soluble proteins followed by intact proteins and insoluble proteins from all the sources used. Protozoal suspension, however, produced ammonia in almost equal amounts from both intact and soluble proteins from all the sources and lesser amounts from insoluble proteins. Maximum amount of ammonia was produced by SRF followed, in order, by cell-free supernatant, bacteria and protozoa from all the intact protein and their fractions.

4.4 Characterization of deaminase from mixed rumen extract (MRBE)

4.4.1 Effect of pH

The pH optima for the deaminase activity in MRBE was

Table 29. Effect of pH on deaminase activity of mixed rumen bacterial extract

pH	μ moles NH_3 produced/mg protein/hr
4.0	0.081
4.5	0.129
5.0	0.142
5.25	0.165
5.5	0.197
5.75	0.221
6.0	0.254
6.25	0.291
6.5	0.341
6.75	0.358
7.0	0.337
7.25	0.308
7.5	0.262
7.75	0.206
8.0	0.148
8.5	0.097
9.0	0.042

FIG.9 EFFECT OF ÞH ON DEAMINASE ACTIVITY OF MIXED RUMEN BACTERIAL EXTRACT
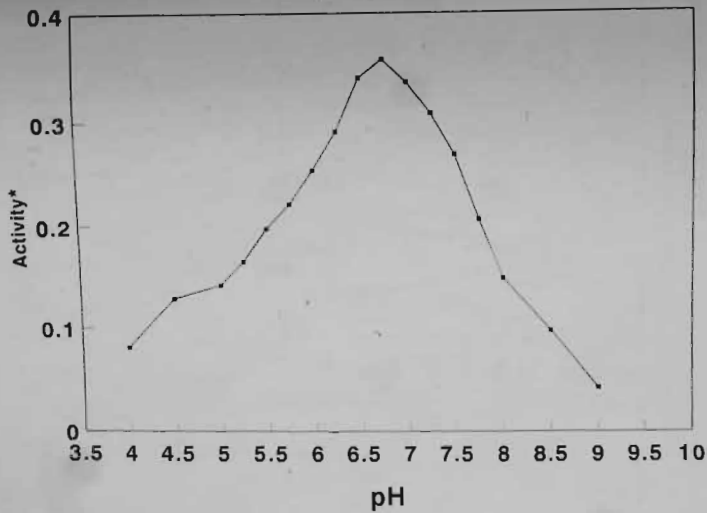
* μmoles NH_3 Produced/ mg Protein/ hr

Table 30. Effect of temperature on deaminase activity of
mixed rumen bacterial extract

Temp.	μmoles NH_3 produced/mg protien/hr
20	0.058
25	0.072
30	0.139
35	0.246
40	0.271
45	0.293
50	0.266
55	0.218
60	0.162

FIG.10 EFFECT OF TEMPERATURE OF INCUBATION ON DEAMINASE ACTIVITY OF MIXED RUMEN BACTERIAL EXTRACT
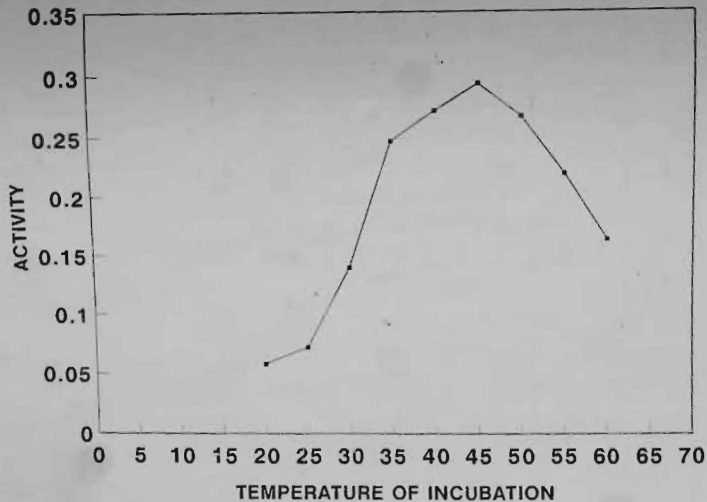
Table 31. Thermal stability of mixed rumen bacterial extract deaminase activity

Temp.	μmoles NH_3 produced/mg protien/hr	Residual activity (%)
25	0.259	100
30	0.261	100
35	0.263	100
40	0.262	100
45	0.265	100
50	0.263	100
55	0.172	65.9
60	0.108	41.4
70	0.054	20.7
80	0.019	7.3
90	0.00	0.00

FIG.11 THERMAL STABILITY OF MIXED RUMEN BACTERIAL EXTRACT

(DEAMINASE ACTIVITY)
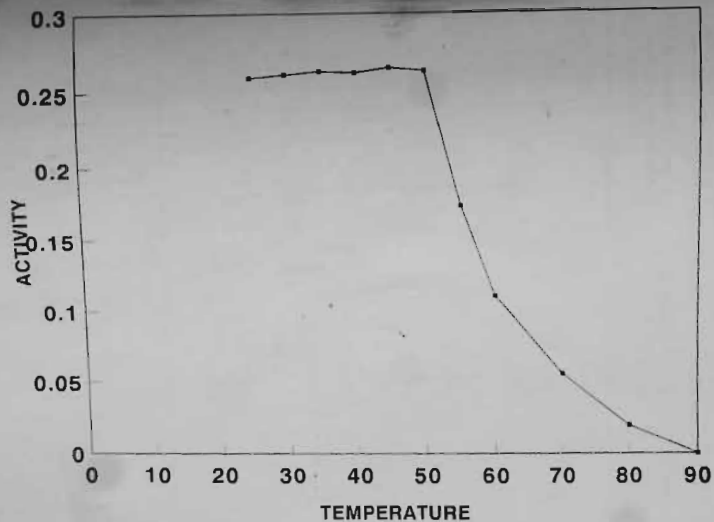
Table 32. Effect of amino acid concentration on deaminase activity of mixed rumen bacterial extract

Concentration of amino acids (mmoles)	μ moles NH_3 produced/mg protein/hr
1.0	0.099
2.0	0.124
3.0	0.177
4.0	0.241
5.0	0.328
6.0	0.351
7.0	0.362
8.0	0.347
9.0	0.329
10.0	0.322
11.0	0.316
12.0	0.304

FIG.12 EFFECT OF AMINO ACID CONCENTRATION ON DEAMINASE
ACTIVITY OF MIXED RUMEN BACTERIAL EXTRACT
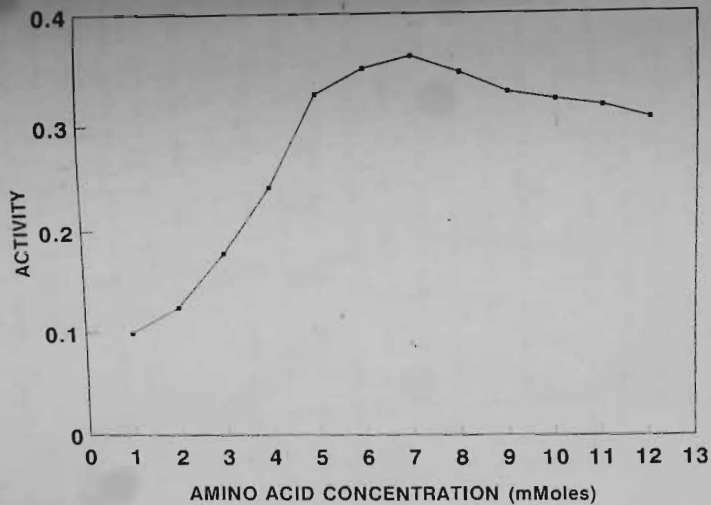
found to be 6.75. The activity decreased at pH both below and above 6.75 (Table 29, Fig.9).

4.4.2 Effect of temperature

Temperature optima for deaminase activity in MRBE was found to be 45°C. At temperature 20°C, 30°C, 37°C, 55°C and 60°C, the activity was found to be 80.2, 52.6, 16.1, 25.6 and 44.7 per cent less, respectively, of the maximum activity (Table 30, Fig.10).

4.4.3 Heat stability of deaminase activity

Deaminase activity was found to be stable upto 50°C and was completely inactivated at 90°C (Table 31, Fig.11).

4.4.4 Effect of amino acid concentration

Effect of concentration of amino acid mixture (1-12 m moles) on the deaminase activity of MRBE was examined. The optimum concentration of amino acids for the deaminase activity was found to be 7.0 m moles. Further increase in concentrations of amino acids did not result in increase in deaminase activity (Table 32, Fig.12).

4.4.5 Effect of metal ions

The results on the effect of certain metal ions on the deaminase activity of MRBE has been given in table 33. Na^+, K^+, Mg^{++}, Mn^{++}, Ca^{++}, Co^{++} at 10 m moles concentration increased the activity by 27.3, 21.4, 7.6, 5.1, 6.1 and 9.6% respectively, over the control value. Ammonium chloride and zinc chloride at 10 m moles concentration decreased the activity by 15.4 and 37.9%, respectively as compared with the

Table 33. Effect of various metal ions on deaminase activity of mixed rumen bacterial extract

Metal ions (mmoles)		μmoles NH₃ produced/mg protein/hr	Enzyme activity (%)
Control		0.312	100
Na Cl	1.0	0.324	103.8
	5.0	0.347	111.4
	10	0.397	127.3
KCl	1	0.322	103.2
	5	0.332	106.4
	10	0.379	121.4
Mg Cl₂	1	0.318	101.9
	5	0.324	103.8
	10	0.336	107.6
Mn Cl₂	1	0.314	100.6
	5	0.317	101.6
	10	0.328	105.1
Ca Cl₂	1	0.311	99.7
	5	0.318	101.4
	10	0.332	106.1
Fe Cl₃	1	0.316	101.2
	5	0.317	101.6
	10	0.321	106.1
Co Cl₂	1	0.331	106.1
	5	0.338	108.3
	10	0.342	109.6
NH₄Cl	1	0.318	101.9
	5	0.286	91.6
	10	0.264	84.6
ZnCl₂	1	0.303	97.2
	5	0.236	75.8
	10	0.194	62.1
AgNO₃	1	0.208	66.7
	10	0.101	32.4
Hg₂Cl₂	1	0.119	38.1
	10	0.00	0.0
PbCl₂	1	0.159	50.9
	10	0.028	8.9
CuSO₄	1	0.196	63.1
	10	0.095	30.6

control. $AgNO_3$, $PbCl_2$, Hg_2Cl_2 and $CuSO_4$ at 1 m moles concentration drastically decreased the activity by 33.3, 36.9 and 69.4%, respectively as compared with the control value.

4.4.6 Effect of various proteinase inhibitors

Most of the proteinase inhibitors examined inhibited the deaminase activity of MRBE to a large extent (Table 34). Chelating agents such as sodium oxalate, sodium citrate, sodium tartarate and EDTA inhibited the activity at all concentrations studied. Decrease in the activity was more at higher concentration of the inhibitors. Sodium oxalate, sodium citrate, sodium tartarate, sodium tungstate and EDTA at 10 m moles concentration inhibited the activity by 20.5, 30.8, 44.9, 29.6 and 45.7% respectively. The reducing agents like dithiothreitol and cysteine hydrochloride increased the deaminase activity at 1.6% and 2.3% at 1 m mole concentration and by 4.1% and 6.9% at 5 m moles concentration. AT 10 m moles level, dithiothreitol, however, increased the activity by 10.2%, while cysteine hydrochloride decreased the same by1.3% compared to control value.

p-chloromercuribenzoate (pCMB) and sodium azide depressed the deaminase activity drastically at all the concentrations used. pCMB and sodium azide at 5 m moles concentration decreased the activity by 83.3 and 73.0% respectively. It was concluded that amongst the chelating agents EDTA depressed the activity to maximum extent and amongst the reducing agents DTT was found to be more effective

Table 34. Effect of various protease inhibitors on deaminase activity of mixed rumen bacterial extract

Inhibitor (mmoles)		μmoles NH_3 produced/mg protein/hr	Enzyme activity (%)
None		0.311	100
Sodium Oxalate	1	0.298	96.1
	5	0.262	84.3
	10	0.247	79.5
Sodium citrate	1	0.294	94.7
	5	0.252	81.3
	10	0.215	69.2
Sodium tartrate	1	0.289	87.4
	5	0.256	74.3
	10	0.218	55.1
Sodium tungstate	1	0.271	93.1
	5	0.231	82.5
	10	0.171	70.4
EDTA	1	0.285	91.8
	5	0.244	78.6
	10	0.168	54.3
Dithiothrutol	1	0.316	101.6
	5	0.323	104.1
	10	0.342	110.2
Cystine hydrochloride	1	0.318	102.3
	5	0.332	106.9
	10	0.306	98.7
p-Chloromercuribanzoate	0.1	0.162	52.4
	1.0	0.129	41.8
	5.0	0.051	16.7
Sodium azide	0.1	0.236	76.1
	1.0	0.162	52.4
	5.0	0.084	27.0

than cysteine HCl in enhancing the deaminase activity.

4.4.7 Effect of various deaminase inhibitors

Effect of various deaminase inhibitors on deeaminase activity was studied at 0.1 and 1.0m moles concentrations except dimethyldiphenyliodonium chloride (DDIC) and phosphoramidon which were used at 10 and 50 ppm levels (Table 35). Hydrazine was found to be most potent inhibitor of deaminase activity. At 0.1 m mole concentration hydrazine, hydrazine sulfate, phenylhydrazine, p-nitrophenylhydrazine, hydroxylamine hydrochloride, sodium arsenate, sodium arsenite, DDIC (10 ppm) and phosphoramidon (10 ppm) decreased the deaminase activity by 33.5, 29.4, 31.7, 32.9, 21.8, 37.7, 38.2, 47.6 and 23.9%, respectively and corresponding per cent inhibition of deaminase activity was 72.9, 68.2, 69.4, 70.6, 43.9, 75.9, 78.7, 80.7 and 52.2% at 1 m mole (50 ppm in case of DDIC and phosphoramidon) concentration. The results show that amongst hydrazine and its derivatives p-nitrophenyl hydrazine was more effective in depressing the deaminase activity. Sodium arsenate is more effective inhibitor than sodium arsenite. DDIC was found to be the most effective deaminase inhibitor.

4.4.8 Effect of various methanogenic inhibitors

The results on the effect of methanogenic inhibitors on the deaminase activity are given in Table 36. Monensin at 25 and 50 ppm concentration depressed the deaminase activity in MRBE by 27.6 and 50.7% respectively, as compared to the control value. Sodium sulfite was found to be a mild inhibitor. It

Table 35. Effect of various deaminase inhibitors on deaminase activity of mixed rumen bacterial extract

Inhibitor (mmoles)	μmoles NH_3 produced/mg protein/hr	Enzyme activity (%)
Control	0.283	100
Hydrazine		
0.1	0.188	66.5
1.0	0.076	27.1
Hydrazine sulfate		
0.1	0.199	70.6
1.0	0.089	31.8
Phenyl hydrazine		
0.1	0.193	68.3
1.0	0.086	30.6
p-nitrophenyl hydrazine		
0.1	0.189	67.1
1.0	0.083	29.4
Hydroxylamine HCl		
0.1	0.221	78.2
1.0	0.158	56.1
Sodium Arsenate		
0.1	0.176	62.3
1.0	0.068	24.1
Sodium arsenite		
0.1	0.174	61.8
1.0	0.061	21.3
DDIC (ppm)		
10	0.148	52.4
50	0.054	19.3
Phosphoramidom (ppm)		
10	0.215	76.1
50	0.135	47.8

Table 36. Effect of various methanogenic inhibitors on deaminase activity of mixed rumen bacterial extract

Inhibitor (mmoles/m)	μmoles NH$_3$ produced/mg protein/hr	Enzyme activity (%)
Control	0.294	100
Monensin (ppm)		
25	0.213	72.6
50	0.144	49.3
Sodium sulfite		
0.1	0.271	92.4
1.0	0.255	87.0
10	0.210	71.5
Chloroform		
0.1	0.226	77.2
1.0	0.171	58.4
10	0.132	45.1
Chloralhydrate		
0.1	0.221	75.1
1.0	0.171	58.4
10	0.108	36.8

Table 37. Effect of various electrolytes on deaminase activity of mixed rumen bacterial extract

Electrolyte (mmoles)		μmoles NH_3 produced/mg protein/h	Enzyme activity (%)
Control		0.296	100
$NaSO_4$	5	0.298	100.1
	10	0.299	101.0
Na_2S	5	0.302	102.1
	10	0.324	109.4
$NaNO_3$	5	0.288	97.3
	10	0.284	96.2
*Na_2HPO_4	5	0.299	101.0
	10	0.304	102.7
*$Na(H_3PO_4)_2$	5	0.298	100.1
	10	0.291	98.3
NaI	5	0.302	102.1
	10	0.311	105.0
$NaOH$	1	0.276	93.2
	5	0.257	86.8
	10	0.226	76.5
$NaOCl$	1	0.264	89.2
	10	0.208	70.3
Na_3CO_3	5	0.303	102.5
	10	0.346	117.1
$NaHCO_3$	5	0.309	104.6
	10	0.371	120.1

*25 mM Tris HCl buffer of pH 7.0 was used instead of 25 mM phosphate buffer of pH 7.0

decreased the deaminase activity by 7.6%, 13.0% and 28.5% at 0.1,1.0 and 10 m moles concentrations, respectively, as compared to the control. Chloroform at similar concentrations decreased the activity by 22.8, 41.6 and 54.9%. In this class, chloral hydrate was found to be most potent inhibitor of deaminase activity. It decreased the activity by24.9, 41.6 and 63.2% at 0.1,1.0 and 10 m moles concentrations, respectively.

4.4.9 Effect of other electrolytes

Effect of various anionic electrolytes on deaminase activity was studied at two levels viz. 5 and 10 m moles except where otherwise mentioned (Table 37). Sodium sulfide, sodium iodide, sodium carbonate and sodium bicarbonate at 10 m moles concentration increased the deaminase activity in MRBE by 9.4, 5.0, 17.1 and 25.1%, respectively, while sodium nitrate, sodium nitrite, sodium hydroxide and sodium hypochlorite at 10 m moles concentration level decreased the activity by 3.8, 9.4, 23.5 and 29.7%, respectively, over the control values. Sodium sulfate, $Na_2(H_3PO_4)_2$ and Na_2HPO_4 were without effect.

4.4.10 Extent of deamination of intact feed proteins and their fractions by MRBE

The data on the degradation of intact proteins and their soluble and insoluble fractions by MRBE are given in Table 38. The per cent degradation and the formation of ammonia are taken as index of extent of protein degradation. The proteins from different sources were degraded by MRBE at different rates. The intact proteins of SF, MC, GC, Soya and

Table 38. Degradation of intact feed proteins and their fractions by mixed rumen bacterial extract

Feed protein	Fraction	μ moles ammonia/mg protein/h	Amount ungraded	Degradation (%)
Groundnut Cake				
i)	Intact	0.141	3.34	33.2
ii)	Soluble	0.213	2.58	48.4
iii)	Insoluble	0.035	4.35	13.1
Mustard Cake				
i)	Intact	0.112	3.48	30.4
ii)	Soluble	0.168	2.76	44.9
iii)	Insoluble	0.064	4.72	15.6
Sunflower seeds				
i)	Intact	0.092	3.59	28.1
ii)	Soluble	0.123	2.97	40.7
iii)	Insoluble	0.052	4.29	14.2
Soyabean seeds				
i)	Intact	0.168	3.02	39.6
ii)	Soluble	0.216	2.61	47.8
iii)	Insoluble	0.136	3.92	21.6
Casein	i) Intact	0.189	2.69	46.1
	ii) Soluble	0.256	2.32	51.6
	iii) Insoluble	0.145	3.90	22.1

Figures represent mean of four observations.
5 mg protein(CP) were added to the assay mixture.

casein were degraded to the extent of 28.1, 30.4, 33.2, 39.6 and 46.1% respectively. The ammonia produced from intact protein of SF, MC, GC, Soya and casein was 0.092, 0.112, 0.141, 0.168 and 0.189 μmoles/mg protein/h, respectively. The soluble fractions of the intact proteins from all the sources used, degraded maximally, while insoluble fractions degraded the least. The extent of degradation of soluble proteins from SF, MC, GC, Soya and Casein was 40.7, 44.9, 48.4, 47.8 and 51.6%, respectively, and the corresponding levels for the degradation of insoluble proteins were 14.2, 15.6, 13.1, 21.6 and 22.1%. The ammonia production from soluble proteins of SF, MC, GC, Soya and Casein was 0.123, 0.168, 0.213, 0.216 and 0.256 μmoles/mg protein/h and the corresponding values for insoluble proteins were 0.052, 0.064, 0.035, 0.136 and 0.145. This data show that soluble proteins were degraded maximally followed by intact proteins and insoluble proteins.

The data also indicated that the extent of degradation of intact, soluble and insoluble proteins was maximum in case of casein followed, in order, by Soya, GC, MC and SF.

4.4.11 Extent of deamination of individual amino acids by MRBE

Exogenously supplied amino acids were catabolized at different rates by MRBE (Table 39). Serine was highest degraded (88.9%) while proline was least degraded (17.9%) amino acid. Serine was followed by aspartate (81.9%) and glutamate (70.3%). Glutamine was degraded by 66.4% followed by threonine (62.1%), cysteine (61.6%) and phenylalanine and

Table 39. Deamination of individual amino acids by mixed
          rumen bacterial extract

Amino acid	Amount added (mg)	Amount undegraded (mg)	Degradation (%)	Ammonia (mg/100 ml RF)
Serine	10.5	1.2	88.9	0.372
Aspertine	13.3	2.4	81.9	0.351
Glutamate	14.7	4.4	70.3	0.294
Glutamine	14.6	4.9	62.1	0.263
Threonine	11.9	4.5	62.1	0.243
Cysteine	12.1	4.6	61.6	0.233
Phenylalaine	16.5	6.5	60.3	0.224
Lysine	14.5	5.0	60.1	0.221
Alamine	8.9	3.8	57.5	0.218
Tyrosine	18.1	9.6	47.1	0.211
Cystine	24.2	12.8	46.6	0.333
Arginine	17.4	9.4	45.9	0.266
Methionine	14.9	8.2	45.2	0.143
Histidine	20.4	12.8	37.2	0.209
Tryptophan	20.4	13.3	34.5	0.201
Valine	11.7	8.1	30.5	0.147
Glycine	7.5	5.2	30.1	0.121
Isoleucine	11.5	8.2	28.5	0.086
Hydroxyproline	13.1	10.6	18.9	0.081
Proline	11.5	9.4	17.9	0.073

Figures represent mean of four observations.

lysine, which were deaminated to a similar extent (60.3 and 60.1%). Alanine was degraded by 57.5% followed by tyrosine (47.1) and cystine (46.6), arginine (45.9) and methionine (45.2) were degraded to almost similar extent. Histidine (37.2%) degradation was higher than that of tryptophan (34.5%), followed by that of valine (30.5) and glycine (30.1) which were degraded to comparable extent. Isoleucine was degraded by 28.5%, followed by hydroxyproline (18.9%) and proline (17.9%). It was concluded that acidic amino acids were degraded to highest extent, followed by branched chain, aromatic and basic amino acids. Neutral amino acids like valine, glycine and isoleucine were degraded to a very small degree. Proline and hydroxyproline being cyclic amino acids were degraded the least. Ammonia produced per mg protein of MRBE per hour followed similar decreasing trend as the extent of amino acid degradation decreased.

4.5 Distribution of deaminase activity in rumen fluid and its fractions

Distribution of deaminase activity in various fractions of rumen fluid was studied and the results are presented in table 40. It was noted that ammonia produced per mg protein of each fraction per hour was maximum for strained rumen fluid (0.358 μmoles) which was taken as 100% for further calculations. Washed protozoal suspension exhibited only 9.1% of the activity of strained rumen fluid with ammonia production as 0.033 μ moles/mg protein/h. Bacterial fraction had 43.7% of

Table 40. Distribution of deaminase activity in rumen fluid and its fractions

Experiment I fraction	μmoles NH_3 produced/mg protein/hr	Enzyme activity (%)
i) Strained rumen fluid	0.358	100
ii) Protozoal suspension	0.033	9.1
iii) Bacterial suspension	0.156	43.7
iv) Cell free supernatant	0.219	61.4
Experiment II fraction		
i) Bacterial pellet	0.156	100
ii) Bacterial pellet <u>sonicated</u>		
Residue (cell debris)	0.041	26.3
iii) Supernatant (cell envelopes & cytosol)	0.228	146.2
iv) Pellet (cell membranes)		
a) Inner mebrane	0.108	69.2
b) Outer membrane	0.012	7.6
c) Peptidoglycan	0.0	-
v) Supernatant	0.149	95.5

the activity associated with it and ammonia production was
0.156 μmoles/mg protein/h. Cell-free supernatant carried the
maximum activity. Ammonia produced was 0.219 μ moles/mg
protein/h and enzyme activity was calculated to be 61.4% in
this case as compared to that of SRF. It was concluded that
major portion of enzyme was released into cell free supernatant
by rumen bacteria. Considerable activity was also contained in
the bacterial cells while protozoa possessed very little
activity.

4.5.1 Localization of deaminase activity in mixed rumen bacterial cell

Efforts were made to localize deaminase activity in
the rumen bacterial cell (Table 40). Bacterial pellet carried
43.7% of the deaminase activity of SRF. When this pellet was
sonicated and centrifuged, most of the enzyme was released into
supernatant and cell debris carried only 26.3% of the total
bacterial pellet activity. When this supernatant was further
centrifuged at higher speed and cell envelopes were separated,
its deaminase activity was decreased from 146.2 to 95.5%. The
pellet, so obtained, was further fractionated into outer cell
membrane, inner cell membrane and middle peptidoglycan protein
layer. Of these, inner cell membrane carried 69.2%, while outer
cell membrane had only 7.6% of the original activity of mixed
bacterial pellet. Peptidoglycan layer exhibited no deaminase
activity. The above data indicated that although major
deaminase activity was located in mixed bacterial population,

most of it (61.4%) was released into rumen fluid. Of the remaining activity associated with rumen bacteria about 69.2% was located in the inside of cell wall and only a fraction (7.6%) was located on the outside.

4.5.2 Effect of various detergents on the release of deaminase activity from mixed rumen bacterial fraction

Effect of various detergents on the release of deaminase activity from mixed rumen bacteria fraction was investigated and the results are given in table 41. All the detergents used helped release of enzyme from the mixed rumen bacteria. Increasing the concentration of detergents from 0.05 to 0.1% increased the release of enzyme activity. AT 0.1% concentration, Triton-X-100, sodium dodecyl sulfate (SDS) and centrimide released 61.1, 51.7 and 34% more enzyme activity as compared with the control. At similar concentration Tween-20, Tween-40, Tween-60 and Tween-80 increased the release of enzyme activity by 17.7, 20.8, 27.1 and 41.7% compared with the control.

It was concluded from above results that Triton-X-100 was most effective in releasing the deaminase activity from bacterial pellet followed by SDS, whereas Tween-20 released it to the least extent of all detergents studied.

Table 41. Effect of various detergents on the release of
deaminase activity from mixed rumen bacteria

Detergent (conc.) (%)		Bacterial pellet (μmole NH_3 produced/mg protein/h	Enzyme activity (%)	Super-natant (μmole NH_3 protein/h	Enzyme activity (%)
Control		0.239	100	0.321	100
Triton x 100					
	0.05	0.153	64.2	0.443	138
	0.1	0.115	48.3	0.517	161.1
Tween-20					
	0.05	0.17	71.2	0.334	104
	0.1	0.135	56.3	0.378	118.7
Tween-40					
	0.05	0.161	67.4	0.356	111.0
	0.1	0.128	53.5	0.388	120.8
Tween-60					
	0.05	0.143	60.1	0.382	119.0
	0.1	0.118	49.3	0.408	127.1
Tween-80					
	0.05	0.131	54.4	0.411	128.0
	0.1	0.097	40.7	0.455	141.7
Cetrimide					
	0.05	0.122	51.0	0.369	114.9
	0.1	0.096	40.1	0.43	134.0
SDS					
	0.05	0.108	45.2	0.395	123.1
	0.1	0.091	38.1	0.487	151.7

4.6 Effect of various deaminase and methanogenic inhibitors on the concentration of TCA-precipitable proteins, ammonia-nitrogen and volatile fatty acids when intact feed proteins were incubated with strained rumen fluid or washed mixed bacterial suspension

After identifying promising deaminase inhibitors, their effect on actual protein biosynthesis by rumenmicroorganisms was examined. But before conducting this experiment the protein biosynthesis by SRF or mixed bacterial suspension from casein hydrolysate and amino acid mixture was exmained (Tables 42,43). It has been observed that the concentration of TCA-precipitable proteins and TVFA in incubation mixture was higher in case of casein hydrolysate as compared to amino acid mixture when incubation was done with SRF or washed bacterial suspension (Tables 42 and 43). SRF was found to be more effective than washed bacterial suspension in degradation of casein hydrolysate or amino acid mixture for production of TVFA or TCA-precipitable proteins. The concentration of ammonia-nitrogen in incubation mixture was slightly higher in case of casein hydrolysate when incubated with SRF as compared to bacterial suspension and it was slightly higher in case of amino acid mixture when incubated with bacterial suspension over SRF. SRF seems to be more active than bacterial suspension.

Tables 44, 45, 46 and 47 represent the partial data on the percent increase or decrease in the concentration of TCA precipitate proteins, ammonia and TVFA when various intact feed

Table 42. Concentrations of TCA-precipitable proteins, ammonia-nitrogen and total volatile fatty acids in incubation mixture when casein hydrolysate and amino acid mixture were incubated with strained rumen fluid

Incubation period (h)	Casein hydrolysate			Amino acid mixture		
	TCA precipitable proteins (mg/100 ml RF)	Ammonia-nitrogen (mg/100 ml RF)	TVFA (mmoles/100 ml RF)	TCA precipitable proteins (mg/100 ml RF)	Ammonia-nitrogen (mg/100 ml RF)	TVFA (mmoles/100 ml RF)
0	36.5±0.61	9.4±0.43	24.3±1.16	33.8±0.72	7.6±0.39	27.1±1.06
4	42.6±0.56 (+16.7)	51.7±0.43	39.8±1.86 (+63.78)	36.3±0.34 (+4.43)	48.7±0.24	41.4±1.77 (+52.76)
8	57.2±0.91 (+56.71)	42.2±0.16	44.5±1.42 (+83.12)	48.9±0.62 (+44.67)	33.8±0.58	46.2±1.89 (+70.47)

Figures represent mean of four observations ± standard deviation
Figures in parenthesis represent per cent increase over control
Conditions of incubation : Same as in Table 3 except casein hydrolysate (5 mg/100 ml SRF) or amino acid mixture (1 mmole/ml SRF) were added in place of intact feed proteins and incubated at 39°C for 4 and 8 h.

Table 43. Concentrations of TCA-precipitable proteins, ammonia-nitrogen and total volatile fatty acids in incubation mixture when casein hydrolysate and amino acid mixture were incubated with washed bacterial suspension

Incubation period (h)	Casein hydrolysate			Amino acid mixture		
	TCA precipitable proteins (mg/100 ml RFE?)	Ammonia-nitrogen (mg/100 ml RFE?)	TVFA (mmoles/100 ml RFE?)	TCA precipitable proteins (mg/100 ml RFE?)	Ammonia-nitrogen (mg/100 ml RFE?)	TVFA (mmoles/100 ml RFE?)
0	32.4±0.58	5.7±0.91	22.4±1.16	30.8±0.66	4.9±0.84	26.1±1.06
4	39.6±0.42 (+22.2)	31.7±0.24	28.3±1.97 (+26.33)	34.8±0.84 (+12.48)	33.2±0.46	30.2±1.23 (+15.7)
8	47.4±0.51 (+46.29)	23.6±0.56	33.2±1.25 (+48.21)	39.6±0.42 (+28.57)	27.5±0.73	34.1±1.49 (+30.65)

Figures represent mean of four observations ± standard deviation
Figures in parenthesis represent per cent increase over control
Conditions of incubation : Same as described in table 27 except 10 ml washed mixed bacterial suspension were added to incubation mixture in place of strained rumen fluid.

proteins were incubated with SRF or washed bacterial suspension in presence of various deaminase and methanogenic inhibitors. In this section only the data for 8 h incubation is given. The remaining data are given in Tables in appendix.

4.6.1 Effect of deaminase inhibitors on the concentration of TCA precipitate proteins, ammonia nitrogen and volatile fatty acid when intact proteins were incubated with strained rumen fluid or washed bacterial suspension

4.6.1.1 Strained rumen fluid

All the deaminase inhibitors used decreased the concentration of ammonia and simultaneously increased the concentration of TCA-precipitable proteins in the incubation mixture (IM) when casein, Soya, GC, MC and SF were incubated with SRF (Table 44). DDIC sodium arsenite and sodium arsenate decreased the concentration of ammonia to greater extent than hydrazine and its derivation. Amongst the hydrazine and its derivatives phenyl hydrazine was more effective and hydrazine was the least effective. Amongst all the inhibitors used sodium arsenite was the highest and hydrazine was the least effective in decreasing the concentration of ammonia-nitrogen. No definite interaction was observed between source of protein and inhibitors on ammonia concentration. The inhibitors which decreased the concentration of ammonia-nitrogen to greater extent i.e. DDIC, sodium arsenate, sodium arsenite and phenylhydrazine also increased the concentration of TCA-precipitable proteins in the IM to maximum extent. In the presence of inhibitors, there was more concentration of TCA-

Table 44. Effect of various deaminase inhibitors on the concentration of TCA-precipitable proteins, ammonia-nitrogen and total-volatile fatty acid when various intact feed proteins were incubated with strained rumen fluid for 8h

Inhibitor (1.0 mmole)	Protein sources				
	Groundnut cake	Mustard cake	Sunflower seed	Soyabean seed	Casein
	Per cent increase (+) or decrease (-) over control				
Hydrazine					
i) TCA precipitable proteins	+35.9	+45.0	+30.5	+17.6	+65.5
ii) NH_3	-12.9	-14.6	-24.0	-20.3	-23.8
iii) TVFA	-49.0	-51.6	-52.4	-29.6	-31.7
Hydrazine sulfate					
i) TCA precipitable proteins	+33.3	+37.8	+35.7	+40.2	+49.5
ii) NH_3	-24.6	-31.8	-26.9	-26.9	-26.1
iii) TVFA	-38.3	-37.5	-37.1	-33.1	-31.1
Phenyl hydrazine					
i) TCA precipitable proteins	+52.3	+62.9	+57.9	+69.3	+79.7
ii) NH_3	-27.0	-33.6	-28.4	-28.8	-29.9
iii) TVFA	-44.9	-51.9	-49.0	-47.1	-49.2
p-nitrophenyl hydrazine					
i) TCA precipitable proteins	+35.5	+42.4	+34.1	+33.8	+51.4
ii) NH_3	-22.3	-21.7	-29.9	-23.1	-23.1
iii) TVFA	-40.2	-39.5	-39.5	-39.6	-41.8

Hydroxylamine, HCl					
i) TCA precipitable proteins	+23.5	+35.9	+30.7	+33.0	+37.6
ii) NH_3	-18.7	-21.1	-18.0	-13.1	-13.7
iii) TVFA	-43.3	-51.4	-39.1	-39.1	38.7
Sodium arsenate					
i) TCA precipitable proteins	+44.5	+50.5	+43.9	+60.4	+80.0
ii) NH_3	-34.0	-38.6	-36.8	-23.0	-28.7
iii) TVFA	-59.5	-63.0	-61.1	-58.9	-59.2
Sodium arsenite					
i) TCA precipitable proteins	+29.4	+48.9	+38.0	+36.0	+54.8
ii) NH_3	-26.0	-67.0	-64.9	-52.6	-56.0
iii) TVFA	-59.9	-67.0	-64.9	-52.6	-56.0
DDIC (50 ppm)					
i) TCA precipitable proteins	+42.6	+51.9	+41.3	+52.0	+76.0
ii) NH_3	-27.4	-37.3	-32.9	-35.1	-32.5
iii) TVFA	-41.9	-43.7	-44.0	-35.6	-46.5

FIG.13 EFFECT OF DEAMINASE INHIBITORS ON TCA-PRECIPITABLE PROTEINS WHEN INTACT FEED PROTEINS WERE INCUBATED WITH STRAINED RUMEN FLUID FOR 8 h.
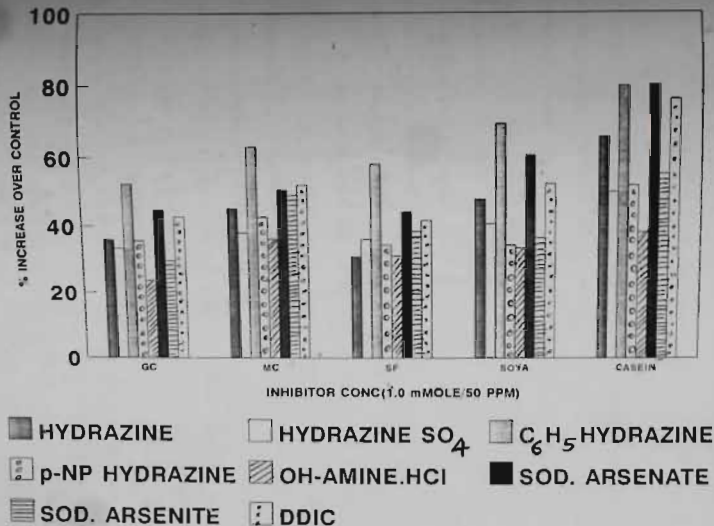
precipitable protein in the IM in case of casein followed, in order, by Soya/MC/SF and GC. The concentration of TCA-precipitable proteins was increased by 79.7, 69.3, 62.9, 57.9 and 52.3% in case of casein, Soya, MC, SF and MC over the control values, respectively, by phenylhydrazine and the corresponding values for sodium arsenate, DDIC and sodium arsenite were 54.8, 36.0, 48.9, 38.0 and 29.4, 76.0, 52.0, 51.9, 41.3 and 42.6 and 80.0, 60.4, 50.5, 43.9 and 44.6%, respectively. All the inhibitors used decreased the concentration of total volatile fatty acids (TVFA) from all the protein sources used.

4.6.1.2 Washed mixed bacterial suspension

As with the SRF, all the deamination inhibitors used decreased the concentration of ammonia and simultaneously increased the concentration of TCA-precipitable proteins in the IM when casein, Soya, GC, MC and SF were incubated with bacterial cell suspension (Table 45). DDIC sodium arsenate and sodium arsenite decreased the concentration of ammonia nitrogen more than the hydrazine and its derivative. Amongst the hydrazine and its derivatives, hydrazine sulfate was found to be more effective and hydrazine was the least effective. Amongst all the inhibitors used, DDIC was the highest and hydrazine was the least effective in decreasing the concentration of ammonia. In general, the decrease in the concentration of ammonia in the presence of inhibitors was maximum in case of casein followed by Soya, GC and MC/SF.

Table 45. Effect of various deaminase inhibitors on the concentration of TCA-precipitable proteins, ammonia-nitrogen and total-volatile fatty acid when various intact feed proteins were incubated with washed mixed bacterial suspension for 8 h

Inhibitor (1.0 mmole)	Protein sources				
	Groundnut cake	Mustard cake	Sunflower seed	Soyabean seed	Casein
	Per cent increase (+) or decrease (-) over control				
Hydrazine					
i) TCA precipitable proteins	+43.4	+64.2	+55.1	+75.2	+97.4
ii) NH_3	-18.0	-17.6	-14.3	-41.1	-50.8
iii) TVFA	-65.2	-62.8	-63.3	-57.1	-56.0
Hydrazine sulfate					
i) TCA precipitable proteins	+74.6	+74.1	+64.37	+78.5	+111.6
ii) NH_3	-41.6	-43.9	-45.6	-44.0	-44.5
iii) TVFA	-75.5	-75.0	-77.3	-76.0	-75.1
Phenyl hydrazine					
i) TCA precipitable proteins	+19.1	+22.3	+23.3	+27.8	+45.2
ii) NH_3	-32.7	-38.0	-32.0	-39.4	-40.6
iii) TVFA	-58.1	-58.7	-58.0	-56.2	-55.5
p-nitrophenyl hydrazine					
i) TCA precipitable proteins	+18.8	+29.4	+25.6	+43.0	+39.1
ii) NH_3	-35.6	-30.7	-33.3	-39.4	-41.3
iii) TVFA	-75.2	-75.1	-76.4	-69.9	-70.2

Hydroxylamine, HCl					
i) TCA precipitable proteins	+26.3	+36.0	+34.8	+48.9	+33.0
ii) NH_3	-42.2	-40.7	-36.2	-44.3	-48.0
iii) TVFA	-56.6	-53.7	-51.3	-47.2	-21.4
Sodium arsenate					
i) TCA precipitable proteins	+52.9	+45.5	+43.5	+50.6	+58.5
ii) NH_3	-60.3	-57.4	-65.4	-61.9	-63.1
iii) TVFA	-82.7	-82.4	-81.9	-77.0	-77.0
Sodium arsenite					
i) TCA precipitable proteins	+33.7	+35.8	+40.1	+60.4	+66.0
ii) NH_3	-52.2	-50.3	-47.9	-49.1	-48.0
iii) TVFA	-83.1	-80.8	-81.4	-82.1	-81.1
DDIC (50 ppm)					
i) TCA precipitable proteins	+43.2	+53.4	+41.6	+52.0	+78.4
ii) NH_3	-61.6	-58.8	-68.2	-63.4	-65.7
iii) TVFA	-31.5	-33.8	-33.3	-36.6	-38.0

FIG.14 EFFECT OF DEAMINASE INHIBITORS ON TCA-PRECIPITABLE PROTEINS WHEN INTACT FEED PROTEINS WERE INCUBATED WITH WASHED BACTERIAL SUSPENSION FOR 8 h.
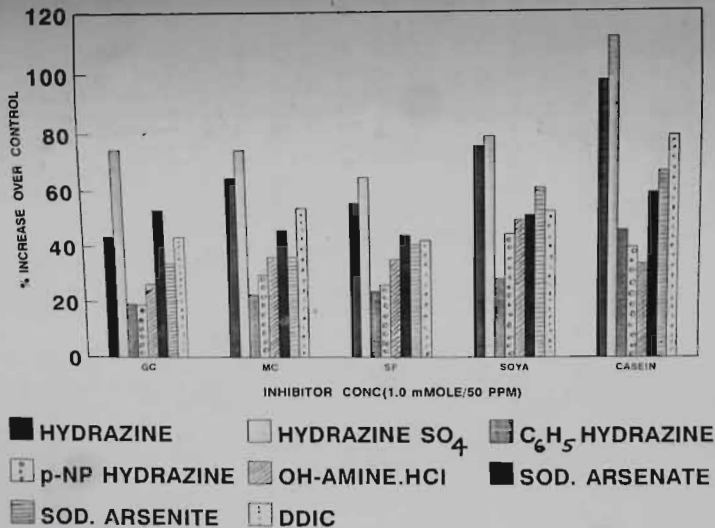
The concentration of TCA-insoluble protein was maximally increased in the presence of hydrazine and hydrazine sulfate. Per cent increase in the concentration of TCA-insoluble proteins in case of hydrazine sulfate was 111.6, 78.5, 74.6, 74.1 and 64.3 when casein, Soya, GC, MC and SF, respectively, were used, over the control values, and the corresponding values for hydrazine were 97.4, 75.2, 43.4, 64.2 and 55.1%. DDIC, sodium arsenate and sodium arsenite followed hydrazine in increasing the TCA-precipitable protein concentration in the IM. In the presence of all the inhibitors used there was more concentration of TCA-precipitable proteins in case of casein and soya as compared to GC, MC and SF. All the inhibitors used also decreased the concentration of TVFA in the IM. Per cent decrease in TVFA concentration was maximum in case of sodium arsenate and sodium arsenite, followed by hydrazine sulfate, hydrazine and its derivatives, DDIC inhibited the TVFA production to the least extent.

The above data also indicated that extent of increase in TCA precipitable proteins was greater in case of washed bacterial suspension as compared with SRF. Similar trend was shown by the effect of deaminase inhibitors on ammonia and TVFA production.

4.6.2 Effect of methanogenic inhibitors on the concentration of TCA-precipitable proteins, ammonia nitrogen and volatile fatty acids when intact proteins were incubated with strained rumen fluid or washed mixed bacterial suspension

4.6.2.1 Strained rumen fluid

Table 46 presents data on the effect of various methanogenic inhibitors on the concentration of TCA-insoluble proteins, ammonia nitrogen and TVFA in IM when intact feed proteins were incubated with SRF.

The decrease in concentration of ammonia-nitrogen was maximum in the presence of chloroform followed, in order, by monensin, chloral hydrate and sodium sulfite. The decrease in the concentration of ammonia-nitrogen in the presence of chloroform was 33.4, 41.8, 37.0, 33.8 and 29.3%, over the control values, in case of GC, MC, SF, Soya and casein and the corresponding values in case of monensin were 29.6, 33.8, 35.1, 28.7 and 24.8%. No definite trend was observed between the kind of inhibitor and the source of protein.

Monensin increased the concentration of TCA-precipitable proteins in the IM to maximum extent followed by chloroform, sodium sulfite and chloral hydrate. The concentration of TCA-precipitate proteins in the 1M was increased by 39.1, 33.8, 42.5, 73.9 and 86.1% in the presence of monensin, over the control value, in case of GC, MC, SF, Soya and casein. The corresponding values for chloroform were 33.9, 32.8, 28.7, 42.2 and 65.6%. In general, the concentration of TCA-insoluble proteins was more in case of

Table 46. Effect of various methanogenic inhibitors on the concentration of TCA-
precipitable proteins, ammonia-nitrogen and total-volatile fatty acid when
various intact feed proteins were incubated with strained rumen fluid for 8 h

Inhibitor (1.0 mmole)	Protein sources				
	Groundnut cake	Mustard cake	Sunflower seed	Soyabean seed	Casein
	Per cent increase (+) or decrease (-) over control				
Sodium sulfite					
i) TCA precipitable proteins	+33.6	+32.6	+36.5	+33.1	+46.0
ii) NH_3	-11.7	-15.9	-13.7	-18.3	-23.2
iii) TVFA	-71.5	-78.3	-75.4	-67.4	-64.5
Chloroform					
i) TCA precipitable proteins	+33.9	+32.8	+28.7	+42.2	+65.6
ii) NH_3	-33.4	-41.8	-37.0	-33.8	-29.3
iii) TVFA	-50.0	-56.1	-53.8	-41.1	-46.2
Chloral hydrate					
i) TCA precipitable proteins	+25.3	+37.6	+30.6	+36.4	+50.0
ii) NH_3	-20.1	-24.5	-21.4	-18.9	-21.3
iii) TVFA	-65.6	-70.3	-68.6	-62.8	-62.8
Monensin (50 ppm)					
i) TCA precipitable proteins	+39.1	+38.8	+42.5	+73.9	+86.1
ii) NH_3	-29.6	-33.8	-35.1	-28.7	-24.8
iii) TVFA	-54.7	-62.4	-58.9	-54.4	-51.6

FIG.15 EFFECT OF METHANOGENIIC INHIBITORS ON TCA-PRECITABLE PROTEINS WHEN INTACT FEED PROTEINS WERE INCUBATED WITH STRAINED RUMEN FLUID FOR 8 h.
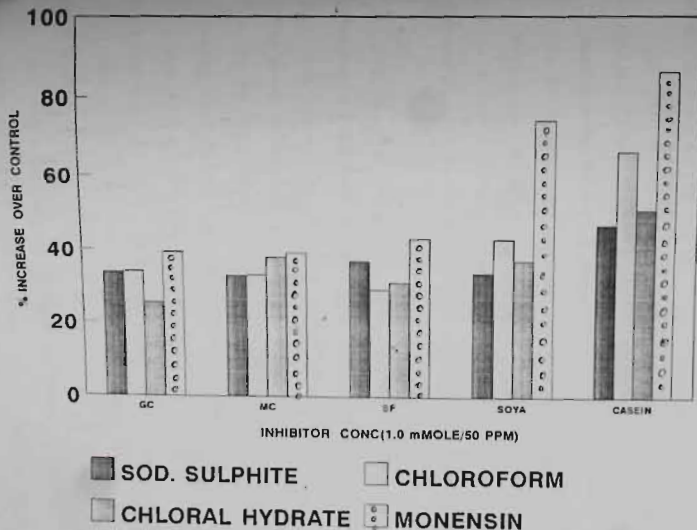
casein and soya as compared to other protein sources in the presence of all the inhibitors used. There was almost no difference between GC, SF and MC.

All the methanogenic inhibitors used decreased the concentration of TVFA in the incubation mixture.

4.6.2.2 Washed mixed bacterial suspension

Data on effect of various methanogenic inhibitors on the concentration of TCA insoluble proteins, ammonia nitrogen and volatile fatty acids in the IM when intact feed proteins were incubated with washed bacterial suspension is presented in table 47.

The ammonia concentration was maximally decreased in the presence of chloral hydrate followed by monensin, sodium sulphite and chloroform in case of all the protein sources used. The ammonia-nitrogen concentration in IM was decreased by 29.1, 36.5, 31.1, 27.9 and 28.8% over the control values. In the presence of chloral hydrate in case of GC, MC, SF, Soya and casein respectively and the corresponding values in case of monensin were 20.2, 25.1, 24.3, 28.0 and 26.4%. As with the SRF, no definite trend was observed between the kind of inhibitors used and the source of protein.

The concentration of TCA-precipitable proteins was also maximally increased in the presence of chloral hydrate followed by monensin, chloroform/sodium sulfite. The concentration of TCA precipitable proteins in the IM was increased by 27.5, 35.7 27.7, 40.8 and 49.7% over the control

Table 47. Effect of various methanogenic inhibitors on the concentration of TCA-precipitable proteins, ammonia-nitrogen and total-volatile fatty acid when various intact feed proteins were incubated with washed mixed bacterial suspension for 8 h

Inhibitor (1.0 mmole)	Protein sources				
	Groundnut cake	Mustard cake	Sunflower seed	Soyabean seed	Casein
	Per cent increase (+) or decrease (-) over control				
Sodium sulfite					
i) TCA precipitable proteins	+17.8	+22.5	+19.1	+20.9	+28.6
ii) NH_3	-20.0	-19.4	-25.3	-23.5	-21.3
iii) TVFA	-71.3	-70.8	-67.9	-63.3	-63.4
Chloroform					
i) TCA precipitable proteins	+19.0	+17.1	+12.3	+22.7	+32.5
ii) NH_3	-21.8	-29.3	-19.2	-10.9	-14.5
iii) TVFA	-65.2	-62.9	-65.9	-59.0	-54.4
Chloral hydrate					
i) TCA precipitable proteins	+27.5	+35.3	+27.7	+40.8	+49.7
ii) NH_3	-29.1	-36.5	-31.1	-27.9	-28.8
iii) TVFA	-39.7	-40.9	-37.7	-43.0	-39.4
Monensin (50 ppm)					
i) TCA precipitable proteins	+16.5	+18.2	+19.2	+34.6	+48.2
ii) NH_3	-20.2	-25.1	-24.3	-28.0	-26.4
iii) TVFA	-43.0	-43.4	-41.8	-41.1	-41.7

FIG.16 EFFECT OF METHANOGENIIC INHIBITORS ON TCA-PRECIPITABLE PROTEINS WHEN INTACT FEED PROTEINS WERE INCUBATED WITH WASHED BACTERIAL SUSPENSION FOR 8 h.
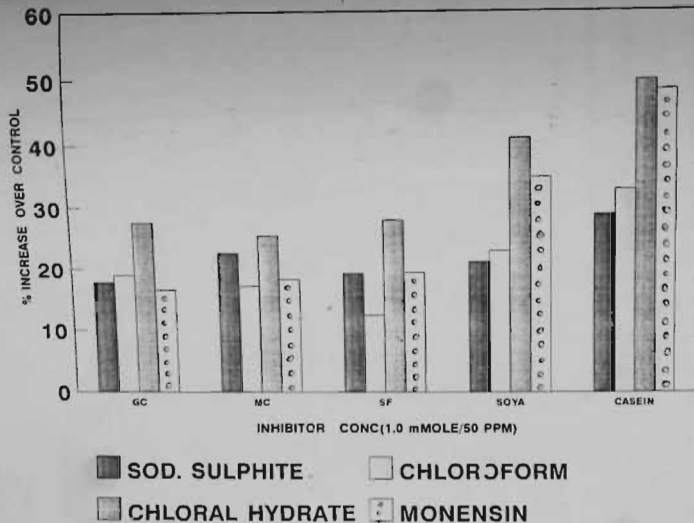
values, in case of GC, MC, SF, Soya and casein, respectively, in the presence of chloral hydrate and the corresponding values in case of monensin were 16.5, 18.2, 19.2, 34.6 and 48.2%. As the concentration of ammonia nitrogen increased, the concentration of TCA-precipitable proteins was maximally increased in case of casein, followed by Soya and GC/SF.MC in the presence of all the inhibitors used.

CHAPTER V

DISCUSSION

Dietary proteins entering the rumen are degraded by microbial proteases to peptides and amino acids (Hobson and Wallace, 1982a,b). Most of the nitrogen of amino acids after their deamination passes through ammonia pool prior to its incorporation into microbial proteins in the rumen (Hungate, 1966 and Chalupa, 1974). During this process, the ammonia produced especially from high quality proteins is not utilized for microbial protein synthesis (Chalupa, 1975). The excess ammonia is absorbed from the rumen and is lost as urea in the urine (Nolan and Leng, 1972). Depending upon various factors like solubility, structure and feed particle in which the protein resides etc. 40 to 60% of dietary proteins are degraded in the rumen (Leng and Nolan, 1984 and Mackie and Kistner, 1985). Undegraded proteins and the microbial proteins are digested post-ruminally and meet the amino acid requirement of the host animal. Quantitative losses upto 55%, however, occur during the conversion of dietary proteins into microbial proteins in the rumen (Chalupa, 1975). For efficient utilization of dietary proteins, it is, therefore, desirable

(1) to decrease the above mentioned loss during the conversion of dietary proteins into microbial proteins by depressing proteolysis, so that more dietary proteins are digested post ruminally, (ii) to depress the deamination of amino acids to ammonia so that either more amino acids are incorporated into microbial proteins or more amino acids leave the rumen undegraded and absorbed post-ruminally or the concentration of ammonia is decreased to avoid its losses through urine and (iii) to maximise utilization of ammonia for microbial protein synthesis. In this investigation, an effort has been made to identify various protein sources which are degraded in the rumen to lesser extent and hence their more utilization post ruminally, and to decrease the deamination of amino acids to ammonia.

The data reported in Tables 3 to 14 (Fig.1 to 4) indicated that intact proteins and their soluble and insoluble fractions of casein were degraded to maximum extent by strained rumen fluid (SRF) and its fractions, followed in order, by soyabean seed meal (Soya), groundnut cake (GC), mustard cake (MC) and sunflower seed meal (SF). Soluble protein fraction from all the feed sources was degraded maximally followed by intact proteins and insoluble protein fractions. The rapid degradation of soluble proteins as compared with insoluble proteins may be due to their more access to rumen microbial proteases. The degradation of insoluble proteins indicate that feed proteins can be hydrolysed directly from the solid phase

without an intervening soluble phase. The data indicated that the rate of degradation of intact proteins, insoluble proteins and even the soluble proteins by rumen microbes differs greatly from different protein sources. These results corroborated the earlier observations made by Nugent and Mangan (1978). Mahadevan et al.(1983) suggested that access to protein by proteolytic enzymes is influenced by the three dimensional structure of the protein molecule. Proteins with extensive cross linking, such as disulfide bonds, are less accessible to proteolytic enzymes and hence relatively resistant to degradation. The data of the present investigation also suggested that in addition to solubility, the structure of protein may also determine the rate of degradation of protein in the rumen. Wallace and Kopecny (1983) while studying the degradation of different types of proteins by rumen bacterial proteases also suggested that while the solubility was an important factor, the secondary and tertiary structure of protein has a major influence on its rate of digestion.

Maximum degradation of intact proteins and their soluble and insoluble fractions from different feed sources by SRF, followed by, cell free supernatant, bacteria and protozoa (Tables 3 to 14 and Figs.1 to 4)) indicated that major proteolytic activity in the rumen fluid is extracellular. Out of cell bound proteolytic activity, major activity is associated with bacteria. Protozoa degraded the proteins to minimum extent. Cotta and Hespell (1986) observed in case of

pure culture of rumen bacteria _Bacteroides fibrisolvens_ that 90% of the proteolytic activity was present in culture fluid and very less activity was associated with the cells. Mackie and White (1990) also indicated that _Bacteroides amylophilus_ produced both cell free and cell bound proteolytic activity, which consistently amounts to 20 and 80%, respectively of the total activity. Kopecny and Wallace (1982) also reported that most of the proteolytic activity was associated with coat and capsular material of mixed rumen bacteria. This activity was removable by gentle physical methods such as shaking and brief blending but without cell disruption. Brock _et al._(1982) indicated that approximately 25% of the proteolytic activity of rumen content was recovered in the SRF and balance of the activity was associated with the particulate fraction. The specific activity of proteases from bacterial fraction was 6 to 10 times higher than that of the protozoal fraction.

The data on the production of ammonia from intact proteins and their fractions by SRF and its fractions (Tables 17 to 28 and Figs.5 to 8) indicated that maximum ammonia was produced from casein, followed, in order, by Soya, GC/MC/SF and SRF was more active in the production of ammonia, followed by cell free supernatant and bacteria/protozoa. This data and that observed on the proteolysis of the proteins (Tables 3 to 14, Figs.1 to 4) suggested that the extent of deamination followed the degradation of proteins.

The data given in Tables 15 and 16 indicated that

different amino acids were degraded at different rates by both SRF and washed bacterial suspension. Depending upon the rate of degradation by SRF, amino acids were grouped into four categories. Serine, cysteine and aspartate (87-92%) were readily degraded, followed by arginine, phenylalanine, threonine, glutamate, glutamine, lysine, tyrosine and cystine (44-70%), tryptophan alanine, methionine and histidine (30-35%) and isoleucine, valine, glycine, hydroxyproline and proline (9-18%). The washed bacterial suspension also catabolised the amino acids approximately in same sequence of magnitude, but the magnitude of catabolism was lower than with SRF. The incubation of individual amino acids with mixed rumen bacterial extract (Table 39) indicated similar pattern of amino acid deamination as with SRF and washed bacterial suspension but the extent of degradation of the last two groups was higher even than that with SRF. This data supported the earlier observations made by Lewis and Emery (1962a), who divided the degradation of individual amino acids by SRF into three groups with regard to their relative rate of deamination. Serine, cysteine, aspartic acid, threonine and arginine were attacked most completely (80-100%), followed by glutamic acid, phenylalanine, lysine and cystine forming the intermediate group (47-75%), and third group in which deamination was much less pronounced was tryptophan Y-amino valeric acid, methionine, alanine, valine, isoleucine, ornithine, histidine, glycine, proline and hydroxyproline (8-37%). They have also

indicated that deamination rates were more rapid and complete in rumen liquor than in washed cell suspension.

With a view to decrease the deamination of amino acids for better animal productivity, the deaminase activity from mixed rumen bacterial extract was characterized. The pH and temperature optima for deaminase activity was found to be 6.75 and 45°C, respectively (Tables 29 and 30, Figs.9 and 10). The enzyme activity was found to be heat stable up to 50°C (Table 31, Fig.11). There is no report available on deaminase from rumen microorganism in this respect but the proteases from rumen bacteria has pH and temperature optima between 5.1 and 7.5 and 50°C, respectively (Kopecny and Wallace, 1982).

Amongst the various cations used, Na^+ and K^+ ions at 10 mmoles concentration increased the deaminase activity by 27.3 and 21.4 per cent respectively, while Zn^{++} and NH_4^+ ions at 10 m moles concentration decreased the deaminase activity by 37.9 and 15.4% respectively (Table 33). The enhancement of activity by Na^+ and K^+ indicated the presence of Na and K activated deaminase in mixed rumen bacterial extract. The inhibition of activity by Zn^{++} suggested the involvement of thiol group at the active centre of the enzyme. The inhibition of deaminase activity by NH_4^+ ions may be due to response of enzyme to increased NH_4^+ concentration (product inhibition). At 10 m moles all the heavy metals like Ag^+, Hg^{++} and Pb^{+2} drastically concentration inhibition the deaminase activity.

The decrease in the activity of deaminase in the

presence of chelating agents such as sodium oxalate, sodium citrate, sodium tartarate, sodium tartarate and EDTA (Table 34) indicated that metallo-enzyme nature of deaminase. The increase in activity of deaminase by various reducing agents like dithiothreitol and cysteine hydrochloride again suggested the involvement of thiol groups for its catalytic activity. The drastic decrease in the activity with p-chloromercuribenzoate, again confirms that the deaminase requires - SH groups for its activity.

Hydrazine and its derivatives strongly inhibited the deaminase activity (Table 35). It has been shown by Sauberlich (1968) that hydrazine and its derivatives are antagonistic to vitamin B_6 probably by reacting with carboxyl group of pyridoxyl and pyridoxal phosphate. Since vitamin B_6 is involved in metabolism of amino acids (Amenta and Johnston, 1963), it is possible that enzyme bound carbonyl group is involved in the catalytic process. Similar suggestions were given by Smith et al.(1967) and Broderick and Baltherp, Jr.(1979). The differences observed in the effectiveness of deamination inhibition by hydrazine and its derivatives may be related to relative differences in attacking the enzyme bound carbonyl group.

Sodium arsenate and sodium arsenite were also found to be very effective deaminase inhibitors (Table 35). It is possible that these compounds decrease the deamination of amino acids by inhibiting the reductive step in stickland type

reactions (Nisman, 1954). The inhibition of deamination by phosphoramidon (Table 35) again confirmed that deaminase is a metalloenzyme. Forsberg et al.(1984) while working on the protease activities of rumen protozoa also observed about 83% inhibition of deamination by phosphoramidon. DDIC at concentrations as low as 50 ppm reduced the deamination by 80.7% (Table 35). An interesting observation made by Broderick and Balthorp, Jr. (1979) that DDIC reduced drastically the alanine degradation and suggested a possible special role of alanine in ruminal deamination. Alanine may be an intermediate in deamination of other amino acids possibly being formed from transamination of these amino acids with pyruvate. Alanine deamination to ammonia and pyruvate with subsequent decarboxylation to acetate and carbon dioxide involves the generation of reducing equivalents (Annison and Lewis, 1959). Oxidation of reducing equivalents (NADH) generated is necessary for further deamination of amino acids. Gatley et al.(1975) actually observed the inhibition of the oxidation of reducing equivalents (NADH) in isolated rat hepatocytes in the presence of DDIC. It is, therefore, possible that DDIC in the present investigation inhibited the amino acids deamination by reducing the regeneration of NADH. The results obtained in the present investigation confirmed the observations made by Broderick and Balthorp, Jr. (1979) in an in vitro experiment that hydrazine and its derivatives like phenylhydrazine, p-nitrophenyl-hydrazine and methylhydrazine, sodium arsenite and DDIC were

the effective inhibitors of amino acids deamination.

It is known that the deamination of amino acids produced ammonia and reduced co-factors (most likely NADH) that must be reoxidized to continue the deamination process. This oxidation can be coupled to hydrogen formation via the enzyme hydrogenase, provided that the partial pressure of hydrogen remains low. Methanogenic rumen bacteria utilize the hydrogen to produce methane and hence keep the partial pressure of hydrogen low enough that hydrogenase activity is feasible (Wolin, 1975). When hydrogenase activity and subsequent methane production is inhibited by various methanogenic inhibitors, there may be accumulation of reduced cofactors. Under these circumstances, highly reduced amino acids like leucine, valine and isoleucine could only be fermented if acceptors of hydrogen, oxidized amino acids, were available (Nisman, 1954). It has been shown by Russell and Martin (1984) that in mixed anaerobic bacterial culture incubations, methanogenesis was a primary hydrogen acceptor process and a reduction of interspecies hydrogen transfer can inhibit the fermentation of highly reduced amino acids. Keeping this fact in view, the effect of various methanogenic inhibitors on the deamination of amino acids was investigated in the present study (Table 36). All the methanogenic inhibitors used (like monensin, chloroform and chloral hydrate) decreased the deamination of amino acids to the extent of 50 to 60%. Russell and Martin (1984) also observed inhibition of amino acid

fermentation to the extent of 50% by carbon monoxide, chloroform, monensin and lasalocid. Similar effect of monensin was also observed by other workers (Van Nevel and Demeyer, 1977; Van Nevel and Demeyer, 1990; Chen and Russel, 1991; Yang and Russel, 1993). Amongst various anions used, sodium hypochlorite at 10 m mole concentration decreased the deaminase activity by 29.1% (Table 37).

In the present investigation, an effort has been made to localise the deaminase activity in mixed rumen bacteria (Table 40). It was observed that 65% of the activity was present in the cytosol and remaining 35% in the membranes. Out of the membrane bound enzyme activity, about 90% was present in the inner membranes. No report is available in literature on this aspect. However, localization of protease activity has been demonstrated. Kopecny and Wallace (1982) observed that major part of the proteolytic activity was localized in the cytosol. The particulate envelope bound activity occurred mainly in the inner membrane of mixed rumen bacteria.

The reduction in deamination in the presence of various inhibitors used, shall spare the amino acids (i) for utilization in ruminal microbial protein synthesis, (ii) for their absorption post-ruminally. In the present investigation, an effort has been made to measure the biosynthesis of proteins from various feed proteins by rumen microorganisms in the presence of some selected inhibitors.

The data given in Tables 44, 45, 46, 47 indicated that

DDIC, **sodium arsenate**, sodium arsenite, hydrazine and its **derivatives** (deaminase inhibitors) and chloroform, monensin, **chloral hydrate** and sodium sulfite (methanogenic inhibitors) **significantly** increased the concentration of TCA-precipitable proteins in the incubation mixture when casein, soya, GC, MC and SF were incubated with strained rumen fluid or washed mixed bacterial suspension increased concentration of TCA-precipitable proteins in the incubation mixture. The presence of these inhibitors may be due to more availability of free amino acids for protein biosynthesis by the rumen microbes. This observation is of great significance because the free amino acids released, which otherwise would have been irreversibly lost after their deamination, are used for protein biosynthesis by rumen microbes in the presence of inhibitors. Those amino acids which are incorporated into microbial proteins shall now be available to the host animal post-ruminally for better animal production. All these inhibitors, however, decreased the concentration of total volatile fatty acids in the incubation mixture. Van Nevel and Demeyer (1990) has also shown that monensin and DDIC significantly decreased the concentration of total volatile fatty acids when casein was incubated _in_ _vitro_ with rumen fluid.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Microbial proteases extensively degrade dietary proteins to peptides and amino acids in the rumen. The free amino acids released are then deaminated to ammonia. The ammonia, so produced, is the main source of nitrogen for protein synthesis by rumen microorganisms. Undegraded dietary proteins and the microbial proteins are digested post-ruminally and meet most of the amino acids requirements of the host animal. All the ammonia produced, however, is not utilized for protein synthesis and the excess is absorbed from the rumen and is lost as urea in the urine. Quantitative losses upto 55% may occur during the transformation of dietary proteins to microbial proteins in the rumen. For efficient utilization of dietary proteins in ruminants, it is desirable that maximum dietary proteins leave the rumen in the undegraded form and lesser deamination of free amino acids in the rumen. In view of the above facts, an effort was made in the present investigation to screen various feed protein sources which are more resistant to ruminal degradation and to depress deamination reaction for better animal productivity.

In vitro degradation of various intact feed proteins and their soluble and insoluble fractions with strained rumen fluid and its fractions viz. bacteria, protozoa and cell-free supernatant

Strained rumen fluid (SRF) was drawn from two rumen fistulated buffalo bulls before feeding in the morning and fractionated into bacterial, protozoal and cell-free fractions. The volume of bacterial suspension, protozoal suspension and cell-free supernatant made was equivalent to the volume of SRF taken for its fractionation. Casein, soyabean seed meal (soya), sunflower seed meal (SF), groundnut cake (GC) and mustard cake (MC) intact proteins and their and soluble and insoluble fractions were incubated with SRF, bacterial suspension, protozoal suspension or cell-free supernatant at 39°C for 0, 2, 4, 6, 8 and 12 h and the incubation mixture (IM) analysed for residual protein and ammonia-nitrogen. In this section results of protein degradation at 12 h and ammonia-N conc. at 4 h of incubation are only given.

The intact feed proteins of casein, soya, GC, MC and SF were degraded to the extent of 62.0, 57.3, 46.8, 38.3 and 38.3% by SRF, 54.3, 51.2, 46.5, 42.5 and 39.5% by cell-free supernatant, 36.2, 34.2, 27.2, 24.5 and 22.2% by bacterial suspension and 14.7, 14.0, 9.2, 6.2 and 5.7% by protozoal suspension, respectively.

The soluble proteins of casein, soya, GC, MC and SF were degraded to the extent of 79.0, 68.0, 73.0, 62.2, and 64% by SRF, 64.0, 58.0, 54.2, 52.0 and 50.7% by cell-free

supernatant, 39.7, 37.0, 32.7, 29.0 and 24.5% by bacterial suspension and 13.0, 12.0, 5.5, 4.5 and 6.0% by protozoal suspension, respectively.

The insoluble proteins of casein, soya, GC, NC and SF were degraded to the extent of 69.5, 57.2, 54.7, 44.0 and 48.2% by SRF, 40.7, 37.2, 32.0, 28.5 and 27.0% by cell-free supernatant, 32.0, 30.2, 19.5, 22.5 and 17.2% by bacterial suspension and 27.2, 24.5, 14.2, 14.5 and 12.5% by protozoal suspension, respectively.

These results indicated that intact, soluble and insoluble proteins of casein were degraded maximally by SRF and its fractions followed, in order, by soya, GC, MC and SF. SRF degraded these proteins to maximum extent followed, in order, by cell-free supernatant, bacterial suspension and protozoal suspension. Soluble proteins were degraded to maximum and insoluble proteins to minimum extent with all the rumen fluid fractions.

The ammonia production from intact feed proteins of casein, soya, GC, MC and SF was 39.6, 30.1, 28.3, 26.9 and 27.1 with SRF, 31.5, 27.5, 22.4, 22.2 and 22.3 with cell-free supernatant, 19.9, 18.7, 15.9, 15.8 and 16.3 with bacterial suspension and 22.4, 20.3, 19.2, 17.8 and 16.6 (8 h) mg ammonia-N/100 ml SRF equivalent with protozoal suspension, respectively.

The ammonia produced from soluble proteins of casein, soya, GC, MC and SF was 45.8, 40.8, 36.8, 34.2 and 32.5 with

SRF, 35.2, 32.8, 27.8, 25.8 and 26.1 with cell-free supernatant, 28.9, 24.8, 18.7, 22.8 and 23.8 with bacterial suspension and 21.2, 19.6, 15.2, 14.6 and 14.4 (8 h) mg ammonia N/100 ml SRF equivalent with protozoal suspension, respectively.

The ammonia production from insoluble proteins of casein, soya, GC, MC and SF was 26.7, 23.4, 18.3, 19.3 and 20.6 with SRF, 23.1, 20.3, 15.8, 13.7 and 14.8 with cell-free supernatant, 17.3, 15.9, 14.2, 13.8 and 13.5 with bacterial suspension and 24.8, 22.3, 20.2, 19.6 and 20.1 (8 h) mg ammonia N/100 ml SRF equivalent with protozoal suspension, respectively.

These results indicated that intact, soluble and insoluble proteins of casein produced more ammonia-N with SRF and its fractions followed, in order, by soya, GC/MC/SF. SRF produced more ammonia-N from all the proteins followed by cell-free supernatant, bacterial/protozoal suspension. Ammonia produced by SRF, cell-free supernatant and bacterial suspension was maximum from soluble proteins followed by intact proteins and insoluble proteins. Protozoal suspension, however, produced ammonia in almost equal amounts from both intact and soluble proteins followed by insoluble proteins. The extent of ammonia produced depended upon the extent of degradation of proteins.

Incubation of individual amino acids with SRF or bacterial suspension for 8 h showed that different amino acids were degraded at different rates. With regard to their relative

rate of degradation, the amino acids were divided into four groups: Serine, cysteine and aspartic more readily degraded (87-92%), followed by arginine, phenylalanine, threonine, glutamate, glutamine, lysine, tyrosine and cystine (44-77%), tryptophan , alanine, methionine and histidine (30-35%) and isoleucine, valine, glycine, hydroxyproline and proline (9-19%). The SRF and bacterial suspension degraded the amino acids approximately in the same sequence, but the magnitude of degradation was more with SRF as compared to bacterial suspension.

Characterization of deaminase activity from mixed rumen bacterial extract

The bacterial pellet, obtained by differential centrifugation of SRF obtained from buffalo bulls 4 h after morning feeding, was sonicated and centrifuged to get the enzyme extract. This extract was termed as mixed rumen bacterial extract (MRBE) and was used to characterize the deaminase activity.

The pH and the temperature optima was found to be 6.75 and 45°C, respectively. The deaminase activity was found to be heat stable upto 50°C.

At 10 m moles concentration Na^+ and K^+ increased the activity by 27.3, 21.4% while NH_4^+ and Zn^{++} decreased the activity by 15.4 and 37.9% respectively, over the control values. Heavy metal ions like Ag^+, Pb^{++} and Hg^{++} at similar

concentrations drastically decreased the activity.

Chelating agents like sodium oxalate, sodium citrate, sodium tartarate and EDTA at 10 m moles concentration inhibited the activity by 20.5, 30.8, 44.9 and 45.7% respectively. p-CMB and sodium azide at 5 mmoles concentration decreased the activity by 88.3 and 73.0%. The reducing agents like DTT (10 m moles) and cysteine-HCl (5 m moles) enhanced the activity by 10.2 and 6.9%, respectively, over the control values.

At 1 m mole concentration hydrazine, hydrazine sulfate, phenylhydrazine and p-nitrophenylhydrazine inhibited the activity by 72.9, 68.2, 69.4 and 70.6%, respectively. Sodium arsenate and sodium arsenite at similar concentration decreased the activity by 75.9 and 78.7%. Hydroxylamine-HCl (1 m mole) DDIC and phosphoramidon (50 ppm) inhibited the deaminase activity by 45.9, 80.7 and 52.2%, respectively, over the control value.

Methanogenic inhibitors like monensin (50 ppm), chloralhydrate, chloroform and sodium sulfite (10 m moles) inhibited the activity by 50.7, 63.2, 54.9 and 28.5% respectively over the control value.

When the activity was traced in various fractions of SRF, it was observed that cell free supernatant, bacteria and protozoa carried 61.4, 43.7 and 9.1 of total activity in SRF (taken as 100). These results indicate that major portion of enzyme was released into cell-free supernatant and considerable activity was also contained in the bacterial cell. It was also

observed that in mixed rumen bacterial cell 65% of the activity was present in the cytosol and remaining 35% was associated with the membranes. Out of the membrane bound activity, about 90% was present in the inner membranes.

Effect of various deaminase and methanogenic inhibitors on protein biosynthesis and volatile fatty acids production when intact proteins were incubated with SRF or bacterial suspension

All the deaminase inhibitors used increased protein biosynthesis when intact proteins were incubated with SRF or bacterial suspension. The order of effectiveness of inhibitors for increasing the TCA-precipitable proteins in the IM was phenylhydrazine > sodium arsenate > DDIC > sodium arsenite > hydrazine > p-nitrophenylhydrazine > hydrazine sulfate > hydroxylamine-HCl when incubated with SRF and hydrazine sulfate > hydrazine > DDIC > sodium arsenate/sodium arsenite > hydroxylamine HCl > phenylhydrazine/p-nitrophenylhydrazine when incubated with bacterial suspension. It has been observed that increase in the concentration of TCA-Precipitable proteins in the presence of inhibitors was more with bacterial suspension than with SRF.

In the presence of all these inhibitors there was more concentration of TCA-precipitable proteins in case of casein and soya as compared to GC, MC and SF. All these inhibitors, however, decreased the concentration of total volatile fatty acids (TVFA) in the IM.

All the methanogenic inhibitors used increased protein

biosynthesis when intact proteins were incubated with SRF or bacterial suspension. The order of effectiveness of inhibitors for increasing the TCA-precipitable proteins in the IM was monensin > chloroform > sodium sulfite > chloralhydrate when incubated with SRF and chloralhydrate > monensin > chloroform/ sodium sulfite when incubated with bacterial suspension. In general, in the presence of inhibitors the concentration of TCA-insoluble proteins in IM was more in case of casein and soya as compared to GC, MC and SF. All these inhibitors also decreased the concentration of TVFA in the IM. In the presence of these inhibitors the per cent increase in the concentration of TCA-precipitable proteins was higher with casein (50-86% with SRF and 33-112% with bacteria) and soya (18-74% with SRF and 28-79% with bacteria) followed by MC (32-62% with SRF and 17-74% with bacteria), SF (30-58% with SRF and 12-64% with bacteria) and GC (25-52% with SRF and 19-75% with bacteria).

It can be concluded from the study that the proteins of GC, MC and SF may be better utilized than soya and casein by ruminants because these proteins were found to be more resistant to ruminal degradation. The proteins of GC may be slightly better than MC and SF. Out of large number of chemicals found to have inhibitory effect on deaminase activity, p-CMB, sodium azide, hydrazine and its derivatives, sodium arsenite, sodium arsenate, DDIC, monensin, chloroform, chloral hydrate and phosphoramidon were proved to be more effective deaminase inhibitors. All these inhibitors (except p-

CMB **and sodium azide** which were not tested in the present investigation) significantly increased the protein biosynthesis and hence may be useful for efficient utilization of dietary proteins by ruminants.

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APPENDIX

e means superscribed by letters a, b, c, d, e, f, g, h, i, j , k,

(P < 0.01) m, n, o, p, q, r, s,t, u, v, w and x(P < 0.05) in the pro-

eeding tables mean the following are significant

, m :	between Column I and II
, n :	between column II and III
, o :	between column I and III
l, p :	amongst the means of column I
e, q :	amongst the means of column II
f, r :	amongst the means of column III
g, s :	between column IV and V
h, t :	between column V and VI
i, u :	between column IV and VI
j, v :	amongst the means of column IV
k, w :	amongst the means of column V
l, x :	amongst the means of column VI

Table 1. Effect of hydrazine on TCA precipitable proteins when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TCA precipitable proteins left after incubation (mg)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	31.2 ± 0.73	$33.1^a\pm0.21$ (+6.21)	$34.8^{bc}\pm0.59$ (+11.39)	27.3 ± 0.28	$36.8^g\pm0.92$ (+34.88)	$37.1^b\pm0.48$ (+35.94)
MC	$28.6^d\pm0.32$	$32.2^{a,q}\pm0.39$ (+12.63)	$33.7^{br}\pm0.47$ (+17.87)	$25.3^j\pm0.43$	$34.3^{g,k}\pm0.63$ (+35.24)	$36.8^{h,i}\pm0.52$ (+45.04)
SF	$29.3^p\pm0.19$	$32.9^{a,q}\pm0.12$ (+12.41)	$33.7^b\pm0.83$ (+15.14)	$27.3^j\pm0.15$	$35.9^{g,k}\pm0.38$ (+31.44)	$35.6^{hl}\pm0.11$ (+30.45)
Soya	$24.1^d\pm0.62$	$26.7^{a,e}\pm0.17$ (+10.82)	$27.1^{bf}\pm0.22$ (+12.61)	$20.1^j\pm0.44$	$30.1^{g,k}\pm0.57$ (+10.1)	$32.1^{h,u,1}\pm0.87$ (+17.56)
Casein	21.3 ± 0.81	$23.7^{a,e}\pm0.46$ (+11.49)	$24.2^{b,f}\pm0.31$ (+13.64)	$17.3^j\pm0.27$	$26.4^{g,k}\pm0.72$ (+52.01)	$28.7^{h,i,1}\pm0.13$ (+65.47)

Figures represent mean of four observations + SD in this and proceeding tables.
Figures in parenthesis represent percent(+) increase or (-) decrease over control in this and proceeding tables.

Conditions of incubation:

200 mg soluble starch, 40 mg intact protein on crude protein basis, 10 ml strained rumen fluid, 9 ml buffer and 0.1 and 1.0 m mole hydrazine in 1 ml buffer were incubated for different periods.

Table 2. Effect of hydrazine on Ammonia produced when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	Ammonia-N (mg/100 ml RF)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	30.4 ± 0.42	$27.4^{a}\pm0.45$ (-10.-4)	$25.3^{b,c}\pm0.37$ (-16.84)	23.4 ± 0.59	$22.1^{s}\pm0.42$ (-5.38)	$20.3^{h,u}\pm0.88$ (-12.94)
MC	$27.6^{d}\pm0.41$	$24.3^{e,a}\pm0.82$ (-11.83)	$22.^{b,f,c}\pm0.14$ (.19.37)	21.39 ± 0.26	$20.3^{k}\pm0.98$ (-4.77)	$18.2^{h,l,u}\pm0.45$ (-14.56)
SF	$28.8^{d}\pm0.23$	$25.1^{a}\pm0.91$ (-12.92)	$24.6^{b,f}\pm0.92$ (-16.6)	$22.8^{f}\pm1.01$	$18.7^{9,k}\pm0.16$ (-17.91)	$17.3^{x,h,i}\pm0.22$ (-24.03)
Soya	$34.6^{d}\pm0.75$	$30.4^{a,}\pm0.22$ (-12.06)	$28.1^{c,b,f}\pm0.19$ (-18.73)	$27.8^{j}\pm0.19$	$26.6^{9,k}\pm0.44$ (-4.24)	$22.1^{h,e,i}\pm0.57$ (-20.31)
Casein	$38.1^{d}\pm0.81$	$32.7^{a,e}\pm0.16$ (-14.24)	$30.6^{c,b,f}\pm0.76$ (-19.84)	$30.3^{j}\pm0.23$	$27.5^{9}\pm0.41$ (-9.19?)	$23.1^{h,x,i}\pm0.36$ (-23.75)

Conditions of incubation: same as in table 1.

Table 3. Effect of hydrazine on Total Volatile fatty acids when various feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TVFA produced (m moles/100 ml RF)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	32.6±0.89	27.5[a]±1.99 (-15.64)	25.9[b,c]±1.87 (-20.55)	34.1±1.62	20.19±1.12 (-41.11)	17.4[h]±1.23 (-49.01)
MC	31.8±1.72	24.3[a]±2.1 (-23.58)	22.7[b]±1.72 (-28.61)	32.2±2.21	18.89±1.09 (-41.16)	15.6[h]±1.49 (-51.55)
SF	32.1±1.46	26.8[a]±1.65 (-16.51)	24.1[b]±1.69 (-24.92)	33.4±1.17	18.99±1.56 (-43.41)	15.9[h,u]±1.44 (-52.39)
Soya	36.3P±2.19	34.6[e]±1.92 (-4.68)	30.4[b,f,c]±1.06 (-16.25)	38.8j±1.63	30.6[9,k]±1.42 (-21.13)	27.3[h]±2.12 (-29.64)
Casein	39.04±1.06	37.2[m,w]±1.12 (-5.58)	32.8[b,r,c]±1.18 (-16.75)	41.9[v]±1.76	31.89±1.29 (-.24.11)	28.6[h,u]±1.77 (~31.74)

Conditions of incubation: same as in table 1.

Table 4. Effect of hydrazine sulphate on TCA precipitable proteins when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TCA precipitable proteins left after incubation (mg)					
	Time of incubation					
	4 h			8 h		
	Concentratioin of inhibitors(m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	30.5 ± 0.87	31.1 ± 0.41 (+2.13)	$33.8^{b,c}\pm0.15$ (+11.09)	$26.6^{c}\pm0.28$	33.19 ± 0.28 (+24.77)	$35.4^{h,i}\pm0.73$ (+33.28)
MC	$27.8^{p}\pm0.91$	$28.6^{a}\pm0.38$ (+2.69)	$29.8^{b,f,o}\pm0.26$ (+7.00)	$23.8^{j}\pm0.16$	$31.1^{k,g}\pm0.63$ (+30.62)	$32.8^{h,l,u}\pm0.87$ (+37.79)
SF	28.6 ± 0.77	$29.8^{m,e}\pm0.26$ (+4.26)	$31.1^{b,r,c}\pm0.19$ (+8.88)	25.1 ± 0.92	$32.8^{k,g}\pm0.34$ (+30.57)	$34.1^{h}\pm0.44$ (+35.66)
Soya	$23.5^{d}\pm0.42$	$25.3^{a,e}\pm0.41$ (+7.31)	$27.8^{b,f,c}\pm0.71$ (+18.22)	$20.5^{j}\pm0.47$	$26.5^{k,g}\pm0.44$ (+29.66)	$28.7^{e,h,i}\pm0.62$ (+40.22)
Casein	$20.8^{d}\pm0.34$	$21.3^{e}\pm0.72$ (+2.39)	$23.8^{b,f,c}\pm0.52$ (+14.19)	$17.6^{f}\pm0.13$	$23.9^{k,g}\pm0.19$ (+35.33)	$26.4^{h,l,i}\pm0.84$ (+49.51)

Conditions of incubation   same as in table 1 except 0.1 and 1.0 m moles of hydrazine
                            sulphate in 1 ml buffer were added to incubation mixture
                            instead of hydrazine.

Table 5. Effect of hydrazine sulfate on Ammonia produced when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	Ammonia-N (mg/100 ml RF)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	35.8 ± 0.41	$33.6^{a}\pm0.38$	$30.3^{b,c}\pm0.33$	27.6 ± 0.41	$24.3^{g}\pm0.32$	$20.8^{h,i}\pm0.14$
MC	30.5 ± 0.32	$28.4^{e,a}\pm0.92$ (-6.69)	$24.4^{f,b,c}\pm0.69$ (-20.02)	$25.8^{j}\pm0.772$	$21.0^{g,k}\pm0.48$ (-18.82)	$17.6^{h,l,i}\pm0.31$ (-31.75)
SF	$33.2^{d}\pm0.42$	$29.8^{a}\pm0.71$ (-10.03)	$27.5^{c,f,b}\pm0.61$ (-17.05)	26.7 ± 0.46	$27.9^{g,k}\pm0.21$ (-14.4)	$19.5^{h,x,i}\pm0.96$ (-26.87)
Soya	$37.5^{d}\pm0.59$	$34.8^{a,e}\pm1.02$ (-7.22)	$30.8^{c,l,f}\pm0.43$ (-17.96)	$30.6^{v}\pm0.71$	$26.4^{g,k}\pm0.33$ (-13.58)	$22.3^{k,l,i}\pm0.34$ (-26.86)
Casein	$40.7^{d}\pm0.44$	$36.3^{a}\pm0.93$ (-10.82)	$32.5^{c,b,r}\pm0.86$ (-19.97)	$32.3^{j}\pm0.18$	$28.5^{g,k}\pm0.27$ (-11.55)	$23.9^{h,l,i}\pm0.61$ (-26.05)

Conditions of incubation : same as in table 4.

Table 6. Effect of hydrazine sulfate on Total Volatile fatty acids production when various intact feed proteins were incubated with SRF for 4 and 8 h

Protein source	TCFA produced (m moles/100 ml RF)					
	Time of incubation					
	4 h	Concentration of inhibitor (m moles)		8 h		
	Conttol	0.1	1.0	Control	0.1	1.0
GC	34.2 ± 1.03	$30.2^{a}\pm1.23$ (-11.69)	$29.2^{b}\pm1.19$ (-14.61)	35.8 ± 1.59	$26.2^{g}\pm0.98$ (-26.81)	$22.1^{h,i}\pm1.17$ (-38.27)
MC	$31.8^{d}\pm1.62$	$26.8^{m,p}\pm1.86$ (-15.72)	$27.5^{n}\pm1.82$ (-13.52)	33.3 ± 1.43	$23.6^{w,q}\pm1.06$ (-29.12)	$20.8^{h,u}\pm1.38$ (-37.5)
SF	32.1 ± 0.45	$27.9^{a}\pm1.61$ (-13.08)	$28.6^{b}\pm1.32$ (-10.9)	34.2 ± 1.72	$25.2^{g}\pm1.85$ (-26.32)	$21.5^{h,u}\pm1.81$ (-37.13)
Soya	$36.9^{d}\pm1.09$	$32.8^{a,e}\pm1.19$ (-11.11)	$30.2^{b,o}\pm1.35$ (-18.15)	38.4 ± 1.66	$30.5^{g,u}\pm2.01$ (-20.57)	$25.7^{h,x,u}\pm2.12$ (-33.07)
Casein	38.5 ± 0.89	$33.6^{a}\pm1.25$ (-12.72)	$31.7^{b}\pm1.49$ (-17.66)	$39.6^{v}\pm1.93$	$32.6^{g}\pm2.13$ (-17.68)	$27.3^{h,u}\pm1.74$ (-31.06)

Conditions of incubation: same as in table 4.

Table 7. Effect of phenyl hydrazine on TCA precipitable proteins when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TCA precipitable proteins left after incubation (mg)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	27.1 ± 0.38	$30.1^{a}\pm0.42$ (+11.2)	$31.6^{b,o}\pm0.51$ (+16.70)	23.5 ± 0.92	$32.7^{g}\pm0.72$ (+39.41)	$35.7^{h,i}\pm0.11$ (+52.27)
MC	$25.4^{P}\pm0.72$	$28.5^{a,q}\pm0.98$ (+12.0)	$29.1^{b,f}\pm0.86$ (+14.72)	$21.2^{g}\pm0.45$	$30.2^{g,k}\pm0.32$ (+42.33)	$34.5^{h,l,i}\pm0.41$ (+62.85)
SF	26.2 ± 0.17	$29.6^{a}\pm0.42$ (+12.9c)	$30.8^{b,r,o}\pm0.64$ (+17.81)	21.8 ± 0.71	$31.6^{g,w}\pm0.12$ (+44.85)	$34.5^{h,i}\pm0.32$ (+57.94)
Soya	$20.6^{d}\pm0.24$	$23.6^{a,e}\pm0.62$ (+13.12)	$25.3^{b,f,o}\pm0.74$ (+24.21)	$17.3^{j}\pm0.36$	$26.4^{j,k}\pm0.28$ (+52.54)	$29.3^{h,i,l}\pm0.65$ (+69.28)
Casein	$17.3^{d}\pm0.81$	$20.2^{a,e}\pm0.79$ (+16.62)	$23.4^{c,b,f}\pm0.16$ (+35.1)	$15.3^{j}\pm0.77$	$24.0^{j,k}\pm0.46$ (+56.93)	$27.5^{l,h,i}\pm0.52$ (+79.71)

Conditions of incubation: same as in table 1 except 0.1 and 1.0 m moles of phenyl hydrazine in 1 ml buffer were added to incubation mixture instead of hydrazine.

Table 8. Effect of phenyl hydrazine on Ammonia produced when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	Ammonia-N (mg/100 ml RF)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
SC	33.4±0.91	31.3[m]±0.44 (-6.1?)	29.4[b,c]±0.33 (-11.85)	25.4±0.53	21.5[g]±0.91 (-15.29)	18.5[h,i]±0.46 (-26.96)
MC	32.2±1.02	28.6[a,e]±0.52 (-11.22)	26.4[b,c,f]±0.65 (-18.07)	22.8[j]±0.46	19.3[g,w]±0.44 (-15.20)	15.2[l,h,i]±0.19 (-33.56)
SF	32.9±0.84	29.2[a]±0.18 (-11.17)	27.5[b,c,f]±0.75 (-16.?3)	23.6±0.72	20.8[w]±0.32 (-11.97)	16.9[l,h,i]±0.28 (-28.42)
Soya	36.4[d]±0.62	33.3[a,e]±0.44 (-8.66)	30.3[b,c,f]±0.97 (-16.84)	29.3[j]±0.66	25.8[k]±0.17 (-11.95)	20.9[l,h,i]±0.64 (-28.82)
Casein	39.2[d]±0.31	36.8[a,e]±0.63 (-6.02)	32.8[b,c,f]±0.31 (-16.29)	31.5[j]±0.18	27.6[k]±0.38 (-12.14)	22.1[l,x,i]±0.34 (-29.88)

Conditions of incubation: same as in table 7.

Table 9. Effect of phenyl hydrazine on Total Volatile fatty acids production when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TVFA produced (m moles/100 ml RF)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
SC	33.8 ± 1.29	$26.7^{a}\pm1.65$ (-21.00)	$24.6^{b,o}\pm1.61$ (-27.21)	35.2 ± 1.21	$21.3^{g}\pm1.93$ (-39.37)	$19.4^{h,b}\pm1.14$ (-44.88)
MC	$30.6^{p}\pm1.85$	$23.2^{a,e}\pm1.74$ (-24.18)	$20.8^{b,c,f}\pm1.77$ (-32.02)	$32.4^{j}\pm1.17$	$17.6^{j,k}\pm1.88$ (-45.68)	$15.6^{h,i}\pm1.67$ (-51.85)
SF	$32.3^{p}\pm1.72$	$24.5^{a}\pm1.39$ (-23.69)	$22.3^{b,o,r}\pm1.58$ (-30.95)	$34.3^{v}\pm1.85$	$19.2^{q,w}\pm1.76$ (-44.02)	$17.5^{h,u,x}\pm1.89$ (-48.97)
Soya	$36.4^{d}\pm1.13$	$29.9^{a,e}\pm1.18$ (-19.23)	$26.2^{b,c,f}\pm1.51$ (-28.02)	$38.4^{j}\pm2.08$	$23.7^{q,k}\pm1.71$ (38.28)	$20.3^{l,u,x}\pm1.12$ (-47.13)
Casein	$39.3^{d}\pm1.39$	$32.4^{a,e}\pm1.46$ (-17.3)	$28.5^{b,c,r}\pm1.67$ (-27.48)	$42.3^{j}\pm1.91$	$25.8^{q,w}\pm1.52$ (-39.00)	$21.5^{l,i}\pm1.38$ (-49.17)

Conditions of incubation: same as in table 7.

Table 10. Effect of p-nitrophenyl hydrazine on TCA precipitable proteins when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TCA precipitable proteins left after incubation (mg)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	28.1 ± 0.14	$30.5^{a}\pm0.81$ (+8.36)	$31.8^{b,c}\pm0.92$ (-13.16)	$26.2^{c}\pm0.98$	33.69 ± 0.86 (+28.44)	$35.4^{h,u}\pm0.72$ (+35.47)
MC	$26.9^{d}\pm0.78$	$27.5^{e}\pm0.55$ (+5.2)	$27.9^{f,n}\pm0.24$ (+6.73)	$23.3^{j}\pm0.64$	$31.5^{w,g}\pm0.39$ (+34.99)	$33.2^{h,l,i}\pm0.38$ (+42.4)
SF	27.5 ± 0.32	$28.1^{f}\pm0.69$	$30.4^{f,n,o}\pm0.86$ (+10.45)	$25.6^{j}\pm0.59$	$32.4^{w,g}\pm0.19$ (+26.24)	$34.4^{h,i,x}\pm0.62$ (+34.04)
Soya	$24.8^{d}\pm0.41$	$25.6^{e}\pm0.72$ (+3.51)	$26.2^{f,n}\pm0.24$ (+5.65)	$22.9^{j}\pm0.51$	$28.1^{k,g}\pm0.91$ (+22.79)	$30.5^{h,i,l}\pm0.51$ (+33.35)
Casein	$22.8^{d}\pm0.26$	$23.2^{e}\pm0.44$ (+1.57)	$24.8^{c,b,f}\pm0.42$ (+8.45)	$20.2^{j}\pm0.33$	$30.5^{h,i}\pm0.11$ (+36.41)	(+51.38)

Conditions of incubation: Same as in table 1 except 0.1 and 1.0 m moles of p-nitrophenyl hydrazine in 1 ml buffer were added to incubation instead of hydrazine.

Table 11. Effect of p-nitro phenyl hydrazine on Ammonia produced when various intact feed proteins were incubated with SRF for 4 and 8 h

Protein source	Ammonia-N (mg/100 ml RF)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	37.3±0.48	34.7[a]±0.44 (-7.00)	32.6[b,c]±0.21 (-12.45)	35.2±0.81	31.2[g]±0.72 (-11.32)	27.3[h,i]±0.42 (-22.3)
MC	34.7[d]±0.92	31.2[e,a]±0.76 (-10.06)	28.1[b,f,c]±0.92 (-18.81)	31.4[j]±0.93	27.3[k,g]±0.18 (-12 91)	24.6[l,h,i]±0.28 (-21.64)
SF	36.5[p]±0.44	33.2[a,e]±0.38 (-8.92)	30.7[b,r,c]±0.76 (-15.97)	37.3[j]±0.24	28.1[w,g]±0.49 (-24.49)	26.1[l,h,i]±0.22 (-29.85)
Soya	38.1[p]±0.68	36.5[e,m]±0.69 (-4.22)	34.7[b,f,o]±0.84 (-9.05)	36.6±0.44	30.7[j,k]±0.31 (-16.2)	28.2[l,h,i]±0.34 (-23.08)
Casein	40.6[d]±0.62	37.3[a,q]±0.23 (-8.20)	35.8[b,c]±0.3 (-11.8)	37.3±0.49	32.6[g]±0.77 (-12.54)	29.4[x,h,i]±0.63 (-21.28)

Conditions of incubation: Same as in table 10

Table 12. Effect of p-nitrophenyl hydrazine on Total Volatile fatty acids production when various intact feed proteins were incubated with SRF for 4 and 8 h

Protein source	TVFA produced (m moles/100 ml RF)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	33.3±1.81	30.4±1.62 (-8.71)	27.6±1.72 (-17.11)	35.6±1.93	26.89±2.13 (-24.72)	21.3[h,u]±1.92 (-40.17)
MC	31.4±1.77	27.6[m]±124 (-12.1)	24.2[n]±1.89 (-22.93)	33.4±1.86	23.39±2.09 (-30.24)	20.2[h]±1.46 (-39.52)
SF	32.1±1.72	28.6[m]±1.33 (-10.9)	25.3±1.96 (-21.18)	34.2±1.98	25.19±1.76 (-26.61)	20.7[h,u]±1.42 (-39.47)
Soya	36.4[p]±1.12	32.8[e,m]±1.21 (-9.89)	30.1[r]±1.94 (-17.31)	38.9[v]±2.24	28.29,[w]±1.38 (-27.51)	23.5[h,x,i]±1.35 (-39.58)
Casein	39.2[p]±1.43	34.5[a]±1.62 (-11.99)	31.1[o]±1.38 (-20.15)	42.6±2.01	29.79±1.44 (-30.28)	24.8[h,b]±1.82 (-41.78)

Conditions of incubation: same as in table 10.

Table 13. Effect of Hydroxylamine on TCA precipitable proteins when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TCA precipitable proteins left after incubation (mg)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	31.3±0.21	32.4^a±0.18 (+3.48)	33.6^{b,c}±0.25 (+7.34)	28.6±0.12	30.6^g±0.66 (+7.28)	35.3^{h,i}±0.94 (+23.52)
MC	29.8^d±0.62	30.6^g±0.89 (+2.51)	31.4^{b,f}±0.72 (+5.32)	26.3^i±0.41	28.2^{g,w}±0.91 (+11.57)	34.4^h±0.77 (+35.93)
SF	30.6±0.87	31.3±0.23 (+2.28)	32.8^{b,r,c}±0.31 (+7.21)	26.3±0.83	29.7^g±0.44 (+12.87)	34.4^h±0.46 (+30.72)
Soya	26.3^d±0.96	27.6^e±0.68 (+4.89)	28.2^{b,f}±0.44 (+7.29)	23.6^j±0.39	25.8^{g,k}±0.27 (+9.63)	31.3^{h,i,x}±0.32 (+33.02)
Casein	29.5^p±0.29	28.5^{a,e}±0.27 (+5.47)	26.3^{b,f}±0.68 (+7.55)	21.5^v±0.92	24.4^{g,w}±0.68 (+13.38)	29.6^{h,i,l}±0.14 (+37.64)

Conditions of incubation: Same as in table 1 except amine HCl in 1 ml buffer in stead of hydrazine. 0.1 and 1.0 m moles of hydroxyl were added to incubation mixture

Table 14. Effect of Hydroxylamine HCl on ammonia produced when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	Ammonia-N (mg/100 ml RF)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	36.0 ± 0.82	$33.1^{a}\pm0.44$ (-7.89)	$31.5^{b,c}\,0.38$ (-12.26)	33.8 ± 0.88	$27.5^{h,i}\pm0.77$ (-7.92)	(-18 69)
MC	$32.4^{d}\pm0.96$	$30.6^{e,p,n}\pm0.29$	$29.5^{b,o,f}\pm0.49$	$31.0^{j}\pm0.21$	$26.2^{g,k}\pm0.29$ (-14.95)	$24.4^{h,i,l}\pm0.41$ (-21.09)
SF	$34.2^{p}\pm0.78$	$31.5^{a,q}\pm0.36$	$30.6^{r,b,o}\pm0.44$	31.5 ± 0.42	$28.3^{g,k}\pm0.24$ (-10.01)	$23.8^{h,i,l}\pm0.38$ (-17.99)
Soya	$38.6^{d}\pm0.69$	$36.4^{e,a}\pm0.21$	$32.5^{b,c,f}\pm0.72$	$36.3^{j}\pm0.92$	$33.1^{g,k}\pm0.38$ (-8.70)	$31.5^{h,i,l}\pm0.22$ (-13.11)
Casein	$40.3^{p}\pm0.91$	$38.3^{a,e}\pm0.72$	$36.0^{b,c,f}\pm0.68$	37.6 ± 0.73	$34.2^{g,k}\pm0.19$ (-9.19)	$32.4^{h,i,l}\pm0.28$ (-13.74)

Conditions of incubation: Same as in table 13.

Table 15. Effect of Hydroxylamine HCl on Total Volatile fatty acids production when various intact feed proteins were incubated with SRF for 4 and 8 h

Protein source	TVFA produced (m moles/100 ml RF)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	30.8 ± 1.42	$24.3^a \pm 1.89$ (-21.1)	$22.8^b \pm 1.17$ (-25.97)	32.6 ± 1.72	$21.3^g \pm 1.27$ (-34.66)	$18.5^h \pm 1.77$ (-43.25)
MC	28.6 ± 1.59	$22.1^a \pm 1.58$ (-22.73)	$20.7^b \pm 2.13$ (-27.62)	31.7 ± 1.69	$18.6^g \pm 1.81$ (-41.32)	$15.4^h \pm 1.43$ (-51.42)
SF	28.9 ± 1.47	$23.4^a \pm 1.52$ (-19.03)	$21.3^b \pm 1.47$ (-26.29)	32.1 ± 1.14	$20.1^g \pm 1.06$ (-37.38)	$15.8^h \pm 1.61$ (-50.77)
Soya	$33.7^d \pm 1.63$	$30.6^{e,m} \pm 0.71$ (-9.2)	$28.6^{b,f} \pm 1.62$ (-15.13)	$36.6^j \pm 1.29$	$25.4^{j,k} \pm 1.77$ (-30.6)	$22.3^{h,l} \pm 2.03$ (-39.07)
Casein	35.3 ± 0.24	32.1 ± 2.03 (-8.81)	$30.2^{b,o} \pm 1.89$ (-14.2)	$31.5^j \pm 1.84$	$27.8^s \pm 1.08$ (-29.62)	$24.2^{h,u,x} \pm 1.44$ (-38.73)

Conditions of incubation: Same as in table 13.

Table 16. Effect of Sodium Arsenate on TCA-precipitable proteins when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TCA precipitable proteins left after Incubation (mg)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	29.1 ± 0.92	$31.2^{a} \pm 0.56$ (+7.00)	$32.7^{b,o} \pm 0.69$ (+12.39)	26.2 ± 0.05	$35.4^{g} \pm 0.15$ (+35.32)	$37.8^{h,i} \pm 0.44$ (+44.57)
MC	$27.2^{d} \pm 0.54$	$28.2^{e,m} \pm 0.19$ (+3.71)	$29.9^{b,c,f} \pm 0.19$ (-9.99)	$23.8^{j} \pm 0.64$	$33.6^{g,k} \pm 0.78$ (+41.49)	$35.8^{h,i,l} \pm 0.38$ (+50.5)
SF	$28.8^{d} \pm 0.61$	$30.6^{m,q} \pm 0.91$ (+6.2)	$31.8^{b,f} \pm 0.46$ (+10.15)	24.8 ± 0.71 (+31.1)	$32.6^{g} \pm 0.82$ (+43.9)	35.8 ± 0.96
Soya	$25.1^{d} \pm 0.55$	$26.8^{e,m} \pm 0.76$ (+6.88)	$28.6^{b,f,o} \pm 0.51$ (+13.69)	$20.5^{j} \pm 0.29$	$29.9^{g,k} \pm 0.61$ (+46.24)	$32.8^{h,i,l} \pm 0.81$ (+60.35)
Casein	$20.2^{d} \pm 0.28$	$22.8^{a,e} \pm 0.84$ (+12.95)	$23.8^{b,f} \pm 0.46$ (+17.86)	17.3 ± 0.33	$27.2^{g,k} \pm 0.54$ (+57.21)	$31.2^{h,i,x} \pm 0.89$ (+80.02)

Conditions of incubation: Same as in table 1 except 0.1 and 1.0 m moles of sodium arsenate in 1 ml buffer were added to incubation mixture instead of hydrazine.

Table 17. Effect of Sodium Arsenate on Ammonia production when various intact feed proteins were incubated with SRF for 4 and 8h.

Protein source	Ammonia-N (mg/100 ml RF)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	34.2 ± 0.44	$31.5^{a}\pm0.62$ (-7.86)	$27.5^{b,c}\pm0.19$ (-19.52)	30.5 ± 0.63	$26.4^{g}\pm0.42$ (-13.41)	$20.1^{h,i}\pm0.23$ (-33.98)
MC	$31.4^{d}\pm0.87$	$29.3^{m,q}\pm0.71$ (-4.93)	$25.2^{b,c,f}\pm0.76$ (-19.97)	$26.8^{j}\pm0.45$	$22.8^{g,k}\pm0.17$ (-15.01)	$16.5^{i,l}\pm0.42$ (-38.57)
SF	$32.8^{p}\pm0.26$	$30.4^{a}\pm0.36$ (-7.14)	$26.7^{b,c}\pm0.84$ (-18.34)	$28.4^{i}\pm0.39$	$24.5^{g,k}\pm0.27$ (-13.93)	$18.0^{h,i,x}\pm0.96$ (-36.81)
Soya	$38.5^{g}\pm0.39$	$36.2^{a,e}\pm0.44$ (-6.13)	$32.4^{b,c,f}\pm0.51$ (-15.84)	$35.8^{j}\pm0.82$	$32.2^{g,k}\pm0.42$ (-10.12)	$27.5^{h,i,l}\pm0.48$ (-23.03?)
Casein	$42.8^{d}\pm0.75$	$40.6^{m,e}\pm0.87$ (-5.25)	$37.3^{b,c,f}\pm0.83$ (-12.84)	$39.8^{j}\pm0.94$	$35.8^{g,k}\pm0.17$ (-10.18)	$28.4^{h,i}\pm0.56$ (-28.72)

Conditions of incubation: Same as in table 16.

Table 18. Effect of sodium Arsenate on Total volatile fatty acids production when various ointake feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TVFA produced (m moles/100 ml RF)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibhitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC.	34.8±1.32	29.7^a±1.24 (-14.65)	24.3^{b,c}±1.49 (-30.17)	36.5±1.91	21.6^g±1.08 (-40.82)	14.8^{h,i}±1.85 (-59.45)
MC	30.3^p±1.77	26.3^m±1.86 (-13.20)	21.5^{r,o}±1.55 (-29.04)	33.2^v±1.28	18.2^{g,k}±0.99 (-45.18)	12.3^{h,i}±1.72 (-62.95)
SF	32.4±1.85	27.4^m±1.75 (-15.43)	22.3^{b,o}±1.82 (-31.17)	34.7±1.77	20.4^{j,w}±0.86 (-14.21)	13.5^{h,i}±1.34 (-61.09)
Soya	37.9^d±1.61	31.9^{a,q}±1.17 (-15.83)	25.6^{b,c}±1.61 (-32.45)	42.8^j±1.31	25.8^{g,k}±1.43 (-39.72)	17.6^{h,i,x}±1.48 (-58.87)
Casein	40.1±1.44	35.3^{a,q}±1.22 (-12.19)	30.4^{b,c,f}±1.38 (-24.38)	45.3±1.12	27.3^g±1.31 (-39.73)	18.5^{h,i}±1.72 (-59.16)

Conditions of incubation: Same as intable 16.

Table 19. Effect of Sodium Arsenite on TCA-precipitable proteins when various intact feed proteins were incubated with SRF for 4 and 8 h

Protein source	TCA-precipitable proteins left after incubation (mg)					
	Time of incubation					
	4h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	30.4±0.39	33.7[a]±0.41 (+10.78)	34.8[b]±0.71 (+22.67)	28.9±0.58	35.6[g]±0.83 (+22.92)	37.4[h,u]±0.49 (+29.41)
MC	27.6[d]±0.79	30.8[a,e]±0.88 (+11.82)	32.5[b,r,o]±0.14 (+17.78)	24.4[j]±0.22	33.8[j,w]±0.91 (+38.61)	36.3[h,i,x]±0.32 (+48.87)
SF	28.9±0.91	32.1[a,q]±0.44 (+11.09)	33.6[b,r,c]±0.44 (+16.18)	26.3[v]±0.96	34.8[g]±0.81 (+32.44)	36.3±0.48 (+37.99)
Soya	26.3[p]±0.85	28.6[a,e]±0.28 (+8.71)	29.4[b,f,o]±0.61 (+11.79)	23.9[j]±0.46	30.8[j,k]±0.14 (+29.12)	32.5[h,i,l]±0.26 (+35.93)
Casein	22.6[d]±0.22	24.4[a,e]±o.36 (+8.19)	26.3[b,f,o]±0.72 (+16.48)	20.1[j]±0.28	27.6[j,k]±0.33 (+36.94)	31.2[h,i,x]±0.79 (+54.76)

Conditions of incubation: Same as in table 1 except 0.1 and 1.0 m moles of Sodium arsenite in 1 ml buffer were added to incubation mixture in stead of hydrazine.

Table 20. Effect of Sodium arsenite on ammonia produced when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	Ammonia-N (mg/100 ml RF)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	31.1±0.42	27.2^a±0.64 (−12.71)	25.6^{b,o}±0.56 (−17.69)	25.2±0.29	22.7^g±0.24 (−10.01)	18.6^h±0.47 (−25.97)
MC	28.6^d±0.69	25.5^{a,q}±0.72 (−10.97)	22.8^{b,c,f}±0.49 (−20.16)	24.7±0.28	19.8^{g,k}±0.77 (−19.88)	16.5^{h,i,l}±0.68 (−33.14)
SF	30.2^p±0.81	26.3^a±0.56 (−12.66)	24.9^{b,f,o}±0.39 (−17.37)	24.7±0.35	21.4^{g,w}±0.92 (−13.58)	17.3^{h,i}±0.19 (29.87)
Soya	35.5^d±0.92	29.1^{a,e}±0.44 (−17.91)	27.2^{b,c,f}±0.22 (−23.52)	32.9^j±0.49	25.8^{g,k}±0.97 (−21.54)	20.2^{h,i,l}±0.53 (−38.74)
Casein	38.8^d±0.16	32.5^{a,e}±0.39 (−16.25)	30.2^{b,c,f}±0.83 (−22.82)	34.7^v±0.92	28.6^{g,k}±0.64 (−17.64)	21.3^{h,i,x}±0.81 (−38.56)

Conditions of incubation: Same as in table 19.

Table 21. Effect of Sodium Arsenite on Total Volatile fatty acids production when Various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TVFA produced (m moles/100 ml RF)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	27.6 ± 1.61	$22.6^{m}\pm1.91$ (-18.11)	$20.8^{n}\pm1.76$ (-24.64)	30.4 ± 1.37	18.69 ± 1.48 (-38.81)	$12.2^{h,i}\pm1.25$ (-59.87)
MC	25.3 ± 1.42	$20.9^{m}\pm1.73$ (-17.39)	$18.3^{n}\pm1.87$ (-27.66)	28.5 ± 1.49	$15.49^{,w}\pm1.16$ (-45.96)	$9.4^{h,i}\pm1.98$ (-67.01)
SF	25.8 ± 1.89	$21.7^{m}\pm1.34$ (-15.89)	$19.1^{n}\pm1.83$ (-25.97)	29.3 ± 1.44	16.39 ± 1.31 (-44.37)	$10.3^{h,i}\pm1.81$ (-64.85)
Soya	$31.6^{d}\pm1.37$	$27.4^{e,m}\pm1.22$ (-13.29)	$24.8^{n,r}\pm1.91$ (-21.52)	34.8 ± 1.51	$21.49^{,k}\pm1.77$ (-38.51)	$16.5^{h,i,l}\pm1.72$ (-52.58)
Casein	$35.4^{P}\pm1.14$	$29.2^{m}\pm1.08$ (-17.51)	$25.9^{n}\pm1.49$ (-26.84)	$39.3^{j}\pm1.33$	23.59 ± 1.52 (~40.2)	$17.3^{h,i}\pm1.44$ (-55.98)

Conditions of incubation: Same as in table 19.

Table 22. Effect of DDIC on TCA-precipitable proteins when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TCA-precipitable proteins left after incubation (mg)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitor (ppm)					
	Control	25	50	Control	25	50
GC	32.6 ± 0.78	$34.9^{m} \pm 0.44$ (+7.1)	$36.8^{b,o} \pm 0.38$ (+12.88)	24.4 ± 0.77	$32.9^{g} \pm 0.61$ (+34.83)	$34.8^{h,u} \pm 0.79$ (+42.62)
MC	31.4 ± 0.42	$33.1^{m} \pm 0.71$ (+5.41)	$35.6^{b,o} \pm 0.24$ (+13.37)	$26.6^{P} \pm 0.41$	$36.8^{g,k} \pm 0.72$ (+38.34)	$40.4^{h,i,l} \pm 0.61$ (+51.87)
SF	$33.1^{P} \pm 0.56$	35.1 ± 0.72 (+6.04)	$37.2^{b,c,r} \pm 0.61$ (+12.38)	27.1 ± 0.38	$35.2^{g} \pm 0.56$ (+29.88)	$38.3^{h,i,l} \pm 0.55$ (+41.32)
Soya	$28.1^{d} \pm 0.77$	$31.0^{a,e} \pm 0.54$ (+10.32)	$33.7^{b,c,f} \pm 0.41$ (+19.92)	$24.6^{d} \pm 0.26$	$34.8^{g} \pm 0.84$ (+49.46)	$37.4^{h,i} \pm 0.34$ (+52.03)
Casein	27.8 ± 0.81	$31.2^{a} \pm 0.24$ (+12.23)	$33.9^{b,o} \pm 0.72$ (+21.90)	$22.1^{P} \pm 0.46$	$33.6^{g} \pm 0.37$ (+52.03)	$38.9^{h,i} \pm 0.7$ (+76.01)

Conditions of incubation : Same as in table 1 except 25 and 50 of DDIC in 1 ml buffer were added to incubation mixture instead of hydrazine

Table23. Effect of DDIC on ammonia produced when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	Ammonia-N (mg/100 ml RF)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitor (ppm)					
	Control	25	50	Control	25	50
GC	32.4 ± 0.96	$30.1^m \pm 0.81$ (-7.09)	$28.4^{b,o} \pm 0.69$ (-12.34)	24.1 ± 0.54	$20.3^g \pm 0.48$ (-15.76)	$17.5^{u,i} \pm 0.51$ (-27.38)
MC	$31.6^p \pm 0.68$	$27.2^{a,e} \pm 0.32$ (-13.92)	$24.8^{b,c,f} \pm 0.81$ (-21.51)	$22.8^v \pm 0.69$	$18.9^{g,w} \pm 0.31$ (-17.10)	$14.3^{h,i,f} \pm 0.34$ (-37.28)
SF	31.9 ± 0.47	$27.8^a \pm 0.41$ (-12.85)	$25.1^{b,o} \pm 0.37$ (-21.31)	23.4 ± 0.87	$19.6^g \pm 0.18$ (-16.24)	$15.7^{h,i} \pm 0.53$ (-32.9)
Soya	$37.8^d \pm 0.55$	$33.6^{a,e} \pm 0.83$ (-11.11)	$30.1^{b,c,f} \pm 0.42$ (-20.37)	$30.2^j \pm 0.76$	$25.8^{j,k} \pm 0.92$ (-14.56)	$19.6^{h,i,l} \pm 0.61$ (-35.09)
Casein	$41.1^d \pm 0.68$	$37.5^{a,e} \pm 0.44$ (-8.75)	$32.8^{b,c,r} \pm 0.77$ (-20.19)	$34.3^j \pm 0.91$	$29.7^{j,k} \pm 0.67$ (-13.41)	$23.1^{h,i,l} \pm 0.78$ (-32.53)

Conditions of incubation: Same as in table 22.

Table 24. Effect of DDIC on Total volatile fatty acids production when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TVFA produced (m moles/100 ml RF)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitor (ppm)					
	Control	25	50	Control	25	50
GC	301±1.61	28.4[m]±1.72 (-5.64)	26.2[b,o]±1.92 (-12.95)	33.2±1.18	23.2[g]±1.23 (-30.1)	19.3[h,i]±1.44 (-41.86)
MC	28.4[p]±1.16	25.1[a,e]±1.36 (-11.61)	21.9[b,c,f]±1.18 (-22.88)	31.6[v]±1.32	20.6[g,w]±1.75 (-34.81)	17.8[h,i,x]±1.62 (-43.67)
SF	29.2±1.72	26.2[a,q]±1.72 (-10.27)	24.1[b,o,f]±1.47 (-17.46)	32.3±1.17	22.8[w]±1.64 (-27.41)	18.1[h,i]±1.92 (-43.96)
Soya	34.7[d]±1.31	31.6[a,e]±1.18 (-8.93)	29.3[b,c,f]±1.49 (-15.56)	37.4[j]±1.82	28.4[j,k]±1.82 (-24.06)	24.1[h,i,l]±1.66 (-35.56)
Casein	38.6[d]±1.21	33.9[a,q]±1.33 (-12.17)	30.6[b,c]±1.38 (-20.72)	41.8[j]±1.69	27.8[g]±1.32 (-33.49)	22.3[h,i,x]±1.17 (-46.65)

Conditions of incubation: Same as in table 22.

Table. 25. Effect of various deaminase inhibitors on TCA-precipitable proteins when casein hydrolysate was incubated with SRF for 4 and 8 h.

Inhibitors	TCA-precipitable proteins left after incubation(mg/100 ml RF)			
	Time of incubation			
	4h		8h	
	concentration of inhibitor (m moles or ppm)			
	0.1(25)	1.0 (50)	0.1(25)	1.0(50)
Control	56.8±0.81		61.7±0.92	
Hydrazine	51.2^a±0.23 (9.85)	46.4^{b,c}±0.52 (18.3)	53.5^g±1.06 (13.29)	43.7^{h,i}±0.64 (21.06)
Hydrazine sulfate	49.9^{a,e}±0.89 (12.41)	44.3^{b,c}±0.91 (22.0)	50.5^g±1.19 (18.15)	47.2^{h,i}±0.23 (23.5)
Phenyl hydrazine	45.8^{a,e}±0.46 (19.36)	39.6^{b,c,f}±0.76 (30.28)	46.9^{g,k}±1.22 (23.9)	40.1^{h,i,l}±0.85 (35.0)
p-nitrophenyl hydrazine	44.6^a±0.41 (21.47)	40.4^{b,c}±0.23 (28.87)	45.3^g±0.34 (26.58)	41.4^{h,i,x}±0.44 (32.25)
Hydroxyamine HCl	53.4^{a,e}±1.01 (5.98)	49.5^{b,c,f}±0.51 (12.85)	55.4^g±1.01 (10.21)	51.9^{h,i,l}±0.58 (15.8)
Sodium Arsenate	43.8^{a,e}±1.06 (22.8)	38.2^{b,c}±0.42 (32.74)	46.5^{g,k}±1.26 (24.63)	39.1^{h,i,l}±0.23 (36.6)
Sodium Arsenite	44.0^a±1.51 (22.53)	39.2^{b,c}±0.38 (30.9)	46.9^g±0.97 (23.98)	41.8^{h,i,x}±1.18 (32.25)
DDIC (in ppm)	42.1^{a,g}±0.94 (25.8)	37.8^{r,b,c}±0.4 (33.45)	44.3^k±0.48 (28.2)	38.7^{h,i,l}±0.65 (37.27)

Conditions of incubation: Same as in table 1 except casein hydrolysate (5 mg/ml SRF) was added to incubation mixture instead of intact feed proteins along with 0.1(25) and 1.0(50) m moles(ppm) of various deaminase inhibitors.

Table 26. Effect of deaminase inhibitors on ammonia production when various intact feed proteins were incubated with SRF for 4 and 8 h.

Inhibitors	Ammonia-N (mg/100 ml RF)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors (m moles of ppm)			
	0.1(25)	1.0(50)	0.1 (25)	1.0(50)
Control	48.7±0.24		45.3±0.87	
Hydrazine	42.3^{a}±0.44 (13.27)	37.6^{b,c}±0.23 (22.95)	32.19±0.44 (28.98)	23.1^{h,i}±1.19 (48.95)
Hydrazine sulphate	43.1^{a}±1.09 (11.46)	38.5^{b,c}±1.01 (21.08)	34.69±1.23 (23.66)	29.3^{h,i,l}±1.02 (35.34)
Phenyl hydrazine	44.0^{g}±1.16 (9.70)	39.3^{b,c}±1.24 (19.42)	33.79±1.91 (25.44)	28.5^{h,u}±1.91 (37.13)
p-nitrophenyl hydrazine	41.3^{a,q}±0.93 (15.24)	37.9^{b,o}±1.18 (22.19)	31.19±1.87 (31.34)	25.8^{h,u}±1.86 (42.90)
Hydroxylamine HCl	43.7^{a,q}±0.77 (10.25)	40.5^{b,o}±1.54 (16.88)	36.69±1.42 (19.06)	32.7^{h,i,l}±1.23 (27.72)
Sodium arsenate	44.7^{a}±0.53 (8 33)	35.9^{b,c,f}±0.77 (26.28)	30.39±1.06 (33.11)	24.2^{h,i,l}±1.51 (46.56)
Sodium arsenite	45.3^{a}±0.46 (6.97)	41.5^{b,c}±1.46 (14.83)	36.59±1.52 (19.28)	27.8^{h,i,x}±0.77 (38.52
DDIC(ppm)	41.9^{a,e}±0.48 (-13.96)	38.72±0.87 (20.53)	28.69±0.92 (36.86)	23.7^{h,i,l}±0.69 (47.68)

Conditions of incubation: Same as in table 25.

Table 27. Effect of deaminase inhibitors on Total Volatile fatty acids production when intact feed proteins were incubated with SRF for 4 and 8 h.

Inhibitors	TVFA produced (m moles/100 ml RF)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors (m moles or ppm)			
	0.1(25)	1.0 (50)	0.II 25)	1.0(50)
Control	37.5$\pm$1.51		42.6$\pm$1.16	
Hydrazine	32.6^a$\pm$1.92 (13.06)	29.4^{b,o}$\pm$1.48 (21.6)	37.3^g$\pm$1.51 (12.44)	33.2^{h,u}$\pm$1.97 (22.06)
Hydrazine sulphate	33.1^a$\pm$1.45 (11.73)	29.9^{b,c}$\pm$1.77 (20.26)	36.5^g$\pm$1.39 (14.32)	34.8^h$\pm$1.22 (18.31)
Phenyl hydrazine	35.4^a$\pm$1.33 (5.6)	32.8^b$\pm$1.33 (13.65)	39.8^{g,w}$\pm$1.94 (6.57)	34.6^{h,i}$\pm$1.44 (18.78)
p-nitrophenyl hydrazine	30.9^{a,e}$\pm$1.48 (17.6)	26.4^{b,c,f}$\pm$1.44 (29.6)	33.8^{g,k}$\pm$1.84 (20.66)	29.9^{h,u,x}$\pm$1.36 (29.81)
Hydroxylamine HCl	352.^{a,q}$\pm$1.51 (6.13)	31.8^{b,b,f}$\pm$1.38 (15.2)	40.6^k$\pm$1.32 (4.69)	37.5^{h,u,l}$\pm$1.72 (11.97)
Sodium Arsenate	30.4^{a,q}$\pm$1.22 (18.93)	24.6^{b,c,f}$\pm$1.17 (34.4)	30.9^{g,k}$\pm$1.19 (27.46)	26.4^{h,i,l}$\pm$1.18 (38.02)
Sodium Arsenite	30.8^a$\pm$1.16 (17.86)	23.2^{b,c}$\pm$1.34 (38.13)	30.3^g$\pm$1.72 (28.87)	25.7^{h,u}$\pm$1.25 (39.67)
DDIC	30.2^a$\pm$1.28 (19.46)	25.4^{b,c,o}$\pm$1.18 (32.26)	31.6^g$\pm$1.46 (25.82)	26.2^{h,i}$\pm$1.61 (38.49)

Conditions of incubation: Same as in table 25.

Table 28. Effect of various deaminase inhibitors on TCA-precipitable proteins when a mixture of amino acids was incubated with SRF for 4 and 8 h

Inhibitors	TCA-precipitable proteins left after incubation(mg/100 ml RF)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors(m moles or ppm)			
	0.1(25)	1.0(50)	0.1(25)	1.0(50)
Control	38.4±1.01		55.1±0.35	
Hydrazine	36.5^m±0.47 (4.94)	32.4^{b,c}±1.08 (15.62)	48.3^g±1.16 (12.34)	44.5^{h,i}±1.01 (19.23)
Hydrazine sulfate	35.8^a±0.63 (6.77)	31.6^{b,c}±0.91 (17.7)	47.7^g±0.68 (13.43)	43.7^{h,i}±1.12 (20.68)
Phenyl hydrazine	34.8^a±0.81 (9.37)	29.4^{b,c,r}±0.46 (23.43)	45.8^{g,w}±0.99 (16.87)	39.4^{h,i,l}±0.68 (28.49)
p-nitrophenyl hydrazine	32.3^{a,q}±0.44 (15.8)	27.6^{b,c,r}±0.85 (28.12)	42.8^{g,k}±0.48 (22.32)	36.7^{h,i,l}±0.53 (33.4)
Hydroxylamine HCl	37.1^a±0.68 (3.38)	34.8^{b,c,r}±1.02 (9.37)	49.7^{g,k}±0.65 (9.8)	46.9^{h,i,l}±0.64 (14.8)
Sodium Arsenate	30.7^{a,e}±0.72 (20.1)	26.3^{b,c,f}±0.99 ((31.51)	41.6^{g,k}±0.72 (24.5)	36.2^{h,i,l}±0.72 (34.3)
Sodium Arsenite	31.2^a±0.16 (18.75)	27.5^{b,c,r}±0.61 (28.4)	39.8^{g,k}±0.91 (27.76)	34.6^{h,i,x}±0.47 (37.2)
DDIC	31.6^a±0.34 (17.7)	27.1^{b,c}±0.45 (29.42)	40.8^{g,k}±0.44 (25.9)	35.4^{h,i}±0.65 (35.75)

Conditions of incubation: Same as in table 1 except 1 m mole amino acid mixture/ml SRF was added to the buffer along with 0.1(25) and 1.0(50) m moles (ppm) of various deaminase inhibitors.

Table 29. Effect of deaminase inhibitors on ammonia production from a mixture of amino acids when incubated with SRF for 4 and 8h.

Inhibitors	Ammonia-N (mg/100 ml RF)			
	4h		8h	
	Concentration of inhibitors (m mole or ppm)			
	0.1 (25)	1.0 (50)	0.1 (25)	1.0 (50)
Control	51.2 ± 0.43		46.3 ± 0.58	
Hydrazine	$48.1^{a}\pm0.33$ (-5.92)	$39.1^{b,c}\pm0.42$ (-23.54)	$41.1^{g}\pm0.25$ (-13.38)	$25.5^{h,i}\pm0.25$ (-44.96)
Hydrazine sulfate	$46.4^{a,q}\pm0.84$ (-9.34)	$38.5^{b,c}\pm0.36$ (-24.68)	$39.3^{g}\pm0.44$ (-15.22)	$27.7^{h,i,l}\pm0.47$ (-40.17)
Phenyl hydrazine	$48.7^{a,q}\pm0.26$ (-4.79)	$41.2^{b,c,f}\pm0.87$ (-19.42)	$42.6^{g,k}\pm0.92$ (-7.96)	$26.0^{h,i,x}\pm0.83$ (-43.78)
p-nitrophenyl hydrazine	$42.8^{a,e}\pm0.71$ (-16.37)	$35.8^{b,c,f}\pm0.33$ (-29.99)	$31.8^{g,k}\pm0.36$ (-31.43)	$22.2^{h,i,l}\pm0.72$ (-52.15)
Hydroxylamine HCl	$49.8^{e,m}\pm0.44$ (-2.69)	$44.4^{b,c,f}\pm0.45$ (-13.31)	$42.2^{g,k}\pm0.31$ (8.94)	$36.4^{h,i,l}\pm0.66$ (-21.37)
Sodium Arsenate	$43.2^{a,e}\pm0.53$ (-15.65)	$36.4^{b,c,f}\pm0.83$ (-28.9)	$31.7^{g,k}\pm0.52$ (-31.62)	$23.3^{h,i,l}\pm0.28$ (-49.91)
Sodium Arsenite	$43.3^{a,q}\pm0.91$ (-11.49)	$38.7^{b,c,f}\pm0.41$ (-24.31)	$33.8^{g,k}\pm0.68$ (-27.01)	$26.0^{h,i,l}\pm0.91$ (-43.91)
DDIC	$42.1^{a,e}\pm0.69$ (-17.77)	$35.2^{b,c,f}\pm0.58$ (-31.25)	$30.6^{g,k}\pm0.46$ (-33.9)	$22.7^{h,i,l}\pm0.71$ (-50.97)

Conditions of incubation: Same as in table 28.

Table 30. Effect of deaminase inhibitors on Total volatile fatty acids production when a mixture of amino acids was incubated with SRF for 4 and 8 h.

Inhibitors	TVFA produced (m moles/100 ml RE)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors (m moles or ppm)			
	0.1(25)	1.0 (50)	0.1(25)	1.0(50)
Control	43.8±1.72		50.2±1.06	
Hydrazine	40.2[m]±1.85 (-8.22)	37.4[b]±1.17 (-14.61)	43.6[g]±1.85 (-13.15)	38.5[h,u]±2.05 (-23.31)
Hydrazine sulfate	40.8±2.13 (-6.85)	36.9[b,o]±1.25 (-15.75)	41.9[g]±1.13 (-16.53)	38.1[h,i]±1.18 (-24.17)
Phenyl hydrazine	41.1±1.71 (-6.16)	38.7[b]±1.88 (-11.64)	44.8[g,w]±1.72 (-10.75)	38.9[h,u]±1.92 (-22.51)
p-nitrophenyl hydrazine	37.3[a,q]±1.87 (-14.84)	31.5[b,c,f]±1.04 (-28.08)	39.4[g,k]±1.06 (-21.51)	32.4[h,i,l]±1.06 (-35.46)
Hydroxylamine HCl	42.5[q]±1.64 (-2.97)	39.8[n,f]±1.76 (-9.13)	46.3[s,k]±1.61 (-7.76)	40.2[h,i,l]±1.97 (-19.92)
Sodium Arsenate	36.4[a,e]±1.13 (-16.89)	28.3[b,s,f]±1.19 (-35.39)	37.2[g,k]±1.32 (-25.89)	32.1[h,i,l]±1.38 (-36.05)
Sodium Arsenite	37.9[a]±1.09 (-13.47)	29.1[b,c]±1.28 (-33.56)	39.3[g]±1.09 (-21.71)	32.8[h,i,x]±1.84 (-34.66)
DDIC	35.8[a,q]±1.17 (-18.26)	29.4[b,c]±1.32 (32.87)	37.8[g]±1.46 (-24.70)	31.1[h,i,x]±1.84 (-38.04)

Conditions of incubation: Same as in table 28.

Table 31. Effect of Sodium sulfite on TCA-precipitable proteins when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TCA-precipitable proteins left after incubation (mg)					
	Time of incubation					
	4h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	28.4 ± 0.49	$29.8^{a}\pm0.12$ (+4.88)	$30.5^{b,o}\pm0.12$ (+7.17)	25.7 ± 0.31	$31.9^{q}\pm0.76$ (+23.91)	$34.3^{w,i}\pm0.52$ (+33.55)
MC	$26.1^{d}\pm0.82$	$27.6^{a,e}\pm0.72$ (+5.85)	$28.4^{n,f}\pm0.86$ (+8.83)	$23.0^{j}\pm0.56$	$29.8^{q,k}\pm0.62$ (+29.81)	$30.5^{h,i,u}\pm0.48$ (+32.59)
SF	27.6 ± 0.77	28.4 ± 0.84 (+2.82)	$29.8^{n,o,r}\pm0.32$ (+7.95)	$24.1^{v}\pm0.49$	$30.5^{q}\pm0.59$ (+26.16)	$33.0^{b,i,l}\pm0.92$ (+36.48)
Soya	$23.3^{d}\pm0.31$	$24.1^{e,m}\pm0.38$ (+3.69)	$25.7^{n,o,r}\pm0.9?$ (+10.43)	$20.8^{j}\pm0.44$	$26.1^{q,k}\pm0.14$ (+25.76)	$27.6^{h,i,l}\pm0.31$ (+33.12)
Casein	$20.8^{d}\pm0.24$	$21.4^{e,m}\pm0.19$ (+2.54)	$23.0^{b,c,r}\pm0.82$ (+10.21)	$16.5^{j}\pm0.39$	$23.3^{q,k}\pm0.32$ (+40.81)	$24.1^{h,u,x}\pm0.34$ (+46.01)

Conditions of incubation : Same as in table 1 except 0.1 and 1.0 m moles of sodium sulfite in 1 ml buffer were added to incubation mixture instead of hydrazine.

Table 32. Effect of sodium sulfite on Ammonia production when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	Ammonia-N (mg/100 ml RF)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	36.5±1.02	34.5[a]±0.32 (-5.53)	33.8[b]±0.99 (-7.44)	31.5±0.44	29.59±0.63 (-6.4)	27.8[h,i]±1.66 (-11.69)
MC	33.8P±0.99	31.6[a,e]±1.21 (-3.13)	30.5[b,c,f]±1.16 (-9.75)	28.4[j]±1.24	26.69[k]±1.21 (-6.26)	23.9[h,i,l]±1.89 (-15.87)
SF	35.3±1.46	33.7[a,q]±1.16 (-4.28)	32.4[b,c,f]±1.26 (-8.16)	29.8[v]±0.92	28.1[s,k]±1.24 (-5.76)	25.8[h,i,x]±1.12 (-13.7)
Soya	39.8[d]±1.21	37.2[a,q]±1.49 (-6.34)	35.8[b,c,f]±1.31 (-9.95)	36.5[j]±1.12	32.49[k]±1.91 (-4.67)	29.8[h,i,l]±1.49 (-18.26)
Casein	42.3[d]±1.14	40.1[a,q]±1.34 (-5.24)	37.8[b,c,f]±1.16 (-10.79)	39.7[j]±1.72	34.59[k]±1.72 (-13.18)	30.5[h,i]±1.62 (-23.16)

Conditions of incubation: Same as in table 31.

Table 33. Effect of Sodium Sulfite on Total volatile fatty acids production when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TVFA produced (m moles/100 ml RF)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	32.3±0.98	18.5^a±1.86 (-42.72)	16.2^b±1.84 (-49.84)	35.4±1.83	12.1^g±1.69 (-64.82)	9.8^h±1.91 (-71.51)
MC	30.4±1.61	16.4^a±1.72 (-46.52)	14.8^b±1.16 (-51.31)	33.1±2.11	10.3^g±1.14 (-68.82)	7.2^h±1.72 (-78.25)
SF	31.6±1.77	17.8^a±1.16 (-43.67)	15.2^b±1.32 (-51.89)	34.6±1.41	11.6^g±1.36 (-66.47)	8.5^{h,u}±1.68 (-75.43)
Soya	36.8^d±1.23	22.3^a±1.22 (-39.4)	20.4^{b,f}±1.43 (-44.56)	38.6^v±1.29	16.8^{g,k}±1.79 (-56.47)	12.6^{h,u,x}±1.01 (-67.35)
Casein	40.2±1.44	26.9^{a,q}±1.41 (-33.08)	23.7^b±1.94 (-41.04)	43.7^j±1.12	20.1^{g,w}±1.06 (-54.00)	13.5^{h,i,x}±1.29 (-64.53)

Conditions of incubation: Same as in table 31.

Table 34. Effect of Chloral hydrate on TCA precipitable proteins when various intact feed proteins were incubated with SRF on 4 and 8 h.

Protein source	TCA-precipitable proteins left after incubation(mg)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	30.3±0.41	31.4[a]±0.59 (+3.93)	32.8[n,o]±0.43 (+8.26)	27.8±0.82	33.5[g]±0.22 (+20.39)	34.8[h,u]±0.91 (+25.25)
MC	27.6[d]±0.33	28.3[a,e]±0.71 (+2.75)	29.1[n,f]±0.92 (+5.54)	24.6[j]±0.91	30.3[g,k]±0.29 (+22.81)	31.4[h,u,l]±0.72 (+27.64)
SF	29.1[d]±0.22	30.3[a,e]±0.43 (+3.97)	31.5[b,c,f]±0.42 (+8.07)	25.6±0.76	32.8[g,k]±0.64 (+28.07)	33.4[h,l] 0.19 (+30.57)
Soya	24.6[d]±0.76	26.3[e,m]±0.33 (+6.65)	27.5[b,c,f]±0.19 (+11.68)	21.3=0.44	28.4[g,k]±0.81 (+32.97)	29.1[h,l]±0.28 (+36.39)
Casein	21.4[d]±0.44	22.7[e,m]±0.71 (+6.13)	23.5[n,f]±0.14 (+9.87)	17.5[j]±0.61	24.6[g,k]±0.26 (+40.64)	26.3[h,i,l]±0.02 (+50.00)

Conditions of incubation: Same as in table 1. except 0.1 and 1.0 m moles of chloral hydrate in 1 ml buffer were added to incubation mixture instead of hydrazine.

Table 35. Effect of chloral hydrate on Ammonia produced when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	Ammonia-N (mg/100 ml RF)					
	Time of incubation					
	4 h			8 h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	34.4 ± 0.93	$31.8^{a}\pm0.41$ (-7.52)	$30.1^{b,c}\pm0.62$ (-12.38)	30.7 ± 0.88	$28.7^{s}\pm0.42$ (-6.32)	$24.3^{h,i}\pm0.67$ (-20.08)
MC	$31.3^{d}\pm0.43$	$29.4^{a,e}\pm0.98$ (-5.79)	$27.9^{b,f}\pm0.74$ (-10.68)	$28.8^{v}\pm0.12$	$26.2^{s,k}\pm0.23$ (-9.32)	$21.8^{h,i,l}\pm0.19$ (-21.45)
SF	$33.2^{d}\pm0.11$	$30.1^{a}\pm0.12$ (-9.13)	$28.7^{b,o}\pm0.99$ (-13.38)	$29.4^{v}\pm0.34$	$27.99^{k}\pm0.46$ (-5.19)	$23.2^{h,i,x}\pm0.45$ (-21.36)
Soya	$37.6^{d}\pm0.92$	$35.4^{m,e}\pm0.57$ (-6.00)	$33.2^{b,o,f}\pm0.81$ (-11.84)	$34.4^{j}\pm0.65$	$31.89^{k}\pm0.79$ (-7.52)	$27.9^{h,i,l}\pm0.34$ (-18.86)
Casein	$39.9^{p}\pm0.32$	36.5 ± 0.44 (-8.31)	$34.4^{b}\pm0.65$ (-13.59)	$36.5^{j}\pm0.18$	$32.69^{q}\pm0.21$ (-10.57)	$28.7^{h,i,x}\pm0.18$ (-21.28)

Conditions of incubation: Same as in table 34

Table 36. Effect of chloral hydrate on Total volatile fatty acids when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TVFA produced (m moles/100 ml RF)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	35.8 ± 1.61	$23.5^{a} \pm 1.44$ (-34.35)	$20.2^{b,o} \pm 1.69$ (-43.57)	38.1 ± 1.47	$18.2^{g} \pm 1.18$ (-52.23)	$13.0^{h,i} \pm 1.21$ (-65.62)
MC	33.5 ± 1.77	$21.4^{a,q} \pm 1.23$ (-36.12)	$18.8^{b} \pm 1.77$ (-43.88)	36.4 ± 1.21	$15.1^{g,w} \pm 1.27$ (-58.51)	$10.8^{h,i} \pm 1.16$ (-70.32)
SF	34.6 ± 1.24	$22.7^{a} \pm 1.88$ (-34.39?)	$19.6^{b} \pm 1.28$ (-43.35)	37.3 ± 1.38	$16.8^{g} \pm 1.77$ (-54.95)	$11.7^{h,i} \pm 1.74$ (-68.63)
Soya	$38.2^{p} \pm 1.16$	$27.6^{a,e} \pm 1.19$ (-27.74)	$23.3^{b,o,r} \pm 1.43$ (-39.00)	$40.1^{v} \pm 1.63$	$21.5^{g,k} \pm 1.62$ (-46.38)	$14.9^{h,i} \pm 1.32$ (-62.84)
Casein	40.8 ± 1.96	$31.8^{a,q} \pm 1.77$ (-22.06)	$27.5^{b,o,r} \pm 1.12$ (-32.35)	$43.6^{v} \pm 1.41$	$23.8^{g} \pm 1.44$ (-45.41)	$16.2^{h,i} \pm 1.39$ (-62.84)

Conditions of incubation: Same as in table 34.

Table 37. Effect of chloroform on TCA-precipitable proteins when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TCA-precipitable proteins left after incubation (mg)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	30.0 ± 0.49	$31.3^{m}\pm0.83$ (+4.13)	$33.2^{n,o}\pm0.72$ (+10.55)	27.5 ± 0.89	$34.0^{g}\pm0.24$ (+23.67)	$36.8^{h,i}\pm0.21$ (+33.89)
MC	$27.5^{d}\pm0.38$	$28.7^{m,e}\pm0.33$ (+4.54)	$30.6^{b,c,f}\pm0.19$ (+11.13)	$25.3^{v}\pm0.76$	$32.0^{g,k}\pm0.39$ (+26.2)	$33.6^{h,i,l}\pm0.38$ (+32.75)
SF	$29.1^{d}\pm0.41$	$29.9^{e}\pm0.94$ (+2.43)	$32.0^{b,c,f}\pm0.22$ (+9.71)	26.4 ± 0.81	$32.5^{g}\pm0.81$ (+22.86)	$34.0^{h,u}\pm0.44$ (+28.72)
Soya	$26.4^{d}\pm0.56$	$29.5^{e}\pm0.39$ (+4.08)	$29.1^{b,c,f}\pm0.68$ (+10.33)	$22.8^{j}\pm0.98$	$30.0^{g,w}\pm0.77$ (+31.49)	$32.5^{h,i,l}\pm0.24$ (+42.18)
Casein	$22.8^{d}\pm0.14$	$23.5^{e,m}\pm0.27$ (-2.76)	$25.3^{b,c,f}\pm0.54$ (+10.99)?	$19.3^{k}\pm1.13$	$27.5^{g,k}\pm0.16$ (+42.41)	$32.0^{h,i,l}\pm0.11$ (+65.61)

Conditions of incubation: Same as in table 1 except 0.1 and 1.0 m moles of chloroform in 1 ml buffer were added to incubation mixture instead of hydrazine.

Table 38. Effect of chloroform on Ammonia produced when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	Ammonia-N (mg/100 ml RF)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	35.5 ± 0.23	$33.1^{m}\pm0.41$ (-6.72)	$30.8^{b,c}\pm0.88$ (-13.19)	32.8 ± 0.88	$26.4^{g}\pm0.38$ (-19.5)	$21.9^{h,i}\pm0.17$ (-33.39)
MC	$33.6^{d}\pm0.72$	$30.3^{a,e}\pm0.34$ (-9.99)	$28.6^{b,o,r}\pm0.52$ (-14.87)	$30.6^{v}\pm0.49$	$24.5^{g,w}\pm0.41$ (-19.75)	$17.8^{h,i,l}\pm0.62$ (-41.76)
SF	34.4 ± 0.89	$31.9^{a}\pm0.77$ (-7.12)	$29.3^{b,o}\pm0.44$ (-14.88)	31.2 ± 0.36	$25.3^{g}\pm0.33$ (-18.64)	$19.6^{h,i,x}\pm0.92$ (-36.97)
Soya	$38.7^{d}\pm0.14$	$35.9^{a,e}\pm0.62$ (-7.34)	$32.2^{b,o,f}\pm0.41$ (-16.34)	$36.6^{j}\pm0.38$	$29.1^{g,k}\pm0.14$ (-20.46)	$24.3^{h,i,l}\pm0.84$ (-34.78)
Casein	$41.8^{d}\pm0.91$	$37.2^{a,q}\pm0.44$ (-10.95)	$35.9^{b,o,f}\pm0.13$ (-14.2)	$38.7^{v}\pm0.74$	$32.6^{g,k}\pm0.39$ (-15.68)	$27.4^{h,i,l}\pm0.84$ (-29.27)

Conditions of incubation: Same as in table 37.

Table 39. Effect of chloroform on Total volatile fatty acids production when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TVFA produced (m moles/100 ml RF)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	34.9±1.24	30.8^m±1.77 (-11.75)	28.4^n±1.83 (-18.62)	36.2±1.38	24.2^g±1.45 (-33.15)	18.1^{h,i}±1.14 (-50.00)
MC	32.4±1.15	27.6^{a,q}±1.65 (-14.81)	25.2^{b,r}±1.77 (-22.22)	35.1±2.11	21.3^g±1.69 (-39.32)	15.4^{h,i}±1.96 (-56.12)
SF	33.5±1.39	28.9^a±1.85 (-13.73)	25.8^n±1.44 (-22.99)	35.9±1.92	22.8^g±1.72 (-36.49)	16.6^{h,i}±2.19 (-53.76)
Soya	37.6^p±1.44	32.2^{a,q}±1.21 (-14.36)	29.1^{b,o,r}±0.99 (-22.61)	39.4^j±1.21	25.7^g±1.16 (-34.77)	21.3^{h,i,x}±1.12 (-41.12)
Casein	39.8^p±1.86	35.6^{m,q}±1.42 (-10.55)	31.4^{b,c}±1.06 (-21.11)	43.1^j±1.18	26.4^g±1.46 (-38.75)	23.2^{h,u}±1.39 (-46.17)

Conditions of incubation: Same as in table 37.

Table 40. Effect of Monensin on TCA precipitable proteins when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	TCA-precipitable proteins left after incubation (mg)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitor (ppm)					
	Control	25	50	Control	25	50
GC	31.4±1.09	34.1[m]±0.86 (+8.63)	36.3[b,c]±0.44 (+15.5)	27.6±0.72	37.6[g]±0.41 (+35.77)	38.4[h]±0.96 (+39.1)
MC	28.4±0.42	32.2[a,q]±0.91 (+13.39)	34.5[b,c,f]±0.25 (+21.12)	25.8[v]±0.77	34.1[g,k]±0.38 (+31.99)	35.9[h,i,l]±0.71 (+38.84)
SF	29.8[p]±0.83	33.4[a]±0.36 (+12.39)	35.2[b,o]±0.92 (+18.21)	26.4±0.84	36.3[g,k]±0.77 (+37.56)	37.6[h,u,x]±0.49 (+42.49)
Soya	23.2[d]±0.76	26.4[a,e]±0.58 (+14.07)	28.4[b,c,f]±0.49 (+22.84)	19.2[j]±0.31	31.1[g,k]±0.53 (+61.85)	33.4[h,i,l]±0.24 (+73.85)
Casein	20.4[d]±0.27	23.7[a,e]±0.52 (+16.31)	27.4[b,c,r]±0.53 (+34.08)	16.6[j]±0.76	27.6[g,k]±0.66 (+66.58)	30.8[h,i,l]±0.97 (+86.06)

Conditions of incubation: Same as in table 1 except 0.1(25) and 1.0(50) of monensin in 1 ml buffer were added to incubation mixture instead of hydrazine.

Table 41. Effect of Monensin on Ammonia produced when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein source	Ammonia-N (mg/100 ml RF)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitor (ppm)					
	Control	25	50	Control	25	50
GC	36.4±0.14	32.5^a±0.68 (-10.79)	30.8^{b,o}±0.55 (-15.22)	32.4±0.98	27.3^g±0.19 (-15.67)	22.8^{h,i}±0.42 (-29.55)
MC	33.9^d±0.23	29.2^{a,e}±0.23 (-13.86)	26.6^{b,c,f}±0.24 (-21.62)	29.8^v±0.74	24.2^{g,k}±0.36 (-18.73)	19.8^{h,i,l}±0.64 (-33.78)
SF	35.2^p±0.81	31.2^{a,e}±0.62 (-11.52)	27.9^{b,c,r}±0.77 (-20.68)	31.2±0.88	25.1^{g,w}±0.42 (-19.58)	20.3^{h,i}±0.81 (-35.14)
Soya	40.6^d±0.69	36.1^{a,e}±0.30 (-11.15)	33.3^{b,c,f}±1.01 (-18.11)	35.2^j±0.41	30.4^{g,k}±0.77 (-13.71)	25.1^{h,i,l}±0.14 (-28.74)
Casein	43.2^d±0.45	38.5^{a,e}±0.91 (-10.93)	35.7^{b,c,r}±0.82 (-17.23)	37.0^j±0.33	32.6^{g,w}±0.63 (-11.8)	27.8^{h,i,x}±0.93 (-24.79)

Conditions of incubation: Same as in table 40.

Table 42. Effect of Monensin on Total volatile fatty acids when various intact feed proteins were incubated with SRF for 4 and 8 h.

Protein Source	TVFA produced (m moles/100 mg RF)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitor (ppm)					
	Control	25	50	Control	25	50
GC	33.2 ± 1.62	$29.7^{m}\pm0.98$ (-10.54)	$24.2^{b,c}\pm1.81$ (-27.11)	36.4 ± 1.18	$21.8^{g}\pm1.06$ (-40.11)	$16.5^{h,i}\pm1.21$ (-54.67)
MC	30.8 ± 0.98	$27.4^{m}\pm1.12$ (-11.04)	$22.4^{b,o,r}\pm1.65$ (-27.27)	35.1 ± 1.72	$18.6^{g,w}\pm1.91$ (-47.00)	$13.2^{h,i,l}\pm1.16$ (-62.39)
SF	32.3 ± 1.46	$28.6^{m}\pm1.66$ (-11.45)	23.3 ± 1.76 (-27.86)	35.8 ± 1.61	$20.1^{g}\pm1.65$ (-43.85)	$14.7^{h,i}\pm1.72$ (½58.94)
Soya	$37.2^{d}\pm1.72$	$34.8^{e}\pm1.84$ (-6.45)	$30.2^{b,o,f}\pm1.61$ (-18.82)	$40.1^{j}\pm1.44$	$24.5^{g,w}\pm1.29$ (-38.9)	$18.3^{h,i,x}\pm1.04$ (-54.36)
Casein	$42.9^{d}\pm1.99$	$38.7^{m,e}\pm2.13$ (-9.79)	$33.4^{b,o,r}\pm1.48$ (-22.14)	$44.8^{v}\pm1.32$	$28.2^{g,w}\pm1.84$ (-37.05)	$21.7^{h,i,x}\pm0.98$ (-51.56)

Conditions of incubation: Same as in table 40.

Table 43. Effect of methanogenic inhibitors on TCA-precipitable proteins when casein hydrolysate was incubated with SRF for 4 and 8 h.

Inhibitor	TCA-precipitable proteins (mg/100 ml RF)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitor (m moles or ppm)			
	0.1(25)	1.0 (50)	0.1(25)	1.0 (50)
Control	38.8±0.72		54.3±0.54	
Sodium sulfite	40.3[m]±0.41 (+3.86)	43.2[b,c]±0.24 (+11.34)	57.5[g]±0.72 (+5.89)	61.8[h,i]±1.02 (+13.81)
Chloroform	39.9±0.23 (+2.83)	41.4[b,o,r]±0.51 (+6.73)	55.8[w]±0.34 (+2.76)	58.7[h,i,l]±0.89 (+7.36)
Chloral hydrate	41.5[a,q]±0.64 (+6.96)	45.8[b,c,f]±0.78 (+18.04)	58.6[g,k]±0.16 (+7.91)	66.2[h,i,l]±0.94 (+21.91)
Monensin (ppm)	43.1[a,q]±0.93 (+11.08)	46.4[b,c]±1.01 (+19.58)	61.3[g,k]±0.43 (+12.89)	70.8[h,i,l]±0.31 (+30.38)

Conditions of incubation: Same as in table 1 except casein hydrolysate (5mg/ml SRF) was added to incubation mixture with 0.1(25) and 1.0(50) m moles (ppm) of various methanogenic inhibitors.

Table 44. Effect of methanogenic inhibitors on Ammonia production when casein hydrolysate was incubated with SRF for 4 and 8 h.

Inhibitors	Ammonia-N (mg/100 ml RF)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors (m moles or ppm)			
	0.1(25)	1.0(50)	0.1(25)	1.0(50)
Control	47.2±0.48		42.2±0.16	
Sodium sulfite	44.7[a]±0.66 (-5.33)	40.3[b,c]±0.51 (-14.58)	39.6[g]±1.14 (-6.02)	36.4[h,i]±0.38 (-13.63)
Chloroform	40.3[a,e]±0.61 (-14.58)	32.8[b,c,f]±0.92 (-30.52)	26.6[g,k]±1.01 (-37.04)	17.3[h,i,l]±0.46 (-58.95)
Chloral hydrate	42.9[a,e]±0.28 (-9.27)	37.2[b,c,f]±0.23 (-21.16)	33.2[g,k]±1.06 (-21.19)	27.8[h,i,l]±1.13 (-34.06)
Monensin (ppm)	41.3[a,e]±0.43 (-12.64)	35.4[b,c,f]±0.76 (-25.08)	30.5[g]±0.91 (-27.75)	20.5[h,i,l]±0.76 (-51.34)

Conditions of incubation: Same as in table 43.

Table 45. Effect of methanogenic inhibitors on Total volatile fatty acids production when casein hydrolysate was incubated with SRF for 4 and 8h.

Inhibitors	TVFA produced (m moles/100 ml SRF)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors (m moles or ppm)			
	0.1(25)	1.0(50)	0.1(25)	1.0(50)
Control	39.8 ± 1.86		44.5 ± 1.42	
Sodium sulfite	38.2 ± 1.51 (-4.02)	$36.4^n \pm 1.91$ (-8.54)	$41.8^s \pm 1.16$ (-6.06)	$37.2^{h,i} \pm 1.44$ (-16.4)
Chloroform	$34.4^{a,q} \pm 1.72$ (-13.57)	$29.2^{b,c,f} \pm 1.88$ (.26.63)	$35.3^{g,k} \pm 1.25$ (-20.67)	$29.8^{h,i,l} \pm 1.23$ (-33.03)
Chloral hydrate	$36.5^m \pm 1.99$ (-8.29)	$32.1^{b,o} \pm 1.76$ (-19.35)	$38.2^g \pm 1.77$ (-14.16)	$34.5^{u,l} \pm 1.76$ (-22.47)
Monensin (ppm)	$36.1^m \pm 1.34$ (-9.29)	$31.4^{b,v} \pm 1.85$ (-21.11)	$37.8^g \pm 1.62$ (-15.06)	$32.6^{h,u} \pm 1.89$ (-26.74)

Conditions of incubation: Same as in table 43.

Table 46. Effect of methanogenic inhibitors on TCA-precipitable proteins when a mixture of amino acids was incubated with SRF for 4 and 8 h.

Inhibitors	TCA-precipitable proteins (mg/100 ml RF)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors (m moles or ppm)			
	0.1 (25)	1.0(50)	0.1(25)	1.0(50)
Control	40.8 ± 0.56		52.6 ± 1.02	
Sodium sulfite	42.2 ± 0.61 (3.43)	$44.9^{b,c}\pm1.32$ (10.04)	$55.1^{g}\pm0.91$ (4.75)	$61.4^{h,i}\pm0.47$ (16.73)
Chloroform	41.5 ± 0.82 (1.71)	$42.3^{n}\pm1.77$ (3.67)	$53.9^{w}\pm1.76$ (2.47)	$55.9^{h,i,l}\pm1.27$ (6.27)
Chloral hydrate	$45.1^{a,e}\pm1.37$ (10.53)	$48.8^{b,c,f}\pm0.49$ (19.6)	$50.7^{g,k}\pm0.48$ (13.49)	$64.2^{h,i,l}\pm1.18$ (22.05)
Monensin (ppm)	$45.9^{a}\pm0.35$ (12.5)	$50.4^{b,c,r}\pm0.84$ (23.52)	$60.8^{g}\pm1.36$ (15.58)	$68.5^{h,i,l}\pm0.31$ (30.23)

Conditions of incubation: Same as in table 1 except a mixture of amino acid 1 m mole/ml SRF) was added to the incubation mixture along with 0.1(25) and 1.0(50) m moles(ppm) of various methanogenic inhibitors.

Table 47. Effect of methanogenic inhibitors on ammonia produced when a mixture of amino acids was incubated with SRF for 4 and 8 h.

Inhibitors used	Ammonia-N (mg/100 ml RF)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors (m moles or ppm)			
	0.1(25)	1.0 (50)	0.1(25)	1.0 (50)
Control	56.7±0.39		48.2±0.47	
Sodium sulfite	54.4[a]±0.91 (-4.19)	48.3[b,c]±1.01 (-14.84)	42.19±0.45 (-12.73)	37.1[h,i]±0.45 (-22.99)
Chloroform	51.8[a,e]±0.47 (-8.63)	43.2[b,c,f]±0.46 (-23.89)	33.29[k]±0.77 (-31.25)	28[h,i,l]±0.58 (-40.01)
Chloral hydrate	53.5[a,q]±1.03 (-5.79)	47.5[b,c,f]±0.74 (-16.21)	38.79[k]±1.31 (-19.65)	32.6[h,i,l]±0.61 (-32.43)
Monensin (ppm)	53.8[a]±0.35 (-0197)	43.4[b,c,f]±0.31 (-20.25)	35.59[w]±1.81 (-26.47)	30.1[h,i,l]±0.19 (-37.71

Conditions of incubation: Same as in table 46.

Table 48. Effect of methanogenic inhibitors on Total volatile fatty acids production when a mixture of amino acids was incubated with SRF for 4 and 8 h.

Inhibitors used	TVFA produced (m moles/100 ml RF)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors (m moles or ppm)			
	0.1(25)	1.0 (50)	0.1(25)	1.0(50)
Control	41.4 ± 1.77		46.2 ± 1.89	
Sodium sulfite	40.2 ± 1.22 (-2.89)	$37.5^n\pm1.86$ (-9.42)	43.2 ± 1.76 (-6.49)	$36.4^{h,i}\pm1.76$ (-21.21)
Chloroform	$37.6^m\pm1.97$ (-9.17)	$30.1^{b,c,f}\pm1.42$ (-27.29)	$37.8^{g,w}\pm1.93$ (-18.18)	$29.1^{h,i,l}\pm1.38$ (-37.01)
Chloral hydrate	39.3 ± 1.42 (-5.07)	$33.4^{b,c}\pm1.34$ (-19.32)	$39.5^g\pm1.24$ (-14.5)	$34.2^{h,i,l}\pm1.44$ (-25.97)
Monensin (ppm)	38.7 ± 1.68 (-6.52)	$31.9^{9,c}\pm1.89$ (-22.94)	$38.3^g\pm1.33$ (-17.09)	$32.8^{h,i}\pm1.61$ (-29.00)

Conditions of incubation: Same as in table 46.

Table 49. Effect of hydrazine on TCA Insoluble proteins when various intact feed proteins were incubated with washed mixed bacterial suspension.

Protein source	TCA Insoluble proteins left after incubation (mg)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	33.38±0.49	36.9^a±0.92 (+10.78)	37.9^{b,o}±0.32 (+13.91)	26.5±0.49	35.9^g±0.96 (+35.53)	38.0^{h,i}±0.42 (+43.38)
MC	31.5^P±0.72	35.1^a±0.71 (+11.09)	36.8^{b,o,r}±0.56 (+16.76)	22.4^j±0.46	35.1^g±0.81 (+56.54)	36.8^{h,u,x}±0.49 (+64.22)
SF	34.0^d±0.81	37.1^{e,a}±0.46 (+9.2)	37.9^{b,o,r}±0.21	25.1^j±0.38	36.8^{g,w}±0.37 (+46.65)	39.0^{h,i,l}±0.28 (+53.09)
Soya	30.9^d±0.28	34.7^{e,a}±0.52 (+12.99)	36.2^{b,c,f}±0.49 (+17.21)	21.0^j±0.77	35.1^{g,w}±0.79 (+66.74)	37.8^{h,u,l}±0.36 (+75.16)
Casein	28.9^d±0.46	34.1^a±0.66 (+17.98)	35.8^{b,o}±0.78 (+23.89)	18.4^j±0.62	36.2^g±0.58 (+97.44)	36.2^h±0.48 (+97.44)

Conditions of incubation: Same as in table 1 except 10 ml washed mixed bacterial suspension was added to incubation mixture instead of SRP

Table 50. Effect of hydrazine on Ammonia produced when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8h.

Protein source	Ammonia-N (mg/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	19.5 ± 0.92	18.4 ± 0.48 (-5.73)	$16.1^{b,c}\pm0.54$ (-17.45)	15.4 ± 0.95	14.3 ± 0.46 (-7.19)	$12.6^{h,u}\pm0.44$ (-18.00)
MC	18.5 ± 1.06	$16.5^{e,m}\pm0.71$ (-10.39)	$15.7^{b}\pm0.62$ (-15.16)	16.4 ± 0.77	$12.5^{g,w}\pm0.61$ (-24.03)	$13.5^{h,u}\pm0.32$ (-17.58)
SF	19.4 ± 0.59	$17.9^{m,q}\pm0.33$ (-8.08)	$14.7^{b,c,r}\pm0.49$ (-24.29)	14.8 ± 0.92	$12.8^{s}\pm0.33$ (-13.07)	$12.6^{l}\pm0.89$ (-14.29)
Soya	$22.2^{b}\pm0.44$	$21.5^{e,q}\pm0.49$ (-3.19)	$12.8^{b,c,f}\pm0.55$ (-42.33)	$17.4^{v}\pm0.96$	$13.5^{g}\pm0.21$ (-21.99)	$10.2^{h,i,x}\pm0.81$ (-40.1)
Casein	$21.4^{P}\pm0.21$	$19.1^{a,q}\pm0.58$ (-10.85)	$13.6^{b,c}\pm0.72$ (-36.34)	$19.8^{v}\pm0.81$	$19.3^{g,h}\pm0.54$ (-28.01)	$9.8^{h,i}\pm0.57$ (-50.83)

Conditions of incubations: Same as in table 49.

Table 51. Effect of hydrazine on Total volatile fatty acids production when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8h.

Protein source	TVFA produced (m moles/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	25.9 ± 1.26	$15.5^{a}\pm1.89$ (-40.15)	$11.7^{b,o}\pm1.19$ (-54.82)	27.9 ± 1.72	$11.00^{g}\pm1.84$ (-60.57)	$9.7^{h}\pm1.77$ (-65.23)
MC	24.1 ± 1.49	$17.9^{a}\pm1.76$ (-25.72)	$12.3^{b,c}\pm1.28$ (-48.96)	28.7 ± 1.99	$11.7^{g}\pm1.86$ (-59.23)	$10.7^{h}\pm2.01$ (-62.77)
SF	29.7 ± 1.28	$15.1^{a}\pm1.51$ (-38.86)	$11.7^{b}\pm1.71$ (-52.63)	28.1 ± 2.1	$13.3^{g}\pm1.56$ (-52.66)	$10.3^{h,i}\pm1.18$ (-63.34)
Soya	$28.3^{p}\pm1.42$	$20.5^{a,e}\pm1.23$ (-27.56)	$14.9^{b}\pm1.65$ (-.47.35)	31.9 ± 1.49	$23.1^{g,k}\pm1.23$ (-27.58)	$13.7^{h,i,x}\pm1.29$ (-57.05)
Casein	28.6 ± 0.96	$21.1^{a}\pm1.44$ (-26.22)	$15.1^{b,c}\pm1.77$ (-47.2)	32.5 ± 1.37	$24.5^{g}\pm1.44$ (-24.61)	$14.3^{h,i}\pm1.45$ (-56.00)

Conditions of incubation: Same as in table 49.

Table 52. Effect of Hydrazine sulfate on TCA-precipitable proteins when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h

Protein source	TCA precipitable proteins left after incubation (mg)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	34.5±0.15	37.3[a]±0.43 (?8.07)	38.3[b]±0.65 (+11.16)	22.1±0.18	35.9[g]±0.11 (+12.12)	38.7[h,i]±0.49 (+74.51)
MC	32.1[d]±0.42	35.8[a,q]±0.41 (+11.29)	36.9[b,r]±0.58 (+14.97)	21.3±0.72	34.5[g,k]±0.23 (+61.67)	37.1[h,i,l]±0.34 (+74.08)
SF	34.5[d]±0.76	38.3[a,e]±0.74 (+11.16)	38.9[b,f]±0.51 (+12.78)	23.5[j]±0.48	36.1[g,k]±0.39 (+54.03)	38.6[h,i,l]±0.18 (+)
Soya	31.3[d]±0.31	33.9[a,e]±0.33 (+8.5)	35.9[b,o,f]±0.72 (+14.8)	20.7[j]±0.71	32.8[g,k]±0.28 (+58.77)	36.9[h,i,x]±0.92 (+78.54)
Casein	27.6[d]±0.82	34.5[a]±0.49 (+25.00)	36.1[b,o]±0.88 (+30.46)	17.5[j]±0.76	33.7[g,w]±0.46 (+92.95)	36.9[h,i]±0.94 (+111.57)

Conditions of incubation: Same as in table   except 0.1 and 1.0 m moles of hydrazine sulfate in 1 ml buffer were added to incubation mixture instead of hydrazine.

Table 53. Effect of Hydrazine sulfate on Ammonia produced when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h.

Protein source	Ammonia-N (mg/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
SC	27.6±0.42	20.3^a±0.31 (-27.66)	17.8^{b,c}±0.39 (-35.44)	21.6±0.84	15.4^g±0.29 (-28.77)	12.6^{h,i}±0.33 (-41.57)
MC	25.4^d±0.54	19.4^{a,q}±0.44 (-23.44)	17.5^{b,c}±0.41 (-30.97)	20.3±0.49	15.4^g±0.18 (-23.96)	11.4^{h,i,x}±0.47 (-43.88)
GF	24.9±0.91	18.2^a±0.65 (-27.21)	16.7^{b,o}±0.86 (-33.07)	20.9±0.17	14.3^g±0.23 (-31.26)	11.4^{h,i}±0.81 (-45.6)
Soya	28.9^d±0.36	20.3^{a,q}±0.61 (-29.87)	18.3^{b,o}±0.72 (-36.69)	23.7^j±0.23	18.4^{g,k}±0.77 (-22.55)	13.3^{h,i,l}±0.27 (-43.97)
Casein	30.8^d±0.23	20.3^a±0.48 (-34.09)	18.9^b±0.33 (-38.51)	25.1^j±0.12	19.8^g±0.86 (-21.01)	14.0^{h,i,x}±0.42 (-44.45)

Conditions of incubation: Same as in table 52.

Table 54. Effect of Hydrazine sulfate on Total volatile fatty acids when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 & 8 h.

Protein source	TVFA produced (m moles/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	27.8 ± 1.42	$12.9^{a} \pm 1.98$ (-53.59)	$8.3^{b,o} \pm 1.44$ (-70.14)	30.6 ± 1.14	$9.2^{g} \pm 1.22$ (-69.93)	$7.5^{h} \pm 1.41$ (-73.49)
MC	26.4 ± 1.66	$12.4^{a} \pm 1.82$ (-53.03)	$8.1^{b,o} \pm 1.79$ (-69.32)	28.8 ± 1.66	$8.5^{g} \pm 1.28$ (-70.49)	$7.2^{h} \pm 1.68$ (-75.00)
SF	23.9 ± 1.59	$11.2^{a} \pm 1.64$ (-53.14)	$7.6^{b,o} \pm 1.38$ (-68.2)	28.2 ± 1.18	$8.1^{g} \pm 1.91$ (-71.28)	$6.4^{h} \pm 1.39$ (-70.3)
Soya	$30.2^{d} \pm 1.33$	$16.8^{a,e} \pm 1.85$ (-44.37)	$10.3^{b,c} \pm 1.21$ (-65.89)	$34.6^{j} \pm 1.23$	$10.4^{g} \pm 1.86$ (-69.93)	$8.3^{h} \pm 1.28$ (-76.01)
Casein	32.1 ± 1.41	$17.5^{a} \pm 1.31$ (-45.48)	$12.4^{bc} \pm 1.25$ (-67.37)	35.3 ± 1.46	$10.8^{g} \pm 1.16$ (-69.4)	$8.8^{h} \pm 1.13$ (-75.07)

Conditions of incubation: Same as in table 52.

Table 55. Effect of Phenyl hydrazine on TCA precipitable proteins when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h.

Protein source	TCA precipitable proteins left after incubation (mg)					
	Time of incubation					
	4h			8h		
		Concentration of inhibitors (m moles)				
	Control	0.1	1.0	Control	0.1	1.0
GC	30.2 ± 0.38	$33.3^{a}\pm0.33$ (+10.5)	$35.3^{b,c}\pm0.41$ (+16.9)	28.6 ± 0.48	$31.2^{g}\pm0.51$ (+9.08)	$34.1^{h,i}\pm0.11$ (+19.13)
MC	$29.2^{p}\pm0.44$	$33.3^{a}\pm0.38$ (+14.02)	$34.5^{b,c,r}\pm0.33$ (+18.08)	27.2 ± 0.45	$30.8^{g}\pm0.84$ (+13.21)	$33.2^{h,i,x}\pm0.38$ (+22.33)
SF	$29.7^{p}\pm0.64$	$31.8^{a,q}\pm0.61$ (+7.31)	$33.9^{b,o}\pm0.84$ (+14.28)	26.3 ± 0.72	$30.2^{g}\pm0.91$ (+14.53)	$32.5^{h,u}\pm0.67$ (+23.26)
Soya	$28.6^{p}\pm0.52$	$30.8^{a,q}\pm0.44$ (+7.44)	$33.3^{b,o}\pm0.76$ (+16.09)	$23.6^{j}\pm0.79$	$28.6^{w}\pm0.72$ (+21.25)	$30.2^{h,u,l}\pm0.16$ (+27.77)
Casein	$25.2^{d}\pm0.91$	$26.8^{a,e}\pm0.22$ (+6.59)	$28.6^{b,c,f}\pm0.71$ (+13.83)	$20.1^{j}\pm0.62$	$25.2^{j,k}\pm0.18$ (+24.92)	$29.2^{h,i}\pm0.77$ (+45.23)

Conditions of incubation: Same as in table   except 0.1 and 1.0 m moles of Phenyl hydrazine in 1 ml buffer were added to incubation mixture instead of hydrazine.

Table 56. Effect of Phenyl hydrazine on Ammonia produced when various intact feed proteins were incubated with washed mixed bacterial suspensioin for 4 and 8 h

Protein source	Ammonia-N (mg/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	23.2±0.44	18.1^a±0.31 (-22.02)	16.4^{b,c}±0.49 (-29.38)	16.1±0.38	14.8^g±0.14 (-8.08)	10.8^{h,i}±0.81 (-32.71)
MC	22.7±0.36	18.1^a±0.61 (-20.2)	16.4^{b,c}±0.56 (-27.72)	15.7±0.41	13.7^{g,k}±0.21 (-12.91)	9.7^{h,i}±0.46 (-37.97)
SF	22.3±0.49	17.4^a±0.78 (-22.33)	15.8^{b,o}±0.33 (-29.12)	14.3±0.63	13.2^{s,w}±0.18 (-7.47)	9.7^{h,i}±0.41 (-31.91)
Soya	25.4^d±0.52	20.8^{a,e}±0.63 (-17.91)	17.4^{b,c,f}±0.41 (-31.65)	17.8^j±0.72	15.6^{g,k}±0.36 (-12.55)	10.8^{h,i,l}±0.24 (-39.38)
Casein	27.8^d±0.39	21.3^a±0.51 (-23.23)	18.1^{b,c}±0.62 (-34.81)	18.2±0.69	15.2^a±0.49 (-16.78)	10.8^{h,i}±0.86 (-40.64)

Conditions of incubation: Same as intable 55.

Table 57. Effect of Phenyl hydrazine on Total volatile fatty acids when various intact feed proteins were incubated with washed mixed bacterial sus for 4 and 8h.

Protein source	TVFA produced (m moles/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	25.9±1.22	18.5^a±1.27 (-28.57)	16.3^b±1.16 (-37.06)	29.1±1.41	15.2^g±1.77 (-47.77)	12.2^{h,u}±1.62 (-58.07)
MC	25.4±1.47	19.3^a±2.08 (-24.01)	16.9^b±1.18 (-24.64)	29.8±1.87	15.8^g±1.64 (-46.98)	12.3^{h,u}±1.79 (-58.72)
SF	26.8±1.39	19.5^a±1.91 (-27.24)	16.6^b±1.41 (-38.06)	30.2±1.62	15.3^g±1.59 (-49.34)	12.7^h±1.44 (-57.95)
Soya	30.4^p±1.21	20.6^a±1.43 (-32.24)	18.1^b±1.33 (-40.46)	32.2±1.43	17.1^g±1.46 (-46.49)	14.1^h±1.13 (-56.21)
Casein	30.6±1.85	22.4^a±1.32 (-26.79)	19.3^b±1.97 (-36.93)	32.6±1.51	20.9^{g,w}±1.55 (-35.88)	14.5^{h,i}±1.22 (-55.52)

Conditions of incubation: Same as in table 57.

Table 58. Effect of p-nitrophenyl hydrazine on TCA precipitable proteins when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8h.

Protein source	TCA-precipitable proteins left after incubation (mg)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	31.8±0.39	33.8[a]±0.31 (+6.8)	34.9[b]±0.86 (+9.74)	27.6±0.84	32.2[g]±0.45 (+16.42)	32.8[h]±0.91 (+18.81)
MC	30.2[d]±0.41	33.4±0.92 (+10.31)	34.9[b]±0.79 (+15.43)	26.1±0.91	31.2[g]±0.16 (+19.5)	33.8[h,u]±0.64 (+29.44)
SF	29.7±0.76	33.4[a]±0.46 (+12.16)	34.3[b,o]±0.18 (+15.19)	26.1±0.62	30.2[g]±0.22 (+15.49)	32.8[h,i]±0.85 (+25.58)
Soya	27.6[p]±0.62	30.2[a,e]±0.38 (+9.44)	32.8[b,c,f]±0.32 (+18.81)	22.5[j]±0.71	29.2[g,w]±0.48 (+29.54)	32.2[h,i]±0.53 (+42.95)
Casein	26.1±0.44	29.7[a]±0.51 (+13.85)	32.3[b,c]±0.61 (+23.15)	21.0[v]±0.69	25.3[g,k]±0.51 (+20.85)	29.2[h,i,l]±0.31 (+39.12)

Conditions of incubation: Same as in table 49 except 0.1 and 1.0 m moles of p-nitrophenyl hydrazine in 1 ml buffer were added to incubation mixture instead of hydrazine.

Table 59. Effect of p-nitrophenyl hydrazine on Ammonia produced when various intact feed proteins were incubated with washed mixed bacterial for 4 and 8 h.

Protein source	Ammonia-N (mg/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	22.7 ± 0.44	$19.5^{a}\pm0.89$ (-14.23)	$16.8^{b,c}\pm0.38$ (-25.87)	14.3 ± 0.49	$11.8^{g}\pm0.81$ (-17.39)	$9.2^{h,i}\pm0.42$ (-35.62)
MC	22.4 ± 0.56	$18.8^{a}\pm0.41$ (-16.14)	$16.6^{b,c}\pm0.46$ (-25.95)	13.7 ± 0.34	$12.2^{s}\pm0.79$ (-10.84)	$9.5^{h,i}\pm0.36$ (-30.71)
SF	23.1 ± 0.51	$19.0^{a}\pm0.33$ (-17.8)	$15.2^{b,c}\pm0.22$ (-34.12)	13.2 ± 0.81	12.2 ± 0.71 (-7.06)	$8.8^{h,i}\pm0.72$ (-33.31)
Soya	$26.2^{d}\pm0.48$	$21.3^{a,e}\pm0.64$ (-18.49)	$18.0^{b,c,f}\pm0.14$ (-31.24)	$16.9^{j}\pm0.79$	$14.3^{g,w}\pm0.42$ (-15.42)	$10.2^{h,i}\pm0.61$ (-39.38)
Casein	$27.4^{p}\pm0.56$	$22.0^{a}\pm0.61$ (-19.91)	$18.4^{b,c}\pm0.72$ (-32.96)	18.3 ± 0.99	$15.2^{g,w}\pm0.37$ (-16.62)	$10.7^{h,i}\pm0.34$ (-41.33)

Conditions of incubation: Same as in table 58 .

Table 60. Effect of p-nitrophenyl hydrazine on Total volatile fatty acids production when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h.

Protein source	TVFA produced (m moles/100 ml RFEq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	22.8±1.39	14.2[a]±1.72 (-37.72)	10.8[b]±1.72 (-52.63)	25.4±1.41	8.19±1.89 (-68.11)	6.3[h]±1.88 (-75.19)
MC	23.2±1.44	14.5[a]±1.41 (-37.5)	10.9[b,o]±1.31 (-53.02)	26.1±1.48	8.69±1.92 (-67.05)	6.5[h]±1.79 (-75.1)
SF	22.9±1.52	14.4[a]±1.33 (-37.12)	10.3[b,o]±1.44 (-55.02)	26.3±1.62	8.29±1 91 (-68.82)	6.2[h]±1.91 (-76.42)
Soya	25.4[P]±1.81	19.2[a,e]±1.62 (-24.41)	16.8[b,f]±1.42 (-33.85)	29.2±1.79	12.49[g,w]±2.04 (-57.53)	8.8[h]±2.02 (-69.86)
Casein	27.3±1.33	21.3[a]±1.69 (-21.98)	17.2[b,o]±1.56 (-36.99)	30.5±1.67	13.99±1.72 (-54.43)	9.1[h,u]±1.38 (-70.16)

Conditions of incubation: Same as in table 58.

Table 61. Effect of Hydroxylamine HCl on TCA precipitable proteins when various intact reed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h.

Protein source	TCA-precipitable protins left after incubation (mg)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	34.7±0.16	35.7[a]±0.27 (+2.94)	36.3[b,c]±0.37 (+4.69)	28.0±0.52	30.5[s]±0.44 (+8.93)	33.5[h,i]±0.91 (+20.26)
MC	31.0[d]±0.22	32.1[a,e]±0.33 (+3.38)	33.6[b,c,f]±0.22 (+8.61)	23.7[j]±0.59	29.8[g]±0.65 (+25.49)	32.3[h,u]±0.92 (+36.03)
SF	31.8[P]±0.31	33.4[a,e]±0.36 (+4.92)	35.2[b,c,f]±0.96 (+10.39)	23.9±0.96	28.9[g]±0.39 (20.54)	32.3[h,i]±0.77 (+34.78)
Soya	30.5[d]±0.28	31.7[q]±0.81 (+4.13)	32.3[n,f]±0.41 (+5.9)	20.5[j]±0.67	25.3[g,k]±0.67 (+23.84)	30.5[h,i,x]±0.72 (+48.9)
Casein	26.5[d]±0.39	28.0[a,e]±0.44 (+5.42)	28.9[b,c,f]±0.33 (+8.78)	19.1[v]±0.72	22.5[g,k]±0.81 (+17.89)	25.3[h,i,l]±0.88 (+33.00)

Conditions of incubation: Same as in table 49 except 0.1 and 1.0 m moles of hydroxylamine HCl in 1 ml buffer were added to incubation mixture instead of hydrazine.

Table 62. Effect of Hydroxylamine HCl on Ammonia produced when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h.

Protein source	Ammonia-N (mg/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	21.4 ± 0.53	$17.3^{a}\pm0.72$ $(-19.\overline{13})$	$15.2^{b,o}\pm0.77$ (-28.84)	17.6 ± 0.61	$12.2^{g}\pm0.82$ (-30.47)	$10.2^{h,u}\pm0.23$ (-42.17)
MC	20.4 ± 0.56	$17.3^{a}\pm0.26$ $(-14.\overline{88})$	$15.9^{b,o}\pm0.79$ (-21.95)	16.1 ± 0.72	$11.9^{g}\pm0.84$ $(-26.\overline{18})$	$9.5^{h,i,x}\pm0.19$ (-40.73)
SF	20.4 ± 0.72	$15.9^{a,e}\pm0.14$ (-21.95)	$14.5^{b,o,r}\pm0.28$ (-28.98)	$13.7^{j}\pm0.24$	$10.2^{g,w}\pm0.44$ (-25.45)	$8.7^{h,u,x}\pm0.26$ (-36.21)
Soya	$24.5^{d}\pm0.65$	$18.2^{a,e}\pm0.18$ (-25.72)	$16.7^{b,c,f}\pm0.33$ (-31.72)	$18.3^{j}\pm0.19$	$11.9^{g,w}\pm0.42$ (-35.17)	$10.2^{h,u,l}\pm0.44$ (-44.34)
Casein	25.1 ± 0.62	$19.3^{a}\pm0.79$ $(-23.\overline{19})$	$16.7^{b}\pm0.46$ $(-33.\overline{37})$	18.3 ± 0.15	$12.3^{g}\pm0.38$ $(-32.\overline{99})$	$9.5^{h,i}\pm0.48$ $(-47.9\overline{5})$

Conditions of incubation: Same as in table 61.

Table 63. Effect of Hydroxylamine HCl on Total volatile fatty acids production when various intact feed poroteins were incubated with washed bacterial suspension for 4 and 8 h.

Protein source	TVFA produced (m moles/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	23.8 ± 1.18	$16.2^{a}\pm1.31$ $(-31.\bar{9})$	$14.3^{b}\pm1.24$ $(-39.\bar{9}1)$	27.2 ± 1.31	$13.2^{g}\pm1.91$ $(-51.\bar{4})$	$11.8^{h}\pm1.42$ $(-56.\bar{6}2)$
MC	23.2 ± 1.65	$18.1^{a}\pm1.39$ $(-21.\bar{9}8)$	$15.1^{b,o}\pm1.41$ $(-34.9\bar{1})$	26.8 ± 1.44	$14.5^{g}\pm1.86$ $(-45.\bar{8}9)$	$12.5^{h}\pm1.39$ $(-53.\bar{3}6)$
SF	25.4 ± 1.72	$16.8^{a}\pm1.42$ $(-33.\bar{8}5)$	$14.8^{b}\pm1.46$ $(-41.\bar{7})$	16.9 ± 1.82	$14.4^{g}\pm1.72$ $(-46.\bar{4}7)$	$13.1^{h}\pm1.36$ $(-51.\bar{3})$
Soya	28.2 ± 1.84	$22.2^{a,e}\pm1.44$ $(-21.28\bar{)}$	$14.9^{b,c}\pm1.83$ $(-47.16\bar{)}$	30.1 ± 1.71	$26.7^{g,k}\pm1.22$ $(-11.29\bar{)}$	$23.8^{h,u,l}\pm1.22$ $(-20.93\bar{)}$
Casein	29.1 ± 1.19	$26.4^{a,q}\pm1.21$ $(-9.28\bar{)}$	$23.6^{b,o,f}\pm1.49$ (-18.9)	30.8 ± 1.94	$26.9^{s}\pm1.13$ $(-12.\bar{6}6)$	$24.2^{h,u}\pm1.17$ $(-21.43\bar{)}$

Conditions of incubation: Same as in table 61.

Table 64. Effect of Sodium Arsenate on TCA precipitable proteins when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h

Protein source	TCA-precipitable proteins left after incubation (mg)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	32.4±0.84	33.8[m,+]±0.75 (+4.54)	35.7[b,c]±0.21 (+10.31)	25.1±0.38	35.7[g]±0.44 (+42.19)	38.4[h,i]±0.12 (+52.94)
MC	31.4±0.25	32.9[m]±0.86 (+4.33)	34.1[b,f]±0.29 (+8.69)	25.9±0.49	36.3[g]±0.52 (+39.92)	37.7[h,u]±0.77 (+45.47)
SF	32.4±0.86	34.1[a,q]±0.12 (+5.47)	34.9[b,o,f]±0.38 (+7.94)	26.7±0.72	36.9[g]±0.65 (+38.13)	38.4[h,u]±0.59 (+43.51)
Soya	27.6[d]±0.92	29.3[m,e]±0.91 (+6.01)	31.4[b,o,f]±0.61 (+13.8)	23.4[j]±0.68	32.5[g,k]±0.38 (+38.68)	35.3[h,i,l]±0.18 (+50.6)
Casein	25.1[P]±1.01	27.7[m,q]±0.79 (+10.31)	29.2[n,o,r]±0.72 (+16.32)	21.8[j]±0.21	32.5[g]±0.42 (48.92)	34.6[h,i,x]±0.59 (+58.5)

Conditions of incubation: Same as in table 49. except 0.1 and 1.0 m moles of sodium arsenate in 1 ml buffer were added to incubation mixture instead of hydrazine.

Table 65. Effect of Sodium Arsenate on Ammonia produced when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 & 8h

Protein source	Ammonia-N (mg/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	28.4±0.36	21.8^a±0.84 (-23.19)	18.7^{b,c}±0.72 (-34.07)	24.5±0.88	14.5^g±0.72 (-40.81)	9.7^{h,i}±0.44 (-60.29)
MC	26.5^d±0.41	20.4^a±0.31 (-23.05)	16.5^{b,c,f}±0.65 (-37.66)	22.8^v±0.31	12.9^{g,w}±0.49 (-43.41)	9.7^{h,i}±0.91 (-57.37)
SF	27.3^p±0.32)	20.4^a±0.68 (-25.25)	15.6^{b,c}±0.33 (-42.77)	23.5±0.42	13.8^g±0.41 (-41.26)	8.1^{h,i}±0.83 (-65.39)
Soya	30.1^d±0.98	23.7^{a,e}±0.52 (-21.2)	19.5^{b,c,f}±0.85 (-35.07)	26.8^j±0.76	15.2^{g,k}±0.33 (-43.14)	10.2^{h,i,l}±0.32 (-61.86)
Casein	32.5±0.31	29.9^a±0.44 (-23.32)	20.8^{b,c}±0.34 (-35.8)	27.7±0.49	17.3^{g,k}±0.81 (-37.5)	10.2^{h,i}±0.44 (-63.11)

Conditions of incubation: Same as in table 64.

Table 66. Effect of Sodium Arsenate on Total volatile fatty acids production when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h.

Protein source	TVFA produced (m moles/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
		Concentration of inhibitor (m moles)				
	Control	0.1	1.0	Control	0.1	1.0
GC	23.7 ± 1.64	$10.8^a\pm1.44$ (-54.43)	$8.8^b\pm1.86$ (-62.87)	25.4 ± 2.05	$7.6^g\pm1.49$ (-70.08)	$4.4^h\pm1.89$ (-82.68)
MC	22.4 ± 1.55	$9.7^a\pm1.38$ (-56.7)	$7.4^b\pm2.06$ (-66.96)	23.3 ± 1.94	$6.5^g\pm1.55$ (-72.1)	$4.1^a\pm1.74$ (-82.4)
SF	22.2 ± 1.21	$9.3^a\pm1.49$ (-58.11)	$7.1^b\pm1.01$ (-68.02)	22.6 ± 1.56	$6.1^g\pm1.46$ (-73.01)	$4.1^h\pm1.72$ (-81.86)
Soya	25.9 ± 1.91	$12.8^{a,q}\pm1.22$ (-50.58)	10.2 ± 1.52 (-60.62)	$27.8^v\pm1.44$	$8.5^q\pm1.18$ (-69.42)	$6.4^h\pm1.76$ (-76.98)
Casein	26.8 ± 1.83	$14.1^a\pm1.16$ (-47.39)	11.5 ± 1.07 (-57.09)	29.6 ± 1.18	$9.2^g\pm1.29$ (-57.57)	$6.8^h\pm1.61$ (-77.03)

Conditions of incubation: Same as in table 64.

Table 67. Effect of Sodium Arsenite on TCA precipitable proteins when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h

Protein source	TCA-precipitable proteins left after incubation(mg)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitor (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	33.4 ± 0.36	$36.2^a\pm0.41$ (+8.46)	$37.1^b\pm0.91$ (+11.13)	27.1 ± 0.48	$34.4^g\pm0.61$ (+27.06)	$36.2^{h,i}\pm0.42$ (+33.66)
MC	$30.2^d\pm0.77$	$36.2^a\pm0.82$ (+20.11)	$37.1^b\pm0.77$ (+23.06)	26.7 ± 0.56	$34.4^g\pm0.69$ (+29.03)	$36.2^{h,u}\pm0.52$ (+35.77)
SF	30.9 ± 0.32	$35.7^a\pm0.91$ (+15.35)	$36.2^b\pm0.67$ (+17.2)	25.9 ± 0.47	$35.3^g\pm0.79$ (+36.41)	$36.2^h\pm0.61$ (+40.12)
Soya	$28.2^d\pm0.44$	$33.4^{a,e}\pm0.16$ (+18.69)	$35.2^{b,c}\pm0.62$ (+25.32)	$22.3^j\pm0.92$	$31.5^{g,k}\pm0.62$ (+41.59)	$35.7^{h,i}\pm0.44$ (+60.43)
Casein	$26.7^d\pm0.38$	$32.5^{a,q}\pm0.24$ (+21.87)	$35.2^{b,c}\pm0.38$ (+32.17)	20.8 ± 0.81	$30.2^{g,w}\pm0.37$ (+45.37)	$34.4^{h,i,l}\pm0.28$ (+65.94)

Conditions of incubation: Same as in table 49 except 0.1 and 1.0 m moles of Sodium Arsenite in 1 ml buffer were added to incubation mixture instead of hydrazine.

Table 68. Effect of Sodium arsenite on Ammonia produced when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h.

Protein source	Ammonia-N (mg/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitor (m moles)					
GC	24.3 ± 0.41	$18.3^{a} \pm 0.36$ $(-24.\overline{64})$	$16.2^{b,c} \pm 0.48$ $(-33.2\overline{6})$	19.9 ± 0.49	13.39 ± 0.31 $(-33.\overline{28})$	$9.5^{h,i} \pm 0.38$ $(-52.1\overline{6})$
MC	$22.8^{p} \pm 0.56$	$17.5^{a} \pm 0.65$ $(-23.\overline{38})$	$16.6^{b} \pm 0.33$ $(-27.\overline{36})$	18.3 ± 0.41	12.59 ± 0.65 $(-32.\overline{04})$	$9.1^{h,i} \pm 0.43$ $(-50.3\overline{})$
SF	22.2 ± 0.91	$17.5^{a} \pm 0.52$ $(-21.\overline{1})$	$162^{b,o} \pm 0.28$ $(-26.7\overline{3})$	17.5 ± 0.33	11.89 ± 0.58 $(-32.\overline{4})$	$9.1^{h,u} \pm 0.91$ $(-47.8\overline{8})$
Soya	$25.3^{d} \pm 0.78$	$19.5^{a,q} \pm 0.98$ $(-23.2\overline{3})$	$17.3^{b,c,f} \pm 0.12$ (-31.64)	$20.5^{j} \pm 0.35$	12.59 ± 0.56 $(-39.\overline{29})$	$10.5^{h,u} \pm 0.94$ $(-49.0\overline{6})$
Casein	27.2 ± 0.41	$19.9^{a} \pm 0.61$ $(-26.7\overline{4})$	$18.1^{b,o,r} \pm 0.38$ (-33.39)	20.8 ± 0.62	13.09 ± 0.18 $(-37.\overline{54})$	$10.8^{h,i} \pm 0.21$ (-48.00)

Conditions of incubation: Same as in table 67.

Table 69. Effect of Sodium arsenite on Total volatile fatty acids production when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h

Protein source	TVFA produced (m moles/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	21.7 ± 1.22	$9.3^a\pm1.45$ (-57.14)	$5.4^{b,o}\pm1.45$ $(-75.1\overline{1})$	24.9 ± 1.58	$6.5^g\pm1.14$ $(-73.\overline{89})$	$4.2^h\pm1.44$ $(-83.\overline{13})$
MC	21.2 ± 1.49	$9.2^a\pm1.61$ $(-56.\overline{6})$	5.1 ± 1.66 (-75.94)	23.4 ± 1.33	$6.2^g\pm1.22$ $(-73.\overline{5})$	4.5 ± 1.38 (-80.77)
SF	20.6 ± 1.19	$10.9^a\pm1.92$ $(-47.\overline{08})$	$5.3^{b,c}\pm1.69$ $(-74.2\overline{7})$	23.1 ± 1.28	$6.2^g\pm1.79$ $(-73.\overline{16})$	4.3 ± 1.06 $(-8\overline{1}.38)$
Soya	$24.3^p\pm1.28$	$16.3^{a,e}\pm1.83$ (-32.92)	$9.9^{b,c,f}\pm1.18$ $(-59,26)$	$28.5^j\pm1.71$	$9.6^g\pm1.61$ $(-66.\overline{31})$	$5.1^{h,i}\pm1.19$ $(-82.1\overline{)}$
Casein	$26.8^p\pm1.33$	$17.8^a\pm1.81$ $(-33.\overline{58})$	$12.6^{b,c}\pm1.63$ (-52.98)	30.1 ± 1.52	$9.9^g\pm1.63$ $(-67.\overline{11})$	$5.7^{h,u}\pm1.46$ $(-81.1\overline{1})$

Conditions of incubation: Same as in table 67.

Table 70. Effect of DDIC on TCA precipitable proteins when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h.

Protein source	TCA precipitable proteins left after incubation (mg)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (ppm)					
	Control	25	50	Control	25	50
GC	29.2 ± 0.68	$31.7^{m}\pm0.99$ (+8.56)	$33.4^{b,o}\pm0.64$ (+14.38)	26.4 ± 0.89	$35.4^{g}\pm0.86$ (+34.09)	$37.8^{h,u}\pm0.49$ (+43.18)
MC	$27.5^{p}\pm0.96$	$29.1^{m,q}\pm0.72$ (+5.81)	$30.4^{b,o,f}\pm0.34$ (+10.54)	$23.8^{j}\pm0.24$	$33.4^{g,w}\pm0.29$ (+40.33)	$36.5^{h,i}\pm0.65$ (+53.36)
SF	28.1 ± 0.37	$30.2^{m}\pm0.81$ (+7.47)	$31.6^{b}\pm0.47$ (+12.45)	$25.3^{v}\pm0.68$	$32.7^{g}\pm0.86$ (+29.13)	$35.7^{h,i}\pm0.39$ (+41.64)
Soya	$23.6^{d}\pm0.92$	$26.2^{a,e}\pm0.66$ (+11.01)	$28.7^{b,o,f}\pm0.63$ (+21.61)	$22.1^{j}\pm0.39$	$31.3^{g}\pm0.74$ (+41.62)	$33.6^{h,u,x}\pm0.97$ (+52.03)
Casein	$20.8^{d}\pm0.72$	$23.4^{a,e}\pm0.42$ (+12.5)	$25.9^{b,c,f}\pm0.99$ (+ 24.51)	$17.6^{j}\pm0.72$	$27.1^{j,k}\pm0.68$ (+53.97)	$31.4^{h,i,x}\pm0.33$ (+78.4)

Conditions of incubation. Same as in table 49. except 25 and 50 DDIC in 1 ml buffer were added to incubation mixture instead of hydrazine.

Table 71. Effect of DDIC on ammonia produced when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h.

Protein source	Ammonia-N (mg/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (ppm)					
	Control	25	50	Control	25	50
GC	27.5 ± 0.92	$20.6^{a}\pm0.75$ (-25.09)	$17.8^{b,c}\pm0.61$ (-35.27)	23.2 ± 0.33	$13.6^{g}\pm0.46$ (-41.37)	$8.9^{h,i}\pm0.38$ (-61.6)
MC	$25.3^{P}\pm0.61$	$19.3^{a}\pm0.25$ (-23.71)	$15.9^{b,c,r}\pm0.38$ (-37.15)	$21.6^{v}\pm0.55$	$12.4^{g}\pm0.61$ (-42.59(	$8.9^{h,i}\pm0.45$ (-58.79)
SF	26.8 ± 0.33	$19.1^{a}\pm0.61$ (-28.73)	$14.7^{b,c}\pm0.81$ (-45.14)	22.3 ± 0.62	$12.9^{g}\pm0.72$ (-42.15)	$7.1^{h,i}\pm0.33$ (-68.16)
Soya	$29.2^{P}\pm0.75$	$22.5^{a,e}\pm0.24$ (-22.94)	$18.8^{b,c,f}\pm0.7.7$ (-35.61)	$25.4^{j}\pm0.54$ (-35.61)	$14.3^{j,w}\pm0.91$ (-43.7)	$9.3^{h,i,x}\pm0.56$ (-63.38)
Casein	$31.7^{d}\pm0.61$	$24.3^{a,q}\pm0.32$ (-23.74)	$19.6^{b,c}\pm0.62$ (-38.17)	26.5 ± 0.61	$14.5^{j}\pm0.77$ (-37.73)	$9.1^{h,i}\pm0.46$ (-65.66)

Conditions of incubation: Same as in table 70.

Table 72. Effect of DDIC on Total volatile fatty acids production when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8h.

Protein source	TVF produced (m moles/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (ppm)					
	Control	25	50	Control	25	50
GC	23.2±0.35	21.7[m]±1.65 (-6.46)	19.6[b,o]±1.22 (-15.5)	25.1±1.16	20.2[g]±0.92 (-19.52)	17.4[h,i]±0.87 (-31.47)
MC	22.1±0.42	19.9[a,q]±0.92 (-9.95)	17.8[b,o,r]±1.01 (-19.95)	22.8[j]±1.12	18.0[g,w]±0.99 (-21.05)	15.1[h,i,x]±0.99 (-33.77)
SF	22.3±0.77	19.6[a]±1.01 (-12.1)	18.1[b]±0.99 (-18.83)	22.5±0.92	17.9[g]±1.11 (-20.44)	15.0[h]±1.06 (-33.3)
Soya	26.4[d]±0.81	23.8[a,e]±0.77 (-9.84)	21.1[b,o,f]±0.67 (-20.07)	28.7[g]±0.75	22.4[g,k]±0.99 (-21.95)	18.2[h,i,l]±1.12 (-36.58)
Casein	27.2±0.99	24.3[a]±0.75 (-10.66)	20.9[b,c]±0.72 (-23.16)	30.5[P]±0.63	22.8[g]±1.01 (-25.24)	18.9[h]±1.34 (-38.03)

Conditions of incubation: Same as in table70.

Table 73. Effect of various deaminase inhibitors on TCA precipitable proteins when casein hydrolysate was incubated with washed bacterial suspension for 4 and 8 h

Inhibitors	TCA precipitable proteins (mg/100 ml RF Eq)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitor (m moles or ppm)			
	0.1(25)	1.0(50)	0.1(25)	1.0(50)
Control	43.6_0.44		48.1+0.64	
Hydrazine	37.2^{a}+0.96 (-14.67)	33.4^{b,c}+0.19 (-23.41)	40.2^{g}+0.69 (-16.83)	36.3^{h,i}+0.95 (-24.53)
Hydrazine sulfate	36.8^{a}+1.02 (-15.59)	33.1^{b,c}+0.44 (-24.1)	36.1^{g,k}+0.72 (-18.8)	35.1^{h,i}+0.61 (-27.02)
Phenyl hydrazine	34.1^{a,q}+0.62 (-21.78)	29.3^{b,c,f}+1.16 (-32.79)	36.1^{g}+0.56 (-24.94)	29.4^{h,i,l}+0.86 (-38.87)
p-nitrophenyl hydrazine	32.4^{a,q}+0.47 (-25.68)	27.4^{b,c,r}+1.02 (-37.15)	33.8^{g,k}+1.29 (~29.72)	28.1^{h,i}+0.91 (-41.58)
Hydroxylamine HCl	40.6^{a,e}+1.06 (-6.88)	36.8^{b,c,f}+0.99 (-15.59)	42.4^{g,k}+0.38 (-11.85)	28.7^{h,i,l}+1.06 (-19.54)
Sodium Arsenate	30.5^{a,e}+0.38 (-30.08)	26.8^{b,c,f}+0.85 (-38.63)	31.4^{g,k}+0.79 (-34.71)	27.3^{h,i,l}+0.38 (-43.24)
Sodium Arsenite	29.8^{a}+0.44 (~31.65)	27.1^{b,o}+0.87 (-37.84)	32.6^{g}+0.81 (-32.2)	27.9^{h,i}+0.44 (-41.9)
DDIC (ppm)	30.8^{a,q}+0.64 (-29.35)	26.5^{b,c}+0.25 (-39.22)	31.0^{g}+0.44 (-35.55)	26.8^{h,i}+1.16 (-44.28)

Conditions of incubation: Same as in table 49, except casein hydrolysate(50 mg) was added to incubation mixture along with 0.1(25) and 1.0(50) m moles(ppm) of various deaminase inhibitors.

Table 74. Effect of deaminase inhibitors on Ammonia produced when casein hydrolysate was incubated with washed mixed bacterial suspension for 4 and 8 h.

Inhibitors	Ammonia-N (mg/100 ml RF Eq)			
	Time of incubation			
	4h		8h	
	Conc. of inhibitors (m moles or ppm)			
	0.1 (25)	1.0 (50)	0.1 (25)	1.0 (50)
Control	31.7 ± 0.24		27.6 ± 0.56	
Hydrazine	$28.1^{a}\pm0.24$ (-11.28)	$25.3^{b,c}\pm0.31$ (-20.14)	$19.38^{g}\pm1.08$ (-29.68)	$13.3^{h,i}\pm0.38$ $(+51.78)$
Hydrazine sulfate	$29.3^{a,q}\pm0.53$ (-7.72)	$26.8^{b,c,r}\pm0.92$ (-15.35)	$20.59^{g}\pm0.96$ (-25.51)	$17.6^{h,i,l}\pm1.01$ $(+36.28)$
Phenyl hydrazine	$30.2^{m}\pm0.87$ (-4.92)	$28.4^{b,c,r}\pm0.24$ (-10.4)	$18.99^{g}\pm0.33$ $(+31.56)$	$14.7^{h,i,x}\pm0.92$ $(+46.59)$
p-nitrophenyl hydrazine	$28.9^{a}\pm0.24$ (-8.86)	$25.1^{b,c,f}\pm1.06$ (-20.77)	$22.89^{g}\pm0.54$ (-17.42)	$15.4^{h,i,l}\pm0.34$ $(+44.23)$
Hydroxylamine HCl	$29.6^{a,q}\pm0.38$ (-6.62)	$27.6^{b,c}\pm1.11$ (-13.05)	$23.59^{g}\pm0.34$ (-14.69)	$20.5^{h,i,l}\pm0.91$ (-25.69)
Sodium Arsenate	$27.1^{a,e}\pm0.53$ (-14.47)	$24.8^{b,c}\pm0.91$ (-21.75)	$21.39^{g,k}\pm0.76$ (-22.6)	$14.8^{h,i,l}\pm0.38$ $(+46.26)$
Sodium Arsenite	$28.1^{a}\pm0.25$ (-11.25)	$26.2^{b,c,r}\pm0.02$ (-17.27)	$22.2^{g}\pm0.41$ (-19.48)	$163^{h,i,l}\pm0.42$ (-40.67)
DDIC (ppm)	$27.6^{a}\pm0.61$ (-12.93)	$24.6^{b,c,r}\pm0.18$ (-22.39)	$20.89^{g,w}\pm0.34$ (-24.63)	$14.2^{h,i,x}\pm0.44$ (-48.55)

Conditions of incubation: Same as in table 73.

Table 75. Effect of deaminase inhibitors on Total volatile fatty acids production when casein hydrolysate was incubated with washed mixed bacterial suspension for 4 and 8h.

Inhibitors	TVFA produced (m moles/100 ml RF Eq)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors (m moles or ppm)			
	0.1(25)	1.0 (50)	0.1(25)	1.0 (50)
Control	31.2±1.52		35.3±1.96	
Hydrazine	28.4±1.36 (-8.97)	24.6^{b,o}±1.32 (-21.15)	30.9^s±1.84 (-12.46)	24.4^{h,i}±1.79 (-30.88)
Hydrazine sulfate	28.8±1.44 (-7.69)	23.8^{b,c}±1.58 (-23.72)	31.3^s±1.76 (-11.33)	25.1^{h,i}±1.86 (-28.89)
Phenyl hydrazine	29.4±1.86 (-5.77)	26.3^b±1.47 (-15.7)	30.8^s±1.81 (-12.74)	22.6^{h,i}±1.77 (-35.97)
p-nitrophenyl hydrazine	28.6±2.16 (-8.33)	23.1^{b,o,r}±1.44 (-25.96)	29.7^g±1.52 (-15.86)	20.2^{h,i}±1.82 (-42.77)
Hydroxylamine HCl	29.2±1.52 (-6.41)	27.1^{b,r}±1.52 (-13.14)	33.2^w±1.63 (-5.94)	28.6^{h,u,x}±2.01 (-18.98)
Sodium Arsenate	25.1^{a,q}±1.98 (-19.55)	21.1^{b,f}±1.89 (-29,81)	23.5^{g,k}±2.14 (-33.43)	13.3^{h,i,l}±1.76 (-52.32)
Sodium Arsenite	25.7^a±1.76 (-17.62)	20.4^{b,o}±1.96 (-34.6)	24.6^g±1.91 (-30.31)	14.2^{h,i}±1.95 (-59.77)
DDIC (ppm)	27.1±1.39 (-13.14)	22.4^{b,o,r}±1.71 (-28.2)	24.2^g±2.01 (-31.44)	15.3^{h,i}±1.88 (-56.65)

Conditions of incubation: Same as in table 73.

Table 76. Effect of various deaminase inhibitors on TCA precipitable proteins when a mixture of amino acids was incubated with washed mixed bacterial suspension for 4 and 8 h

Inhibitors	TCA precipitable proteins (mg/100 ml RF Eq)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitor (m moles or ppm)			
	0.1(25)	1.0(50)	0.1(25)	1.0(50)
Control	35.6±0.49		46.3±0.81	
Hydrazine	31.9[a]±0.52 (-10.39)	29.3[b,o]±1.12 (-17.69)	39.8[g]±0.98 (-14.03)	36.3[h,i]±0.61 (-21.59)
Hydrazine sulfate	31.6[a]±0.42 (-11.23)	29.1[b,o]±1.24 (-18.25)	39.3[g]±0.44 (-15.11)	35.8[h,i]±0.37 (22.67)
Phenyl Hydrazine	30.7[a]±0.26 (-13.76)	27.5[b,c,r]±0.77 (-22.75)	36.1[g,k]±0.61 (-22.03)	31.2[h,i,l]±0.76 (-32.61)
p-nitrophenyl Hydrazine	28.6[a,q]±0.91 (-19.66)	25.2[b,c,r]±0.91 (-29.21)	33.8[g,k]±0.92 (-26.9)	26.7[h,i,l]±0.49 (-42.33)
Hydroxylamine HCl	32.8[a,e]±0.55 (-7.86)	31.4[b,f]±0.26 (-11.79)	41.6[g,k]±0.41 (-10.15)	36.8[h,i,l]±0.52 (-20.5)
Sodium Arsenate	27.4[a,q]±0.24 (-23.03)	24.2[b,c,f]±0.89 (-32.02)	30.1[g,k]±0.72 (-34.98)	25.3[g,i,l]±0.36 (-45.35)
Sodium Arsenite	28.1±0.66 (-21.06)	24.9[b,q]±0.76 (-30.05)	31.0[g]±0.61 (-33.04)	26.2[h,i]±0.51 (-43.4)
DDIC (ppm)	26.4[a,q]±0.62 (-25.84)	22.8[b,c,r]±0.42 (-35.9)	28.7[h,k]±0.88 (-38.01)	25.1[h,i]±0.46 (-45.75)

Conditions of incubation: Same as in table 49 except a mixture of amino acids (10 m moles) were added to incubation mixture along with 0.1(25) & 1.0(50) m moles(ppm) deaminase inhibitors

Table 77. Effect of deaminase inhibitors on ammonia produced when a mixture of amino acids was incubated with washed mixed bacterial suspension for 4 and 8h.

Inhibitors	Ammonia–N (mg/100 ml RF Eq)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors (m moles or ppm)			
	0.1 (25)	1.0 (50)	0.1(25)	1.0(50)
Control	32.2±0.46		30.5±0.37	
Hydrazine	30.1[a]±0.48 (-6.46)	26.6[b,c]±0.82 (-17.43)	22.1[g]±1.64 (-27.34)	17.5[h,i]±1.32 (-42.50)
Hydrazine sulfate	29.3[a,q]±0.77 (-9.07)	24.3[b,c]±1.79 (-24.35)	20.8[g]±0.16 (-31.57)	16.5[h,i]±1.13 (-45.91)
Phenyl hydrazine	30.6[q]±1.82 (-5.00)	27.0[b,o]±1.02 (-16.06)	21.5[g]±0.23 (-29.54)	17.6[h,i]±0.91 (-42.17)
p-nitrophenyl hydrazine	27.1[a,q]±0.49 (-15.68)	21.5[b,c,f]±0.38 (-33.27)	18.0[g,w]±1.72 (-40.99)	12.1[h,i,l]±0.76 (-60.12)
Hydroxylamine HCl	31.8[q]±0.86 (-1.34)	29.6[b,o,f]±1.45 (-7.89)	27.7[k]±2.05 (-9.22)	23.1[h,i,l]±0.22 (-24.25)
Sodium Arsenate	25.5[a,e]±1.93 (-20.75)	19.5[b,c,f]±1.33 (-39.54)	20.4[k]±0.74 (-33.02)	15.4[h,i,l]±1.83 (-49.46)
Sodium Arsenite	27.4[a,q]±0.58 (-14.78)	20.9[b,c]±1.17 (-35.19)	21.5[g]±0.38 (-29.50)	15.8[h,i]±1.21 (-47.98)
DDIC (ppm)	26.3[a]±0.47 (-18.32)	20.2[b,c]±0.87 (-37.26)	17.4[k]±0.81 (-42.95)	11.3[h,i,l]±0.33 (-62.95)

Conditions of incubation: Same as in table 6

Table 78. Effect of deaminase inhibitors on Total volatile fatty acids production when a mixture of amino acids was incubated with washed mixed bacterial suspension for 4 and 8 h.

Inhibitors	TVFA produced (m moles/100 ml RF Eq)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors (m moles or ppm)			
	0.1 (25)	1.0(50)	0.1(25)	1.0(50)
Control	46.7±1.08		54.3±1.67	
Hydrazine	42.8±3.08 (-8.35)	37.2^{b,c}±1.29 (-20.34)	44.6^g±1.16 (-18.61)	28.1^{h,i}±1.61 (-48.72)
Hydrazine sulfate	41.6^a±1.48 (-10.92)	36.6^{b,o}±2.41 (-21.62)	43.8^g±1.89 (-20.07)	26.4^{h,i}±2.31 (-51.82)
Phenyl hydrazine	42.6^a±1.22 (-8.78)	37.8^{b,o}±1.58 (-19.06)	44.2^g±2.16 (-19.34)	27.8^{h,i}±3.01 (-49.27)
p-nitrophenyl hydrazine	38.5^{a,e}±1.28 (-17.56)	31.1^{b,c,f}±1.72 (-33.19)	33.8^{g,k}±1.23 (-38.32)	21.5^{h,i,x}±1.19 (-60.76)
Hydroxylamine HCl	44.6^e±1.68 (-4.49)	41.7^{b,f}±1.77 (-10.71)	48.7^{g,k}±1.52 (-11.13)	40.9^{g,i,l}±1.26 (-25.36)
Sodium Arsenate	40.2^{a,q}±1.47 (-13.92)	30.4^{b,c,f}±1.02 (-34.9)	32.1^{g,k}±2.49 (-41.42)	20.4^{h,l,l}±1.57 (-62.77)
Sodium Arsenite	40.9^a±1.04 (-12.42)	32.1^{b,c}±1.86 (-31.26)	33.4^g±1.08 (-39.05)	20.7^{h,i}±1.41 (-62.22)
DDTC (ppm)	37.5^{a,e}±1.18 (-19.7)	29.7^{b,c,f}±1.61 (-36.4)	31.4^{g,w}±1.92 (-42.17)	19.1^{h,i}±1.39 (-70.34)

Conditions of incubation: Same as in table 76.

Table 79. Effect of Sodium sulfite on TCA precipitable proteins when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8h.

Protein source	TCA precipitable proteins left after incubation (mg)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	33.1±0.84	33.7±0.58 (+0.86)	34.2^{b,o}±0.23 (+3.01)	26.7±0.92	29.6±0.14 (+10.85)	31.5^{h,i}±0.86 (+17.81)
MC	31.9±0.76	32.6±0.77 (+2.01)	33.3^{n,r}±0.48 (+4.29)	26.7±0.81	30.1^{g,w}±0.19 (+12.8)	32.7^{h,i}±0.49 (+22.53)
SF	32.6±0.41	33.7^{m}±0.65 (+3.56)	34.2^{b,r}±0.31 (+4.91)	27.5±0.64	31.8^{g,w}±0.27 (+15.79)	32.7^{h}±0.81 (+19.14)
Soya	26.4^{d}±0.56	27.2^{e}±0.57 (+2.72)	27.8^{f}±0.92 (+5.33)	21.3^{j}±0.47	24.8^{g,k}±0.31 (+16.61)	25.8^{h,l}±0.69 (+20.88)
Casein	24.9^{p}±0.48	25.5^{e}±0.31 (+2.69)	26.1±0.81 (+4.94)	19.1^{j}±0.88	22.9^{g,w}±0.21 (+19.7)	24.7^{h,i,x}±0.52 (+28.94)

Conditions of incubation: Same as in table 49 except 0.1 and 1.0 m moles of sodium sulfite in 1 ml buffer were added to incubation mixture instead of hydrazine.

Table 80. Effect of Sodium sulfite on Ammonia produced when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8h.

Protein source	Ammonia-N (mg/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
		Concentration of	inhibitors (m moles)			
	Control	0.1	1.0	Control	0.1	1.0
GC	30.4 ± 0.77	$28.4^m\pm0.44$ (-6.83)	$26.4^{b,o}\pm0.81$ (-13.11)	27.6 ± 0.77	$25.4^s\pm0.84$ (-8.07)	$22.1^{h,i}\pm0.44$ (-19.98)
MC	29.1 ± 0.92	$26.6^{m,q}\pm0.72$ (-8.48)	$24.8^{b,o,r}\pm0.58$ (-14.77)	$25.4^j\pm0.34$	$23.1^{g,w}\pm0.49$ (-8.97)	$20.5^{h,i,l}\pm0.32$ (-19.41)
SF	$29.7^p\pm0.66$	$27.8^m\pm0.75$ (-6.62)	24.8 ± 0.47 (-16.57)	$26.3^v\pm0.41$	$24.3^{g,w}\pm0.31$ (-7.52)	$19.6^{h,i,x}\pm0.55$ (-25.34)
Soya	32.2 ± 0.59	$30.9^q\pm0.91$ (-4.01)	$29.1^{b,o,f}\pm0.23$ (-9.48)	$29.9^j\pm0.92$	$27.6^{g,k}\pm0.12$ (-7.49)	$22.8^{h,i,l}\pm0.59$ (-23.5)
Casein	$35.4^d\pm0.52$	$33.0^{m,q}\pm0.88$ (-4.99)	$30.6^{b,c,f}\pm0.19$ (-13.69)	30.9 ± 0.61	$28.9^{g,w}\pm0.76$ (-6.66)	$24.3^{h,i,x}\pm0.41$ (-21.33)

Conditions of incubation: Same as in table 79.

Table 81. Effect of sodium sulfite on Total volatile fatty acids production when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h.

Protein source	TVFA produced (mg/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	27.8±1.85	18.9^a±1.72 (-32.01)	14.3^{b,o}±1.45 (-48.49)	29.3±1.84	10.3^g±1.24 (-64.85)	8.4^h±0.99 (-71.33)
MC	25.3±1.91	19.7^a±1.41 (-22.13)	11.8^{b,c}±1. (-53.36)	28.1±1.67	10.5^g±1.61 (-62.63)	8.2^h±1.44 (-70.82)
SF	24.9±1.72	19.2^a±1.42 (-22.89)	10.6^{b,c}±1.62 (-57.43)	26.8±1.61	10.6^g±1.55 (-60.45)	8.6^h±1.86 (-67.91)
Soya	30.8^d±1.65	23.2^{a,q}±1.59 (-24.67)	15.3^{b,c,f}±1.44 (-50.32)	32.4^j±1.52	12.3^g±1.49 (-62.04)	11.9^h±1.92 (-63.27)
Casein	32.1±1.66	26.1^a±1.38 (-18.69)	17.6^{b,c}±1.72 (-45.17)	33.6±1.76	14.8^g±1.44 (-55.45)	12.3^h±1.12 (-63.39)

Conditions of incubation: Same as in table 79.

Table 82. Effect of chloroform on TCA precipitable proteins when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8h.

Protein source	TCA precipitable proteins left after incubation (mg)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	30.2 ± 0.52	30.7 ± 0.41 (+1.45)	31.1 ± 0.38 (+2.74)	28.1 ± 0.31	32.59 ± 0.39 (+15.5)	$33.4^{h,i}\pm0.71$ (+18.95)
MC	29.7 ± 0.61	30.2 ± 0.96 (+1.98)	$31.8^{n}\pm0.26$ (+7.08)	27.3 ± 0.68	31.19 ± 0.62 (+14.06)	$32.0^{h,u}\pm0.18$ (+17.06)
SF	$31.1^{p}\pm0.48$	31.9 ± 0.81 (+2.7)	$32.7^{r}\pm0.49$ (+5.21)	$29.8^{j}\pm0.41$	$31.1^{s}\pm0.71$ (+4.56)	$33.4^{h,i,l}\pm0.22$ (+12.28)
Soya	$26.7^{d}\pm0.42$	$25.3^{m,q}\pm0.52$	$26.1^{n,f}\pm0.61$	$24.3^{j}\pm0.18$	$28.29^{,k}\pm0.79$ (+16.1)	$29.8^{h,u,l}\pm0.48$ (+22.69)
Casein	$23.4^{d}\pm0.33$	$24.2^{m}\pm0.44$ (+3.33)	$25.0^{n}\pm0.72$ (+6.79)	$21.3^{j}\pm0.81$	$26.49^{,w}\pm0.64$ (+23.68)	$28.2^{h,u,l}\pm0.56$ (+32.5)

Conditions of incubation: Same as in table 49 except 0.1 and 1.0 m moles of chloroform in 1 ml buffer were added to incubation mixture instead of hydrazine.

Table 83: Effect of chloroform on Ammonia produced when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h.

Protein source	Ammonia-N (mg/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
		Concentration of inhibitor (m moles)				
	Control	0.1	1.0	Control	0.1	1.0
GC	28.7 ± 0.44	$27.1^a\pm0.38$ (-5.4)	$26.1^b\pm0.74$ (-8.86)	22.2 ± 0.76	$20.4^s\pm0.25$ (-7.76)	$17.3^{h,i}\pm0.69$ (-21.84)
MC	$27.5^p\pm0.56$	$26.4^m\pm0.41$ (-4.14)	$25.2^n\pm0.63$ (-8.39)	23.8 ± 0.84	$21.2^s\pm0.72$ (-11.04)	$16.8^{h,i}\pm0.61$ (-29.27)
SF	$26.1^p\pm0.49$	25.5 ± 0.89 (-2.6)	24.4 ± 0.27 (-6.54)	$21.4^j\pm0.52$	20.3 ± 0.66 (-5.5)	$17.3^{h,i}\pm0.35$ (-19.22)
Soya	$30.9^d\pm0.32$	$28.3^{a,e}\pm0.27$ (-8.45)	$27.3^{b,c,f}\pm0.16$ (-11.53)	$27.6^j\pm0.49$	$26.5^k\pm0.81$ (-3.9)	$24.5^{h,i,l}\pm0.44$ (-10.92)
Casein	$32.2^d\pm0.18$	$32.7^e\pm0.91$ (-1.36)	$30.3^{b,c,f}\pm0.22$ (-8.47)	$31.7^j\pm0.12$	$29.2^{g,k}\pm0.77$ (-7.92)	$27.1^{h,i,l}\pm0.32$ (-14.45)

Conditions of incubation: Same as in table 82.

Table 84. Effect of chloroform on Total volatile fatty acids production when various intact feed proteins were incubated with washed mixed bacterial suspension doe 4 and 8 h.

Protein source	TVFA produced (mg/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	23.4 ± 1.42	$18.3^a\pm1.25$ $(-21.\overline{79})$	$16.4^b\pm1.41$ $(-29.\overline{91})$	25.6 ± 1.67	$13.2^g\pm1.62$ $(-48.\overline{44})$	$8.9^{h,u}\pm1.79$ $(-65.2\overline{3})$
MC	$25.1^P\pm1.61$	$19.7^a\pm1.23$ $(-21.\overline{51})$	$17.6^b\pm1.54$ (-29.88)	27.2 ± 1.94	$15.6^g\pm1.18$ $(-42.\overline{65})$	$10.1^{h,i}\pm1.81$ $(-62.87\overline{)}$
SF	26.2 ± 1.67	$18.0^a\pm1.16$ $(-31.\overline{29})$	$16.3^b\pm1.59$ $(-37.\overline{78})$	27.6 ± 2.13	$14.8^g\pm1.29$ $(-46.\overline{38})$	$9.4^{h,i}\pm1.45$ $(-65.9\overline{4})$
Soya	29.1 ± 1.84	$25.1^g\pm1.69$ $(-13.\overline{7})$	$23.7^{b,f}\pm1.48$ $(-18.56\overline{)}$	$32.4^j\pm1.76$	$20.1^{g,k}\pm1.14$ $(-37.04\overline{)}$	$13.3^{h,i,x}\pm1.35$ (-58.95)
Casein	30.4 ± 1.52	27.2 ± 1.89	$26.1^b\pm1.33$	33.3 ± 1.84	$22.4^{g,w}\pm1.06$ $(-32.73\overline{)}$	$15.2^{h,i}\pm1.33$ $(-54.35\overline{)}$

Conditions of incubation: Same as in table 82.

Table 85. Effect of chloral hydrate on TCA precipitable proteins when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h.

Protein source	TCA precipitable proteins left after incubation (mg)					
	Time of incubation					
	4h			8h		
	Control	Concentration of inhibitors (m moles)		Control		
		0.1	1.0		0.1	1.0
GC	31.9 ± 0.72	32.64 ± 0.86 (+2.19)	$35.4^{b,c}\pm0.71$ (+10.8)	28.8 ± 0.44	33.39 ± 0.31 (+15.65)	$36.8^{h,i}\pm0.52$ (+27.54)
MC	$30.4^{P}\pm0.61$	31.35 ± 0.59 (+3.06)	$33.3^{b,o,r}\pm0.81$ (+9.33)	$25.5^{j}\pm0.92$	32.69 ± 0.49 (+28.2)	$34.4^{h,u,l}\pm0.81$ (+35.31)
SF	$31.9^{b}\pm0.52$	$32.85^{e}\pm0.24$ (+2.85)	$34.1^{b,c}\pm0.52$ (+7.01)	$27.5^{v}\pm0.31$	32.99 ± 0.12 (+19.6)	$35.2^{h,i}\pm0.76$ (+27.69)
Soya	$28.5^{d}\pm0.72$	$29.7^{e}\pm0.86$ (+4.43)	$30.1^{n,f}\pm0.34$ (+5.59)	$23.2^{d}\pm0.62$	$30.79^{g,k}\pm0.24$ (+32.18)	$32.7^{h,i,l}\pm0.69$ (+40.76)
Casein	$26.8^{P}\pm0.31$	28.1 ± 0.92 (+4.81)	$29.7^{b}\pm0.77$ (+10.81)	$21.3^{v}\pm0.66$	30.19 ± 0.71 (+40.89)	$31.9^{h,u}\pm0.41$ (+49.65)

Conditions of incubation: Same as in table 49 except 0.1 and 1.0 m moles of chloral hydrate in 1 ml buffer were added to incubation mixture instead of hydrazine.

Table 86. Effect of chloralhydrate on Ammonia produced when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h.

Protein source	Ammonia-N (mg/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	26.9 ± 0.41	$23.4^{a} \pm 0.84$ (-12.76)	$22.0^{b} \pm 0.72$ (-18.27)	23.7 ± 0.91	$20.2^{g} \pm 0.84$ (-15.01)	$16.8^{h,i} \pm 0.77$ (-29.13)
MC	$25.3^{p} \pm 0.86$	$21.3^{a,q} \pm 0.76$ (-15.64)	$20.3^{b,r} \pm 0.41$ (-19.87)	$21.0^{v} \pm 1.01$	$18.5^{w} \pm 0.86$ (-11.77)	$13.3^{h,i,l} \pm 0.72$ (-36.51)
SF	21.6 ± 0.72	$21.3^{a} \pm 0.31$ (-18.35)	$19.8^{b,o} \pm 0.68$ (-24.36)	21.4 ± 1.06	$19.2^{s} \pm 0.42$ (-10.26)	$14.8^{h,i,x} \pm 0.81$ (-31.12)
Soya	$29.3^{d} \pm 0.56$	$27.3^{a,e} \pm 0.23$ (-6.8)	$25.8^{b,o,f} \pm 0.61$ (-11.78)	$25.5^{f} \pm 0.84$	$22.1^{g,k} \pm 0.51$ (-13.14)	$18.4^{h,i,l} \pm 0.74$ (-27.85)
Casein	$31.2^{d} \pm 0.41$	$29.4^{a,e} \pm 0.18$ (-5.62)	$28.2^{b,o,f} \pm 0.59$ (-9.63)	$27.1^{v} \pm 0.79$	$23.4^{g,w} \pm 0.39$ (-13.82)	$19.3^{h,i} \pm 0.81$ (-28.79)

Conditions of incubation: Same as in table 85.

Table 87. Effect of chloral hydrate on Total volatile fatty acids when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8h.

Protein source	TVFA produced (m moles/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (m moles)					
	Control	0.1	1.0	Control	0.1	1.0
GC	27.8±2.07	24.2^m±1.41 (−12.$\overline{95}$)	20.3^{b,o}±1.94 (−26.98)	30.5±2.08	20.9^9±1.06 (−31.$\overline{47}$)	18.4^h±1.49 (−39.67)
MC	25.6±1.84	22.9^{a,q}±1.23 (−10.54)	19.6^{b,o}±1.86 (−23.44)	27.9±1.91	20.6^9±1.15 (−26.$\overline{16}$)	16.5^{h,i}±1.41 (−40.86)
SF	26.3±1.33	23.4^m±1.47 (−11.$\overline{03}$)	18.4^{b,c}±1.77 (−30.04)	27.3±1.72	19.2^9±1.32 (−29.67)	17.2^h±1.38 (−36.$\overline{99}$)
Soya	31.1^d±1.21	27.6^{m,q}±1.21 (−11.25)	22.1^{b,c,r}±1.19 (−28.94)	33.7^j±1.53	22.7^9±1.44 (−32.$\overline{64}$)	19.2^{h,u}±1.19 (−43.03)
Casein	33.4±1.16	29.9^{a,q}±0.96 (−10.48)	24.4^{b,c}±1.61 (−26.95)	35.3±1.42	25.6^9±1.97 (−27.$\overline{48}$)	21.4^{h,u}±1.72 (−39.38)

Conditions of incubation: Same as in table 85.

Table 88. Effect of Monensin on TCA precipitable proteins when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h.

Protein source	TCA precipitable proteins left after incubation (mg)					
	Time of incubation					
	4h			8h		
		Concentration of inhibitors (ppm)				
	Control	25	50	Control	25	50
GC	34.2 ± 0.42	$35.5^{a}\pm0.39$ (+3.83)	$37.2^{b,o}\pm0.92$ (+8.88)	32.1 ± 0.18	$36.19^{g}\pm0.26$ (+12.41)	$37.4^{h,i}\pm0.44$ (+16.48)
MC	32.8 ± 0.78	$34.2^{m,e}\pm0.17$ (+4.17)	$36.2^{b,c}\pm0.37$ (+10.17)	$30.6^{v}\pm0.72$	$35.29^{k}\pm0.29$ (+15.16)	$36.2^{h}\pm0.79$ (+18.2)
SF	33.5 ± 0.34	$35.0^{a}\pm0.45$ (+4.51)	$36.2^{b,o}\pm0.33$ (+8.13)	31.4 ± 0.61	$35.89^{k}\pm0.12$ (+14.1)	$37.4^{h,u}\pm0.85$ (+19.19)
Soya	$28.6^{d}\pm0.98$	$30.8^{a,e}\pm0.77$ (+7.66)	$32.7^{b,c,f}\pm0.18$ (+14.49)	$25.3^{j}\pm0.56$	$33.49^{k}\pm0.44$ (+31.89)	$34.1^{h,l}\pm0.72$ (+34.61)
Casein	$25.3^{d}\pm0.89$	$28.6^{a,e}\pm0.16$ (+12.79)	$30.8^{b,c,f}\pm0.23$ (+21.14)	$22.2^{j}\pm0.66$	$30.89^{k}\pm0.35$ (+38.81)	$32.8^{h,i}\pm0.06$ (+48.19)

Conditions of incubation: Same as in table 49 except 25_ and 50 monensin in 1 ml buffer were added to incubation mixture instead of hydrazine

Table 89. Effect of Monensin on Ammonia produced when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h

Protein source	Ammonia-N (mg/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (ppm)					
	Control	25	50	Control	25	50
GC	28.2 ± 0.34	$26.5^{a}\pm0.49$ (-6.04)	$23.8^{b,c}\pm0.75$ (-15.3)	21.6 ± 0.27	$19.6^{g}\pm0.43$ (-9.33)	$17.3^{h,i}\pm0.81$ (-20.15)
MC	$26.3^{d}\pm0.46$	$24.1^{a,e}\pm0.25$ (-8.24)	$21.3^{b,c,f}\pm0.66$ (-18.96)	$20.2^{v}\pm0.67$	$17.5^{j,k}\pm0.85$ (-13.250)	$15.2^{h,i,l}\pm0.54$ (-25.06)
SF	$27.2^{p}\pm0.81$	$25.4^{a}\pm0.38$ (-6.86)	$22.9^{b,c,f}\pm0.32$ (-15.82)	20.9 ± 0.63	$18.9^{s,w}\pm0.76$ (-9.88)	$15.8^{h,i}\pm0.51$ (-24.34)
Soya	$31.7^{d}\pm0.92$	$29.2^{a,e}\pm0.61$ (-7.88)	$26.3^{b,c,f}\pm0.41$ (-17.21)	$26.8^{j}\pm0.59$	$23.2^{j,k}\pm0.49$ (-13.74)	$19.3^{h,i,l}\pm0.43$ (-27.97)
Casein	$34.6^{d}\pm0.71$	$32.1^{a,e}\pm0.33$ (-7.25)	$28.5^{b,c,f}\pm0.85$ (-17.58)	$28.3^{j}\pm0.34$	$25.8^{j,k}\pm0.11$ (-8.7)	$20.8^{h,i,l}\pm0.24$ (-26.36)

Conditions of incubation: Same as in table 88.

Table 90: Effect of Monensin on Total volatile fatty acids production when various intact feed proteins were incubated with washed mixed bacterial suspension for 4 and 8 h.

Protein source	TVFA produced (m moles/100 ml RF Eq)					
	Time of incubation					
	4h			8h		
	Concentration of inhibitors (ppm)					
	Control	25	50.	Control	25	50
GC	25.3 ± 1.38	23.3 ± 1.91 (-5.53)	$18.6^{b,c} \pm 1.48$ (-26.48)	28.4 ± 1.85	19.89 ± 1.55 (-30.28)	$16.2^h \pm 2.03$ (-42.96)
MC	$21.4^P \pm 1.21$	21.3 ± 1.18 (-0.47)	$17.5^{b,o} \pm 1.55$ (-18.22)	25.6 ± 2.01	18.39 ± 1.68 (-28.51)	$14.5^{h,u} \pm 1.86$ (-43.36)
SF	23.2 ± 1.49	22.8 ± 1.49 (-1.72)	$18.8^{b,o} \pm 1.72$ (-18.96)	26.3 ± 1.18	17.59 ± 1.52 (-33.46)	$15.3^h \pm 1.44$ (-41.82)
Soya	$28.6^d \pm 1.66$	$26.4^w \pm 1.72$ (-7.69)	$19.2^{b,c} \pm 1.96$ (-32.86)	$30.9^v \pm 2.06$	$22.89^{,w} \pm 1.91$ (-26.21)	$18.2^{h,u} \pm 1.59$ (-41.1)
Casein	30.2 ± 1.72	28.8 ± 1.31 (-4.63)	$22.6^{b,c} \pm 1.81$ (-25.16)	33.1 ± 1.99	$25.39^{,k} \pm 1.57$ (-23.56)	$19.3^{h,i} \pm 1.61$ (-41.69)

Conditions of incubation: Same as in table 88.

Table 91. Effect of various methanogenic inhibitors on TCA precipitable proteins when casein hydrolysate was incubated with washed mixed bacterial suspension for 4 and 8 h.

Inhibitors	TCA precipitable proteins (mg/100 ml RF Eq)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors (m moles or ppm)			
	0.1 (25)	1.0 (50)	0.1 (25)	1.0 (50)
Control	37.6 ± 0.42		52.9 ± 0.79	
Sodium sulfite	$39.2^m \pm 0.81$ (+4.25)	$42.2^{b,c} \pm 1.04$ (+13.8)	$56.3^g \pm 0.52$ (+6.42)	$61.2^{h,i} \pm 0.86$ (+15.68)
Chloroform	38.4 ± 0.42 (+2.21)	$40.6^{b,o,r} \pm 0.72$ (+7.97)	$54.1^{s,w} \pm 0.55$ (+2.26)	$57.8^{h,i,l} \pm 0.89$ (+9.26)
Chloral hydrate	$40.3^{a,q} \pm 0.64$ (+7.18)	$44.8^{b,c,f} \pm 0.36$ (+19.14)	$58.6^{9,k} \pm 0.16$ (+7.91)	$65.3^{h,i,l} \pm 0.68$ (+24.38)
Monensin (ppm)	$42.9^{a,q} \pm 0.77$ (+14.09)	$45.3^{b,c} \pm 0.58$ (+20. 47)	$61.3^{9,k} \pm 0.43$ (+12.89)	$70.1^{h,i,l} \pm 0.44$ (+32.51

Conditions of incubation: Same as in table 43 except 10 ml of washed mixed bacterial suspension were added in incubation mixture instead of SRF.

Table 92. Effect of methanogenic inhibitors on Ammonia produced when casein hydrolysate was incubated with washed mixed bacterial suspension for 4 and 8 h.

Inhibitors	Ammonia-N (mg/100 ml RF Eq)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors (m moles or ppm)			
	0.1(25)	1.0(50)	0.1(25)	1.0(50)
Control	33.2±1.22		31.4±0.71	
Sodium sulfite	30.8[a]±0.24 (-7.02)	26.5[b,c]±0.84 (-20.07)	25.7[g]±1.16 (-18.24)	23.2[h,u]±1.01 (-26.26)
Chloroform	27.2[a,e]±0.16 (-18.08)	21.6[b,c,f]±0.37 (-34.78)	19.1[g,k]±1.21 (-39.02)	13.3[h,i,l]±0.72 (-57.7)
Chloral hydrate	29.4[a,e]±0.33 (-11.51)	25.8[b,c,f]±0.52 (-22.15)	24.4[g,k]±0.93 (-22.41)	20.8[h,i,l]±0.35 (-33.64)
Monensin (ppm)	28.6[a,q]±0.41 (-13.68)	23.5[b,c,f]±0.21 (-29.26)	21.8[g,k]±0.38 (-30.55)	17.5[h,i,l]±0.22 (-44.43)

Conditions of incubation: Same as in table 91.

Table 93. Effect of methanogenic inhibitors on Total volatile fatty acids production when casein hydrolysate was incubated with washed mixed suspension for 4 and 8 h.

Inhibitors	TVFA produced (m moles/100 ml RF Eq)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors (m moles or ppm)			
	0.1 (25)	1.0 (50)	0.1 (25)	1.0 (50)
Control	28.3 ± 1.97		33.2 ± 1.25	
Sodium sulfite	27.6 ± 1.23 (-2.47)	25.2 ± 1.76 (-10.95)	$30.4^{s}\pm1.16$ (-8.43)	$23.8^{h,i}\pm2.14$ (-28.31)
Chloroform	$22.8^{a,e}\pm1.54$ (-19.43)	$19.1^{b,o,f}\pm1.81$ (-32.5)	$25.2^{g,k}\pm1.59$ (-24.09)	$18.6^{h,i,x}\pm2.07$ (-43.97)
Chloral hydrate	$26.2^{a,q}\pm1.31$ (-7.42)	$22.3^{b,c,r}\pm1.23$ (-21.2)	$27.4^{g}\pm1.98$ (-17.46)	$21.6^{h,i}\pm1.98$ (-34.94)
Monensin (ppm)	$24.3^{m}\pm1.25$ (-14.13)	$21.7^{b,o}\pm1.43$ (-23.32)	$26.8^{g}\pm1.14$ (-19.28)	$19.3^{h,i}\pm1.51$ (-41.86)

Conditions of incubation: Same as in table 91

Table 94. Effect of various methanogenic inhibitors on TCA precipitable proteins when a mixture of amino acids was incubated with washed mixed bacterial suspension for 4 and 8 h.

Inhibitors	TCA precipitable proteins (mg/100 ml RF Eq)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors (m moles or ppm)			
	0.1(25)	1.0(50)	0.1(25)	1.0(50)
Control	41.3±0.63		53.7±0.38	
Sodium sulfite	43.1^m±0.61 (+4.35)	47.2^{b,c}±0.92 (+14.28)	57.4^9±0.38 (+6.89)	62.2^{h,i}±0.79 (+15.82)
Chloroform	42.6±0.77 (+3.14)	44.7^{b,c,f}±0.85 (+21.5)	55.3^{8,k}±0.82 (+2.97)	59.0^{h,i,1}±0.66 (+9.86)
Chloral hydrate	46.3^{a,e}±0.96 (+12.1)	50.2^{b,c,f}±0.58 (+21.5)	58.7^{9,k}±0.44 (+9.31)	67.2^{h,i,1}±0.38 (+25.13)
Monensin (ppm)	46.8^a±0.57 (+13.31)	51.6^{b,c}±0.72 (+24.93)	64.1^{9,k}±0.55 (+19.36)	71.1^{h,i,1}±0.82 (+33.51)

Conditions of incubation: Same as in table 46 except 10 ml of washed mixed bacterial suspension were added to incubation mixture instead of SRF

Table 95: Effect of methanogenic inhibitors on ammonia produced when a mixture of amino acids was incubated with washed mixed bacterial suspension for 4 and 8 h.

Inhibitors	Ammonia-N (mg/100 ml RF Eq)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors (m moles or ppm)			
	0.1(25)	1.0(50)	0.1 (25)	1.0 (50)
Control	30.5±0.28		27.5±0.83	
Sodium sulfite	30.1±0.87 (-1.18)	27.2^b±1.19 (-10.86)	23.6^g±0.76 (-14.28)	20.8^{h,i}±0.83 (-24.32)
Chloroform	28.4^{a,q}±0.74 (-6.66)	24.8^{b,o}±1.62 (-18.44)	19.1^g±1.24 (-30.46)	14.4^{h,i,l}±0.28 (-47.51)
Chloral hydrate	29.1^a±0.34 (-4.66)	25.3^{b,c}±1.73 (-16.84)	21.5^{g,w}±0.38 (-21.99)	17.6^{h,i,l}±1.13 (-35.84)
Monensin (ppm)	28.8^m±1.23 (-5.61)	26.2^{b,o}±1.04 (-17.33)	20.1^g±1.33 (-26.97)	15.7^{h,i}±0.98 (-42.82)

Conditions of incubation: Same as in table 94.

Table 96. Effect of methanogenic inhibitors on Total fatty acids production when a mixture of amino acids was incubated with washed mixed bacterial asuspension for 4 and 8h.

Inhibitors	TVFA produced (mg/100 ml RF Eq)			
	Time of incubation			
	4h		8h	
	Concentration of inhibitors (m moles or ppm)			
	0.1(25)	1.0(50)	0.1(25)	1.0(50)
Control	30.2 ± 1.23		34.1 ± 1.49	
Sodium sulfite	30.5 ± 1.76 (+0.99)	29.1 ± 1.83 (-3.64)	31.1 ± 2.11 (-6.74)	$27.2^h\pm1.93$ (-20.23)
Chloroform	27.8 ± 1.89 (-7.94)	$24.3^{b,o,r}\pm1.64$ (-19.53)	$25.39^k\pm1.28$ (-2.8)	$19.4^{h,i,l}\pm1.18$ (-43.11)
Chloral hydrate	30.6 ± 1.26 (+1.32)	$27.4^o\pm1.81$ (-9.27)	27.19 ± 1.25 (-20.52)	$22.7^{h,u,x}\pm1.74$ (-33.43)
Monensin (ppm)	28.8 ± 1.41 (-4.63)	$25.7^n\pm2.07$ (-14.9)	$26.59^n\pm1.76$ (-22.28)	$20.5^{h,i}\pm1.65$ (-39.88)

Conditions of incubation: Same as in table 94.